

**PERENNIAL CEREAL CROPS FOR THE COLD TEMPERATE ZONE: AGRONOMY,
PHYSIOLOGY, SINK REGULATION AND DISEASE RESISTANCE**

By

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ABSTRACT

PERENNIAL CEREAL CROPS FOR THE COLD TEMPERATE ZONE: AGRONOMY, PHYSIOLOGY, SINK REGULATION AND DISEASE RESISTANCE

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Within the last few decade, renewed attention has been devoted to the development of high yielding, herbaceous perennial plants that can meet the demand for food, forage and fiber in environmentally sustainable ways. While woody plants and annual herbaceous plants have been successfully selected to achieve high levels of reproductive allocation, herbaceous perennial plants with very high levels of sexual reproduction do not presently exist. The lack of high yielding herbaceous perennials is of intense interest to agronomists, horticulturalists, evolutionary biologists, population ecologists, ecophysiologicalists, and breeders.

However, perennial cereal crops face challenges if they are to be economically feasible, including genetic incompatibilities, resource tradeoffs between sexual reproduction and multi-year survival, and potential for disease buildup. In a series of studies between 2008 and 2012, I explore these issues within the context of three perennial cereal species: perennial wheat (*Triticum aestivum* x *Thinopyrum* spp.), perennial rye (*Secale cereale* x *montanum*) and intermediate wheatgrass (*Thinopyrum intermedium*).

Chapter 1 of this dissertation is a literature review dealing with the history of perennial cereal breeding, potential environmental benefits, and challenges that perennial cereals face. Chapter 2 reports on a two-year agronomic study of four accessions of perennial wheat and one accession of perennial rye. Chapter 3 describes a series of experiments exploring differences in

photosynthetic rates, and other traits affecting resource accumulation, between perennial and annual cereals. Chapter 4 explores the extent to which photosynthesis and metabolism in perennial cereals is responsive to changes in carbohydrate supply / demand ratio and whether this responsiveness differs between perennial and annual cereals. Finally, Chapter 5 explores the resistance of three perennial cereal species to the fungal disease *Fusarium* head blight (FHB).

In brief, we find the following.

- Perennial wheat achieves grain yields of approximately 50% of annual wheat, while perennial rye achieves 75% of annual rye. Both species show an ability to maintain these yields into their second year, and show later flowering than annuals.
- Perennial wheat, perennial rye and intermediate wheatgrass show 10-60% higher photosynthetic rates than their annual analogues. Intermediate wheatgrass shows declining photosynthetic rates with increasing plant age. These differences are driven primarily by biochemical rather than hydraulic changes.
- Perennial wheat and rye are more sink-limited than their annual analogues, and show an ability to mostly compensate for moderate source/sink changes. The perennial species appear to show a more conservative reproductive strategy than their annual relatives.
- Intermediate wheatgrass shows high resistance to FHB, while perennial rye is moderately susceptible and perennial wheat accessions vary in susceptibility.

Thus our studies provide novel contributions to the growing literature on perennial cereals, illustrating some physiological traits of perennial cereals as well as some of the problems they face. We hope that our results can contribute to advancing efforts to achieve high yielding herbaceous perennials, as well as to improving our understanding of how life history, source-sink balance and whole-plant age interact to affect resource acquisition rates.

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CHAPTER 1.

POTENTIAL BENEFITS AND CHALLENGES FACING PERENNIAL CEREAL CROPS

Overview

In recent years, considerable and increasing attention has been paid to the goal of trying to introduce greater perennality into human-managed ecosystems (Crews, 2005). In both tropical and temperate environments, techniques such as agroforestry, silviculture, planting shrubs and perennial herbs, and moving to perennial-dominated pastoral systems have for many years been promoted to achieve better functioning of ecosystem services within agricultural, forest, and semi-managed ecosystems (Ewel, 1999). These strategies can be seen as examples of a general trend towards increasing the amount of perennial vegetation within agroecosystems, as there are numerous environmental benefits that are hypothesized to correlate with increased perennality.

However, while agroforestry is a widely practiced technique with tremendous ecosystem-service benefits as well as some economic costs, as yet relatively little attention has been paid to trying to introduce perennial plants as main staple food crops. Woody perennials have an important role in food production systems across the world: they include sources of oil-rich nuts (walnut, hazelnut, apple, cashew, citrus), fruit trees (apple, cherry, pear, tamarind, mango and many others), fruiting shrubs (raspberry, blackberry), and in a few cases trees harvested for green leaves (e.g. *Moringa*). However, while these sources of fruits, vegetable products and oilseeds are critical nutritional components of human diets, there are currently very few perennial species which occupy the role of main food staples. The few perennial cereals or legumes that are widely grown include forage legumes like alfalfa (*Medicago*), pigeon pea (*Cajanus*), and a handful of others, yet their contribution to food production systems is marginal and/or limited to specific

regions of the world (*Cajanus*, for example, is a weak perennial mainly limited to India, the Caribbean and parts of Africa, and even there is often grown as an annual).

Trees and shrubs, because of their slow growth and long establishment period, face some inherent limitations in terms of their utility as staple food crops. While the role of woody perennials in agriculture can be expected to grow in future, and there are good nutritional as well as environmental reasons for shifting production land from staple grains to perennial fruits and vegetables, it is almost certain that for the near future herbaceous plants like cereals, oilseeds and legumes will occupy a key role in global food production systems. In recent years the case has been made that unless we begin growing perennial grasses, forbs and legumes as replacements for our staple food and forage crops, ultimately our ability to create sustainable agroecosystems that carry out more ecosystem services is limited.

Since about the mid-1990s, plant breeders have been trying to develop perennial crops that could supplement or replace grain, oilseed, and legume crops that we use today (Cox et al., 2006). In some cases they are making efforts to domesticate existing perennial grasses or legumes, such as intermediate wheatgrass (*Thinopyrum intermedium*), gamagrass (*Tripsacum dactyloides*), Illinois bundleflower (*Desmanthus*) and others. In other cases they are attempting to combine perennial traits with existing crops through hybridization. Millet, rice, wheat, rye, sorghum, and other grains (as well as sunflower) have wild perennial relatives with which they are interfertile, and potentially a successful hybrid cross might (as well as being reproductively feasible and fertile) combine high yielding genes of one parent with the genes for perenniality from the other. Some perennial plants are known to be highly productive (alfalfa, pigeon pea, etc.) at least for a few years (relative to shrubs or trees, these are better called ‘weak perennials’, and it is likely that perennial grains will be used as weak perennials as well.

The dearth of perennial cereals and legumes reflects an underlying reality of plant population ecology: currently, there are virtually no perennial herbaceous plants which achieve levels of reproductive allocation equivalent to the most productive perennial trees, perennial shrubs or annual herbaceous plants. In other words, the combination of extremely high reproductive allocation (e.g. over 20% of annual assimilation), herbaceous growth form and perennial life history represents a ‘missing’ combination of traits that is extremely rare either in nature or in cultivated agroecosystems (Van Tassel et al., 2010). Agroecosystems can be viewed as a novel ecological niche, created by human-directed artificial selection, and characterized by high fertility, regular disturbance, and management of herbivory.

Artificial selection has resulted in very high levels of reproduction in many cultivated annual cereals: for example, barley can achieve harvest index of over 60% (Kemanian et al., 2007). Wheat has achieved a harvest index of up to 55%, while corn has achieved over 50%. Some woody cultivated perennials can also achieve very high levels of reproductive allocation, once established. Though reproduction is typically low for the first few years, mature apple trees (*Malus domestica*) can achieve reproductive allocation of up to 80% (Xia et al., 2009). Blueberry (*Vaccinium corymbosum*) can achieve 55% (Pritts and Hancock, 1985) while olive (*Olea europaea*) can achieve 60% (Villalobos et al., 2006) and oil palm (*Elaeis guineensis*) can achieve 54% (Corley, 1983).

However, no perennial herbaceous plants with comparable levels of reproductive allocation are currently known. It is as yet unknown whether this reflects unknown metabolic and energetic tradeoffs in the perennial herbaceous life history, or rather because their combination of traits did not favor early domestication (Van Tassel et al., 2010). Thus, as plant breeders and agronomists attempt to develop sustainable and productive cropping systems based on herbaceous perennials,

it is critical to gather evidence as to the current levels of productivity in perennial cereals, grasses and legumes, as well as the potential for breeding to improve them. Equally, it is necessary to gain a better understanding of what factors influence perenniality vs. productivity tradeoffs from the ecological and physiological points of view, and to learn what physiological mechanisms high yielding herbaceous perennials could use to mitigate and negotiate these tradeoffs.

This review will first consider some of the promising current prospects for perennial cereal and legume crops, especially those adapted for the cold temperate, deciduous forest/prairie zone, and some of the environmental benefits that might be associated with increased perenniality in agroecosystems. We will then consider inherent ecological and physiological challenges that perennial cereals might face, and what mechanisms perennial cereals might use to reduce tradeoffs between seed production and long term survival. Finally, we will briefly outline how some hypotheses regarding perennial cereal physiology might be tested, and introduce the questions we will be exploring in the rest of this dissertation as well as methods we will be using.

Intermediate Wheatgrass: A Case Study in Domestication

Many of the world's most important staple food crops are either C3 or C4 grasses: rice, wheat, corn, barley, millet, sorghum, rye, and others. Thus, some of the initial efforts to develop perennial cereal crops have focused on developing new perennial grasses for food production, either through domestication of wild species or through hybridization between existing crops and wild relatives. One of the most prominent candidates for domestication is the perennial grass intermediate wheatgrass, *Thinopyrum intermedium* (formerly *Elytrigia* or *Agropyron*). Intermediate wheatgrass is a relatively high-yielding, cross-pollinated, rhizomatous grass with an autoallohexaploid genome, whose native range stretches from the Balkans across southern

Russia to Pakistan. It produces erect stems with erect basal leaves, of a bright green to bluish-green color: plants begin growing fairly late in spring, but grow quickly to a maximum height of 1-1.5 m (Smoliak, 1981). It was introduced into the United States as a forage grass in 1932, and grows well in moderately dry to mesic areas with temperate climates and at least 350 mm of rainfall. It can survive over 50 years, although when grown as a dryland forage grass it is encouraged not to be grown for more than 5 years.

Intermediate wheatgrass today grows as a naturalized grassland plant and forage/hay crop throughout the western and Great Plains states, as well as in New York, Massachusetts and Georgia (Berdahl and Redfearn, 2007; Malinowski et al., 2003; Moore et al., 1979). It has intermediate vegetative competitiveness, able to maintain itself against weed pressure without becoming a noxious weed itself; under moderate herbicide levels in areas infested with downy brome, emergence is between 300-400% better with intermediate wheatgrass than with another closely related forage, crested wheatgrass (Evans et al., 1967). Indeed both “Reliant” and pubescent subspecies of intermediate wheatgrass have been used to reduce leafy spurge prevalence by up to 72% three years after planting (Lym and Tober, 1997). Other perennial wheatgrasses (e.g. *T. ponticum*, *T. bessarabicum*) have been found to have considerable salt tolerance, being able to survive up to 2.5 to 7.5% NaCl solutions; introgressions of *T. ponticum* chromosomes into bread wheat have been shown to improve salt tolerance, resulting in only 15% declines in seed yield at salt levels which cause a 50% decline in normal bread wheat (Colmer et al., 2006). It remains to be seen to what extent intermediate wheatgrass has similar salt tolerance in the field; greenhouse experiments in Texas (Miyamoto et al., 2004) found that some cultivars of *T. intermedium* were moderately salt-tolerant grass, registering only a 25% reduction in shoot growth at salt levels corresponding to about 6-8 dS m⁻¹ (roughly 3000-4500 ppm NaCl, or 0.3-

0.45% salt). Finally, intermediate wheatgrass is a reasonably high-yielding grass. The older cultivars (e.g. ‘Greenar’ in the 1930s) averaged 200-400 kg ha⁻¹ of seed yield, but more recent cultivars have achieved up to 900 kg ha⁻¹ under ideal conditions.

Although the benefits of intermediate wheatgrass as a forage crop are known (moderately high biomass yield, large seed, competitiveness with weeds, salt tolerance, strong perenniality), it has not yet been selected as a grain crop for high seed yield. As currently seed yields are under 900 kg ha⁻¹, much more selection needs to be undertaken in order to make it an economical grain crop. Knowles (1977) was able to increase seed yield in a population by 10% per cycle by using mass selection, picking the 5% highest yielding plants at each cycle; when pollination was controlled by removing selected plants to the greenhouse during the winter, he was able to achieve 20% gains in seed yield per cycle. Further efforts were made to select intermediate wheatgrass for higher yields during the mid 1990s; it was selected as a promising candidate for domestication after scanning about 100 perennial grasses on the basis of the following criteria: palatability of seeds, threshability, medium to large seed size, synchronous seed maturity, resistance to shattering, tendency not to lodge, seedheads held above the foliage level, drydown of seed stalks at maturity, high potential for mechanical harvest, and vigorous perennial growth (Wagoner and Schauer, 1990). Wagoner (1995) cross-pollinated 20 of the highest yielding accessions (out of 300) and eventually derived 14 accessions which had yields 25% higher than the original population mean.

Hybridization of Grain Crops (Poaceae) with Perennial Wild Relatives

Improving productivity of perennial grasses through repeated selection will presumably be a long and slow process. The development of modern, high-yielding annual crops from their wild forebears took place over thousands of years, and few additional crops have been developed in recent centuries. However, it has been argued that a 'shortcut' is available to the development of high-yielding perennial grain and grass crops, through hybridizing existing annual crops with perennial wild relatives (Cox et al., 2002). In this case, the theory is that since the hard work of selecting for high yield has already been done, the introgression of part of the genome from the perennial parent could introduce perenniality into succeeding generations while still maintaining reasonably high yields. It is possible, however, that introducing perennial chromosomes into an existing annual cereal could result in large declines in yield, if they result in the loss (or simply lower levels of expression) of QTLs which contribute to high grain yield.

Many annual grain crops are known to have perennial wild relatives with which they are interfertile, and in some cases these wild relatives have been used in hybridizations in the past (usually to introduce some desirable trait such as reduced lodging, increased salt tolerance, pest resistance, etc.). Some potential or actual crosses of crops and their wild relatives are as follows: all are known to be interfertile but a * indicates that there are few problems with sterility or embryo rescue.

Rice (*Oryza sativa*) and *Oryza rufipogon**

Sorghum (*Sorghum bicolor*) and johnsongrass (*Sorghum halepense*)*

Sorghum (*Sorghum bicolor*) and *Sorghum propinquum*

Oats (*Avena sativa*) and *Avena macrostachya*

Corn (*Zea mays*) and eastern gamagrass (*Tripsacum dactyloides*)

Wheat (*Triticum aestivum*) and wheatgrass species (*Thinopyrum intermedium*, *Th. elongatum* or *Th. junceum*)*

Millet (*Pennisetum glaucum*) and Napiergrass (*Pennisetum purpureum*)

Rye (*Secale cereale*) and *Secale montanum**

(Cox et al., 2002).

Perennial Wheat and Rye: Case Studies in Grass x Cereal Hybridization

In parallel with the efforts to develop intermediate wheatgrass into a commercially viable perennial cereal crop, efforts have been made in the last few decades to develop a hybrid perennial wheat species, through hybridizing annual wheat (*Triticum aestivum*) with various *Thinopyrum* species (*Th. intermedium*, *Th. elongatum* and *Th. junceum*). In addition, breeding efforts have successfully developed perennial cultivars of rye through hybridizing annual rye (*Secale cereale*) with *S. montanum*.

Annual wheat has varying degrees of cross-fertility with *Thinopyrum* species, and over the last century breeding efforts have repeatedly crossed wheat with one or more *Thinopyrum* species in order to introgress specific traits of interest into the wheat cultivar. Wheat has been crossed with *Th. junceum* in order to achieve greater salt tolerance (Wang et al., 2003), while crosses with *Th. bessarabicum* and *Th. elongatum* have been made to achieve combined resistance to high salinity and low oxygen (Akhtar et al., 1994). Crosses with *Th. bessarabicum* have also been made in order to introduce resistance to copper, aluminum or manganese toxicity. Likewise, crosses with *Th. intermedium* have been made in order to achieve resistance to leaf rust, stem rust, stripe rust and Fusarium head blight. The popular Chinese cultivar Xiao Yan 6 has been developed through two chromosomal substitutions from *Thinopyrum elongatum* (a

congeneric of intermediate wheatgrass) and combines high yield potential with resistance to oxidative stress and photoinhibition, resistance to *Septoria tritici* and stem rust, and tolerance of high temperatures, and is considered a successful Chinese cultivar (Li et al., 2008).

The long period of development of cultivars like Xiao Yan 6 (the *Thinopyrum*-wheat cross was made in 1960 and the variety finally released in 1977) illustrates that intergeneric hybridization is a complex and challenging procedure (Li and Wu, 2006; Li et al., 2008). However, the example of these past crosses does show that many wild relatives can be successfully hybridized with wheat, and that there are few immediate barriers to intergeneric hybridization. While such hybrids are usually self-sterile, fertility can be restored either through back-crossing to wheat or to the wild parent (e.g. Murphy et al., 2010) or by using colchicines to double the genome.

Until recently, however, little effort has been devoted to hybridizing wheat and its wild relatives for the specific objective of developing a perennial cereal species. Perenniality is a more difficult trait to achieve than resistance to a particular pathogen, as it is a complex life history adaptation involving changes in many genes, and has profound effects on patterns of allocation, senescence and growth. However, attempts to develop perennial wheat began with Soviet breeding efforts in the 1900s through 1930s. Unfortunately, these efforts were later abandoned and little literature on perennial wheat crosses was communicated to the scientific establishment in western countries. In general, they showed poor perennial regrowth and yielded good harvests in the first year (Tsitsin, 1965). In the 1960s, efforts to breed perennial wheat went on at the University of California, Davis, but were abandoned by 1965 due to insufficient yields. The genotype ‘MT-2’, developed through hybridization between *Th. intermedium* and *Triticum turgidum*, suffered from low kernel mass and unreliable persistence (Schulz-Schaeffer and

Haller, 1987) while similar crosses in Sweden also suffered from low yields. Most lines required reseeded after 2-3 years, and suffered from reduced winter hardiness, high mortality rates, and rapidly declining yields in the second and third years. Thus efforts to breed perennial wheat remained of marginal interest to the broader scientific and agronomic communities (Wagoner, 1990). By the 1990s, however, it became clear that perennial wheat might be a feasible crop in spite of achieving lower yields than annual wheat, if it could compensate for lower yields through improved ecosystem services. In addition, breeding efforts on wild Triticeae grasses (for forage purposes) had helped produce higher yielding, strongly perennial accessions that might be good perennial parents for developing truly perennial hybrid cereals.

Since the late 1990s, efforts have been ongoing at Washington State University (Pullman, WA) and the Land Institute (Salina, KS) to develop amphiploid hybrids of wheat with *Thinopyrum* relatives, which combine acceptably high yields with perennial life history. Wild grass species which have been used as perennial parents in crosses with wheat included intermediate wheatgrass (*Th. intermedium*), Russian wheatgrass (*Th. junceum*) and tall wheatgrass (*Th. elongatum*). Early attempts at perennialization of wheat included crosses with *Th. intermedium* (Banks et al, 1993; Scheinost, 2001), as well as the cross ‘AgCS’ with *Th. elongatum*, which displayed perenniality. A few years later, genetic analysis of ‘AgCS’ found that the ability for perenniality was linked to chromosome 4E of *Th. elongatum* (Lammer et al., 2004). A QTL on this chromosome allows the capacity for post sexual cycle regrowth to be turned on or off, although loci on other chromosomes may contribute to the degree of perenniality, and indeed the amount of post sexual cycle regrowth (PSCR) may be linked to the amount of perennial ancestry.

Because of its smaller chromosome complement, which will be described in further detail

below, *Thinopyrum elongatum* generally is more likely to produce stable hybrids with wheat than either *Th. junceum* or *Th. intermedium*. For this reason, beginning in the late 1990s, efforts were undertaken at Washington State University (Pullman, WA) to hybridize *Th. elongatum* with perennial wheat, with the goal of developing a robust, highly productive perennial hybrid. Both for purposes of improving fertility and gaining more productivity-related traits from annual wheat, it was decided to back-cross the amphiploids to annual wheat (Wagoner et al., 1990). In 1998, the spring wheat cultivar ‘Chinese Spring’ was crossed with *Th. elongatum*, and the resulting hybrid line was backcrossed to the winter wheat ‘Madsen’. Approximately 500 single plants were selected from the F₂ generation of these hybrids, and were grown out (including self fertilization) to increase seed for two more generations. Out of these 500 lines, 31 were selected for field experiments in 2005-2006 (on the basis of showing acceptable regrowth). These lines were tested for seed yield and regrowth, as well as nutritional characteristics, at Washington State (Murphy et al., 2009, 2010) and also form the source of the lines used in studies to be described later in this dissertation.

Simultaneously, efforts have been ongoing at the Land Institute (Salina, KS) to hybridize various perennial wheatgrass species with wheat, as well as at various other locations in China, Argentina, Russia, Mexico and India. Perennial parents have included *Th. junceum*, *Th. intermedium*, *Th. elongatum* and the decaploid *Agropyron elongatum*, while annual parents included *Triticum aestivum*, *Triticum durum*, and *Triticum carthlicum*. A range of over 150 of these accessions was tested for seed production, disease resistance and perennial regrowth in Australia between 2009 and 2011 (Hayes et al., 2012), compared against the annual cultivars ‘Wedgetail’, ‘Naparoo’ and ‘Ventura’ as well as perennial grasses *Th. intermedium* and *Secale montanum*. A concurrent study in China examined morphological characters and perennial

regrowth in a hybrid of wheat x *Elymus*, backcrossed to *Th. intermedium* (Shi et al., 2011).

Similar breeding efforts, with somewhat greater success thus far, have been devoted to breeding a perennial version of rye. Annual rye (*Secale cereale*) is an important cereal crop in cold-temperate regions of the world, used for food, cover, and forage, and particularly important as a food crop in parts of Europe. Since the early 1980s, efforts have been ongoing to hybridize annual rye with a perennial wild relative, *Secale montanum*. *S. montanum* is known to have desirable traits including cold tolerance, ability to tolerate high levels of heavy metals, weed-competitiveness, and high protein content (Tang et al., 2011; Kubiczek et al., 1981). *S. montanum* has been found to have smaller yields and kernel weights than annual rye, but also to have 50% higher protein content, higher mineral content and adequate baking qualities (Füle et al., 2005).

Various efforts have been made over the past decades to hybridize *S. cereale* with *S. montanum*, and have generally been somewhat more successful than similar efforts with perennial wheat. However, *S. montanum* itself has only 20% the grain yield of annual rye, and thus it has been difficult to identify hybrid lines that are both strongly perennial and high yielding (Cox et al., 2002). Previous experiments with breeding perennial rye found that perennial growth habit required homozygosity for a few critical chromosomes from the perennial parent; however, such homozygosity was rarely achieved, and those lines which were homozygous for the desired chromosomes showed low yields due to their preponderance of *S. montanum* genetics. Efforts were made to develop a new cultivar, “Black Mountain”, through backcrossing the amphiploid parent twice to *S. montanum*. However, though perennial, this cultivar showed declining grain yields over time, and was useful primarily as a forage (Oram 1996).

Reimann-Philipp (1995) took a new approach, using colchicine to double the chromosome number of the *S. cereale x montanum* amphiploid, and develop a tetraploid rye, 'Permontra'. This cultivar achieved a better combination of high grain yield and perenniality than previous lines. Reimann-Philipp (1986) found yields of 2.2 in the first year and 2.4 tons ha⁻¹ in 'Permontra' perennial rye, thus demonstrating good ability for regrowth and yield stability over at least two years. In a second study, however, yields declined over time due to weed pressure. A later study involving both "Permontra" perennial rye and intermediate wheatgrass (*Thinopyrum intermedium*) found that pure stands of perennial rye yielded 1.7 – 2.7 tons ha⁻¹ in its first year (Weik et al., 2002). By the second year, however, yields decreased by 73-88 %, most likely due to weed pressure and nitrogen limitation (for example, in pure stands of perennial rye, weed biomass increased 2500% between the first and second years). Yield stability was somewhat higher in mixed stands of perennial rye and lupin, in which "Permontra" yields declined only 50% between the first and second year. These studies would seem to indicate that both nitrogen limitation and weed competition play a role in the ability of perennial cereals to regrow.

More recently, intensive efforts to develop a commercially viable perennial rye species have been ongoing in Canada. Acharya et al. (2003) developed the new forage cultivar 'ACE-1' from a *Secale cereale x montanum* cross. In a multi-year study, the ACE-1 perennial rye cultivar produced 32% more biomass than annual rye when averaged over 2 years, but 16% less biomass than annual rye when averaged over three years, which is evidence of a rapid decline in forage yields over time (Acharya et al, 2003). In a subsequent study, *Secale cereale x montanum* 'ACE-1' produced around 55% of annual rye ('Kodiak') yields when averaging across 1-year old, 2-year old, and 3-year old plants; biomass yields of the perennial rye were between 5% and 190% higher than annual rye (Acharya et al., 2004).

Potential Environmental Benefits of Perennial Agroecosystems

One of the primary reasons for the interest in large-scale perennialization of agriculture, is the belief that perennial ecosystems can maintain higher levels of ecosystem services. It has been hypothesized that perennial systems might have numerous advantages in terms of ecosystem services, which could include 1) lower nutrient leaching, 2) less soil erosion, 3) lower use of agricultural chemicals, 4) higher populations of pollinator and predatory insects, and 5) speculatively, more efficient and mutualistic relationships with mycorrhizae. Some key pieces of evidence supporting some of these hypotheses is outlined below.

Nutrient Usage and Leaching

There is some evidence that perennial systems could greatly reduce nitrogen leaching to almost zero (Dowdell and Webster, 1980; Crews, 2005) could effectively sequester carbon into long-term, recalcitrant organic pools in the soil, and stabilize the soil. There is also evidence that perennial grasses, if developed into grain crops, could be more efficient users of nutrients. Ridley et al. (2001) compared Australian pastures sown with annual (clover/ryegrass) and perennial (clover/perennial grass) mixtures, subjecting each pasture to rotational sheep grazing and allowing annual plants to replace themselves over the subsequent two seasons through natural seed dispersal. They found that annual nitrogen losses were 33% lower in unlimed perennial pastures and 45% lower in limed perennial pastures as compared to annuals; this is, of course, a highly conservative estimate of the difference since the “annual” pastures included a certain percentage of clover cover which may have increased over the three years of the study. Tallgrass

prairie in Kansas, as an example of a largely perennial grassland system, showed nitrogen leaching of less than $1 \text{ kg ha}^{-1} \text{ yr}^{-1}$, while conventionally grown continuous corn grown with 187 kg of N led to leaching losses of $60 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (over 30%); under similar conditions barley grown with 120 kg of N lost 8%, or $10 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Crews and Peoples, 2005; Crews, op. cit.). Fescue and alfalfa systems are known to have 86% less nitrate leaching during the lifespans of the plants, but nitrate leaching undergoes a temporary burst as soon as the land is tilled (Bergstrom, 1987).

Potential mechanisms of reduced nutrient leaching in perennial cropping systems, especially perennial polycultures, include nutrient retranslocation, and deeper, more long-lived root systems (Crews, 2005). Comparisons of closely related annual and perennial grass species (Roumet et al., 2006) and of ecologically similar intermountain perennial and annual grasses (Arredondo et al., 1998) have found that perennial grasses tend to have thicker roots that are less efficient at nutrient uptake but longer-lived. Some perennial species, for example intermediate wheatgrass have much deeper and larger root systems than closely related annuals (Cox et al., 2006), and in these cases the deeper root systems could recover nutrients from deeper soil levels.

Nevertheless, even when perennial grasses are relatively shallow-rooted, the year-round presence of living roots enables more nutrients and water to be retained by the soil. A study of perennial and annual grasses in grasslands in Spain found that perennial grasses took up about 25% more nitrogen over the course of a growing season. Interestingly, N uptake by perennials was 82% of the total 'potentially available' nitrogen in the early part of the growing season (October-January), while N uptake by annuals was only 52% of the potentially available N (the potentially available N levels being similar for annuals and perennials). The authors took this as

evidence of leaching: “[t]he difference between the nitrogen effectively absorbed and the nitrogen likely to be absorbed by the annuals was probably due to partial leaching of the nitrates following the autumn rains....” (Joffre, 1990). Net mineralization in soils supporting perennial grasses was higher throughout the autumn rainy period than in soils supporting annual grasses; since net mineralization is negatively related to leaching, this is evidence that leaching was lower as well.

Perennial grasses can also be expected to use nutrients more efficiently through nitrogen recycling. In a study of fescue (*Festuca rubra*) and common bent (*Agrostis capillaris*), labeled ^{15}N was used to determine the provenance of the nitrogen in next year’s regrowth. It was found that 70% and 82%, respectively, of the nitrogen used in early spring regrowth was from nitrogen retranslocated and stored from the last year’s leaves. This declined to 35% and 45% respectively by June (Bausenwein *et al.*, 2001). Spring growth of perennial grasses under N-limited conditions has been found to be related to levels of nitrogen availability in the previous fall (Gloser, 2005).

The phenomenon of nitrogen retranslocation would reduce leaching if nitrogen is retranslocated out of senescing leaves instead of remaining in the leaves as they die, decay, and are gradually mineralized: the amount of mineralized nitrogen in the soil would be lower, and less could be vulnerable to leaching. Indeed, perennial grasses are able to control rates of nitrogen loss through the litter: under low-nitrogen conditions, they can increase retranslocation of nitrogen from senescing leaves to roots. In an aquatic (hydroponic) analogue of nitrogen-stress conditions for perennial grasses, wheatgrass (*Agropyron dasystachyum*) were first supplied with labeled nitrogen fertilizer and then transferred to either high- or low- N conditions. Under low nitrogen conditions, plants had much more labeled N in new leaf growth, suggesting that the

labeled N had been effectively conserved and translocated to growing leaves: in the high-N treatment, the lower N translocation to leaves corresponded to increasing N escaping into the solution (presumably equivalent to leaching under terrestrial conditions). Low-N plants leaked no measurable amount of labeled N into their surrounding solutions, while high N plants leaked about 12% of their total stored nitrogen into the surrounding solution. If these results hold true in natural, field conditions as well as in the greenhouse, they constitute powerful evidence that perennial grasses are able to reduce leaching losses, when necessary, to almost zero through effective nutrient recycling (Li et al., 1992). Perennial grasses under low nitrogen conditions have been found to have lower N losses through leaf litter and higher N losses through root senescence and exudation (suggesting increased translocation and storage of nitrogen in roots), while overall nitrogen losses are lower than under high-nutrient conditions- in other words, the decreased leaf losses more than counteract the increased root losses (Vasquez de Aldana et al., 1996). Gusewell (2005) found similar evidence of nitrogen translocation, but in contrast to the previously cited paper, found that rates of nitrogen resorption were linked to phosphorus, not nitrogen concentrations: under low P conditions, wetland grasses increased their efficiency of both nitrogen and phosphorus use by increasing resorption of both nutrients. Drought is yet another stress which can increase nitrogen translocation from leaves to rhizomes and roots (reducing leaf N concentrations by up to 40% and increasing rhizome concentrations by 20-100%) in warm-season C4 perennial grasses (Heckathorn and DeLucia, 1994).

Beyond the reduced risk of leaching, however, improved nitrogen translocation means that less applied fertilizer would be needed for early season regrowth: since only 20-30% of the plant's nitrogen needs are being met by taking up nitrogen from the soil, growers of perennial grain forages or crops could potentially reduce nitrogen fertilizer applications in the early spring.

The effects of perennality on nitrate leaching may interact with rainfall effects. In a study of leaching under fully perennial and fully annual grass pastures (with no legume mixtures) Ridley *et al.* (1999) found that perennial pastures had lower levels of nitrate leaching in dry years, but not in wet years: apparently increased precipitation was enough to overcome the ability of the deeper root systems to retain nitrate ions. The effects of perennality may also depend on how long the perennial crop is allowed to survive: studies on alfalfa indicate that nitrate concentrations in deep soil layers are lowest after four years, and start increasing again in the fifth and six years, as roots begin to senesce (Entz *et al.*, 2001). In general, comparisons between annual and perennial systems are fairly sparse but seem to indicate that perennial systems have great potential to minimize leaching: to optimize these benefits however, we need more information on perennial grass life cycles and how long these crops should be left in the soil.

Improved Carbon Sequestration

Perennial grain crops could potentially carry on photosynthesis later in the fall than annuals, and begin photosynthesizing earlier in the spring (due to faster early-spring growth fueled by belowground reserves). It is possible that due to extra photosynthesis early and late in the growing season, more total CO₂ could be assimilated on a yearly basis by perennials than by annuals (Cox *et al.*, 2006). For example, intermediate wheatgrass and Illinois bundleflower maintain a large and photosynthetically active leaf area during August-September, a time when wheat is not growing at all. The higher amounts of total seasonal assimilation in perennials would be enhanced even more if perennial species maintained higher photosynthetic rates per unit time, than their annual relatives. While this is not the case in many annual vs. perennial species comparisons, some perennial species are known to maintain higher photosynthetic rates

than closely related annuals. These questions will be examined in detail, through both a review of relevant literature and an experimental study of three perennial cereals, in Chapter 5 of this thesis.

In addition, perennials may have larger and deeper root systems, and their root systems can be expected to be longer-lived (since they must support the plant's survival through each winter). Indeed, Cox et al. (2006) found that only 25-40% of perennial wheatgrass roots die back each winter as compared to 100% of annual wheat roots. The lower root turnover rates can be expected to lead to a slower rate of organic carbon influx into the soil, and lower rates of soil respiration. Perennial systems would also be incompatible with frequent tillage, which is a major contributor to the breakdown and loss of soil organic carbon- e.g. West and Post (2002) found that a switch from conventional tillage to no-till farming, across a variety of cropping systems, resulted in an average of $57 \text{ g C m}^{-2} \text{ yr}^{-1}$. Thus it is reasonable to expect that, for one or more of these reasons, carbon assimilation will be higher and/or soil respiration lower under perennial cropping systems than among annual systems, therefore total carbon sequestration would be higher. In an era of increased concern about climate change and rising CO_2 levels, which are expected to reach 560 ppm by 2050, perennial cropping systems could be a valuable means for societies to reduce their total contribution to global climate change and for farmers to benefit from receiving 'carbon credits'.

To what extent is the hypothesis of increased carbon sequestration borne out in reality? As yet, perennial grain crops are still in the process of development, so there is little assessment of their contribution to CO_2 fluxes in the field. Some evidence, however, is forthcoming from - perennial forage or energy crops sequester $320 \text{ to } 440 \text{ kg ha}^{-1}$ of carbon per year, while the

annual crops studied fixed from 0-300. Zan et al., (2001) found that switchgrass crops grown for bioenergy purposes resulted (after 4 years of production) in soil organic C concentrations comparable to those of willow trees (the most carbon-negative system), while annual corn fields had lower levels than willow plantations. Liebig et al. (2008) found that net carbon sequestration under switchgrass ranged from -0.6 (i.e. net CO₂ production) to 4.3 Mg C ha⁻¹ over the course of a year, thus generally this system was a carbon sink. Location was found to strongly influence the differences in soil organic carbon that developed over eight years between a restored perennial grassland (including various wheatgrass and needlegrass species) and a wheat-fallow rotation; these differences varied between footslopes, midslopes and shoulder areas as well as between sites (Nelson et al., 2008). Perennial grasslands sequestered up to 120% more carbon at one location, and about 15% less carbon at another site; in general, however, since carbon sequestration was higher in the grassland treatment at 6 of the 9 locations, we can tentatively conclude that carbon sequestration may be improved in perennial grain systems.

One caveat to the goal of improved carbon sequestration under perennial systems, is posed by the fact that buildup organic carbon is quickly erased by single tillage events. Thus in order to optimize carbon sequestration in perennial cereal systems, it may be necessary to grow these species in reduced-tillage systems.

Research is ongoing at Kellogg Biological Station of Michigan State University, to test both of the above hypotheses: that perennial cereal species show lower rates of nutrient leaching, and higher rates of carbon sequestration, than closely related annual species (Culman S. et al., unpublished data; Sprunger C., unpublished data). An ongoing four year experiment compares annual wheat (*Triticum aestivum* cv. 'Caledonia') to a closely related, cool-season perennial cereal species, intermediate wheatgrass (*Thinopyrum intermedium*). The wheatgrass accession

(‘TLI-C1) has been bred at the Land Institute (Salina, KS) for higher productivity, threshability and palatability, while ‘Caledonia’ is a wheat variety well adapted to Michigan and considered the ‘variety to beat’ in the mid-2000s (Lewis JM, personal communication). These systems are being compared over a period of four years, with the wheatgrass growing for a four year cycle and the wheat being replanted every year. Rates of nitrogen leaching are being monitored with lysimeters, while rates of organic carbon buildup are being assayed through a permanganate oxidation method (Culman S. et al., unpublished data).

Improved Soil Quality

Increased soil organic carbon, of course, would not merely be beneficial from the point of view of offsetting greenhouse gas emissions, but also from the point of view of increasing soil quality. Higher levels of soil organic matter could improve nutrient retention, water retention, soil bulk density, soil structure, and soil microbe populations. If significant areas of land in, for example, the Great Plains were dedicated in the long term to perennial cropping systems, soil organic matter could increase over time to levels comparable to those before the advent of modern agriculture. This could allow for eventual rotation of perennial and annual cropping systems, with the increased levels of soil organic matter built up by the perennials able to support high productivity by the annuals.

Islam and Weil (2000) found that perennial grasslands planted with Napier grass or wild sugarcane, had comparable soil bulk density, aggregate stability, and N content to natural fores, and were superior to cultivated fields including rice, cotton, and sugarcane. While this experiment was carried out in a tropical environment, it is interesting in part because both perennial grasses that they looked at are congeneric relatives of existing annual crops (millet and

sugarcane) and are currently under investigation as contributing to potential perennial crops. A comparison of perennial kikuyugrass (*Pennisetum clandestinum*), native grassland and annual ryegrass (*Lolium multiflorum*) in South Africa found similar results in a Mediterranean climate. Soil organic C, potassium sulfate-extractable C, microbial biomass C, and soil aggregate stability were lower under the annual than under the perennial treatments. In the case of aggregate stability, values were higher under the perennial by 33%-100% depending on the site (Milne and Haynes, 2004). Haynes and Francis (1993) found similar results to temperate environments, and found that perennial ryegrass pastures produced higher levels of soil aggregate stability than annual pastures.

The ability of perennial grass species to improve soil quality and soil physical properties as well as improving the efficiency of nutrient use, suggests that perennial grain crops could do similar things. These are essentially partly perennial, high-yielding grass or forbs, and they follow perennial or weakly perennial growth habits. If the improvements in soil quality under perennial pastures are related to perenniality itself, and not merely species effects, then it is reasonable that perennial grain crops could potentially result in improved soil quality and be an important part of soil restoration and sustainable agriculture efforts in future.

Resistance and Tolerance to Herbivory and Insects

Insect and pest pressure is an important environmental stress that most agricultural systems have to deal with. It represents a particularly difficult stress to overcome since it is a 'moving target' unlike, say, nitrogen deficiency. Insects and other pests evolve to become resistant to common pesticides, and have been found to develop resistance in as little as five years. Because insect pressure is such an important stress to overcome, it is important to know

how perennial cropping systems might vary from annual ones in terms of the ability to resist insect attack, tolerate herbivory, or facilitate biological control of pests.

In recent years, more attention has been paid to biological control, i.e. encouraging and fostering natural enemy populations, as a means of crop protection. Natural enemies (primarily predatory or parasitoid insects or arachnids) have many advantages over the use of many chemical insecticides. Natural enemies are usually specific for the desired pest, and to some extent self-limiting as their populations track those of their prey; they have the advantage of biological reproduction, so that a population can be started from a very small seed population, and they do not introduce broad-spectrum poisons into the environment. While in recent years the use of genetically modified (Bt) crops has overcome some of the problems with older, less specific insecticides, these varieties are only available for a few crops and it seems likely that encouraging natural enemies will continue to be a valuable strategy in the future.

There is some reason to believe that diverse, perennial grain-based polycultures could support larger, healthier and more diverse populations of natural enemies than current, low-diversity and high-productivity annual systems. It has been argued that perennial crop systems would favor conservation biological control through providing habitat to predators and parasitoids through a longer portion of the year, and because they are subject to lower levels of disturbance (Landis et al., 2000). Perennial grasses provide a habitat where natural enemies can overwinter, and are able to re-colonize rapidly in the spring, quickly establishing control over the pest population; strips of perennial grasses have been used to increase predator habitat in annual cereal fields (Thomas et al., 1992). Perennial grain crops may foster growth of predatory beetle populations by providing continuous ground cover (Clark et al., 1997). There is evidence that perennial grain crops could be more resistant to herbivory (Gutman et al., 2001), more able

to adapt to rapidly changing environments by altering the balance between aboveground and belowground investment (*ibid.*), more able to produce anti-herbivore toxins, and more able to attract natural enemies of herbivores (Tschartnke and Greiler, 1995; Bommarco, 1999; Fiedler and Landis, 2007), and potentially more supportive of pollination (Tuell et al., 2008). Pollination and biological control services are critical to agriculture as well as to the health of natural ecosystems, and if perennial grain crops can support higher levels of these beneficial insects then they could be an important aspect of sustainable managed and semi-managed ecosystems in future, which could ensure higher levels of ecosystem services not merely on perennial grain lands themselves but also on landscapes as a whole.

Challenges to Perennial Cereal Crops

Perennial cereal crops face a number of challenges, if they are to become viable crops which are economically feasible for farmers to grow, while yielding significant environmental benefits. For the remainder of this review we will be focusing on three major distinct challenges which face perennial herbaceous crops in general, but we will specifically consider each topic through the lens of three perennial cereals: perennial rye, perennial wheat and intermediate wheatgrass. Each of these species is under development as an alternative food crop for the cold temperate zone, and while the issues that they face are to some extent common to other herbaceous perennial species, they can be most usefully considered with reference to the specific challenges posed by the genetics, agronomy and physiology of these particular species. In this review, we shall briefly outline issues of genetic incompatibility, disease pressure, and yield vs. perenniality tradeoffs, with reference to our three perennial species. While the remainder of this dissertation will not explicitly address genetic and breeding issues, Chapter 2 will address one

important aspect of pathogen resistance (specifically, resistance to *Fusarium graminearum* head blight). Chapter 3 will address the remaining challenge (yield vs. perenniality tradeoffs).

Chapters 4 and 5 consider basic questions of resource acquisition and allocation in perennial cereals, which should help cast further light on the extent to which high yield and perennial life history might be compatible.

Genetic Incompatibility

Any wide cross between widely differing lineages faces potential issues of genetic incompatibility. The fact that the offspring contain only half the genetic complement of each parent could result in insufficient expression of some traits necessary to metabolism, defence, reproduction or resource acquisition. This may be somewhat less of a problem in a polyploid species, where homeologous genes on different chromosomes may fulfil the same or similar roles, but it can still pose a challenge. Any cross between the two will probably result in a loss of some yield potential, for several reasons: some genes affecting yield may be incompletely dominant so that the heterozygote has lower yield, while some genes from the perennial parent may somewhat downregulate allocation to seed production. It will be necessary to select for a number of generations to get yield up to an acceptable level. Moreover, in the F₂ and later generations, there is potential for heterozygosity, which means that the offspring may segregate for different traits and selection will be necessary to identify which offspring have the desired trait. Finally, sometimes the chromosome number between parents may differ, which creates problems for chromosome pairing and meiosis in the F₁ generation unless chromosome doubling occurs (e.g. with colchicine treatment: Jauhar, 1995; Anamthawat-Jonsson, 1996).

Challenges specific to the *Triticum x Thinopyrum* cross include difficulties in chromosome pairing, unstable chromosome number, as well as potentially high genetic load. *Triticum aestivum* ($2n = 42$) and *Thinopyrum intermedium* ($2n = 42$) have the same number of chromosomes, but pairing can generally not occur because of the *Ph* locus in wheat, which allows only homologous chromosomes (as opposed to homoeologous chromosomes) to pair during meiosis. This means that the hybrids act functionally as diploid hybrids between two completely different genomes, each comprising 21 chromosomes. Pairing cannot occur, which usually results in the hybrids being self-sterile.

In making crosses with *Thinopyrum* spp., plant breeders have found varying ways to deal with this problem. Some breeders have used Chinese Spring *ph1* mutants, or other *ph1*-deficient cultivars (e.g. Angas, Australian). The inhibition of pairing trait in wheat is recessive, thus only one nonfunctional copy is needed (Marais, 1992; Han et al., 2003). Artificial doubling of the genome, using agents like colchicine or dimethyl sulfoxide (DMSO) can also produce self-fertile plants with twice the number of chromosomes (Anamthawat-Jonsson, 1996). This approach has been successfully used with other *Thinopyrum* crosses but not yet with *Th. intermedium*. The self-fertile plants are often only female-fertile, as aneuploidy tends to be more of a problem for male gametes (complete male sterility) than for female gametes, This means that the F1 plants can be used as female parents in backcrosses to either wheat or the perennial parent. In perennial wheat breeding efforts at Washington State University (Murphy et al., 2009; Murphy et al., 2010) the wheat x *Thinopyrum* crosses have been back-crossed to annual wheat.

Sometimes wheat x *Thinopyrum* hybrids may ‘naturally’ produce a certain number of unreduced gametes, which contain a complete set of each haploid genome; these can combine with gametes from either parent to produce a BC1 plant. There may also be ‘natural’ genome

reduction as certain chromosomes are eliminated; this is a common result of wheat-*Thinopyrum* crosses. While this is not a problem for those breeders who are looking to introgress a specific trait into wheat, since only one or two chromosomes are needed, it is a problem for people interested in developing a new perennial wheat species, and these plants (with less than a full set of *Thinopyrum* chromosomes) cannot be used in further breeding.

The unreduced gametes approach is the approach that breeders in Washington and Kansas have used thus far in producing perennial wheat lines (Anamthawat-Jonsson et al., 1997; Cox et al., 2002). The breeders have used cold temperatures during pollination and embryo development to promote the formation of unreduced gametes- this results in gametes with twice the 'normal' number of chromosomes ($n = 42$) so that pairing and meiosis can occur. These plants, with unreduced gametes, are self-fertile (which has been used to develop lines in Kansas). They can also be used as the female parent in a back-cross with wheat (as has been done in Washington) and produce self fertile offspring. However, the high chromosome number is unstable, and some of the *Thinopyrum* chromosomes tend to be spontaneously eliminated so that after several generations the chromosome number stabilizes around $2n = 56$ (42 from wheat, 14 from wheatgrass). This results in segregation for traits, including perenniality (as some of the plants have eliminated the 4E chromosome which is linked to perenniality) and is clearly not ideal (Cox et al., 2002). As a future strategy, breeders in Kansas are attempting to try to back-cross the F1 generation to the perennial parent (*Th. intermedium*) and develop a new crop species that will have at least as much *Thinopyrum* ancestry as wheat (Anamthawat-Jonsson et al., 1997).

An additional issue is the unstable chromosome makeup in *Thinopyrum* x wheat hybrids (Dvorak, 1976). Annual wheat has 42 chromosomes, while durum (tetraploid) wheat has 28: *Thinopyrum intermedium* has 42, while *Th. elongatum* has 14 (Cox et al., 2002) and *Th. junceum*

has 42 (Wang et al., 2010). Combining the genomes, following doubling or lack of reduction of gametes, would lead to $2n = 84$ chromosomes for *Th. intermedium* x *T. aestivum* hybrids. However, this chromosome number is unsustainably large (Schulz-Schaeffer and Haller, 1987; Schulz-Schaeffer and Haller, 1988;) and thus the chromosome number generally stabilizes around $2n = 56$, with the loss of some chromosomes (Jones et al. 1999; Banks et al., 1993), though $2n = 42$ hybrids are more stable meiotically. However, this chromosome loss takes place somewhat randomly, with the elimination of both wheat and *Thinopyrum* chromosomes. Thus the progeny of any given cross will be extremely variable, without even having the same chromosomes, and many will suffer from extremely deleterious traits, since they may be missing large amounts of critical genetic material. The cross ‘AT-3425’, for example, derived from a wheat / *Thinopyrum* cross for the purpose of *Cephalosporium* resistance, had 36 wheat chromosomes, 14 *Thinopyrum* chromosomes, and 6 derived from translocations between the two (Cai et al., 1998). Another perennial amphiploid line, ‘AgCS’, with a stable chromosome number of $2n = 56$, was derived through hybridization of wheat and *Th. elongatum* (Cai et al., 2001).

Because of the smaller genome of *Th. elongatum* compared to *Th. intermedium* or *Th. junceum*, wheat hybrids with *Th. elongatum* are more stable, and can in fact stabilize at the threshold of $2n = 56$ without any chromosome loss at all (though as this is still above the ideal for meiotic stability, some chromosome loss may take place in succeeding generations). For this reason, in the mid-2000s, breeding efforts at Washington State University began to focus on *Thinopyrum elongatum* as a source of perennial parentage in wheat x wheatgrass amphiploids (Murphy et al, 2009; Murphy et al, 2010; Jauhar, 1992; Jauhar, 1995; Cox et al., 2002).

A third genetic challenge to developing wheat x *Thinopyrum* hybrids is that wheat is a mostly self-pollinated, self-compatible crop (and thus has very few deleterious recessive alleles and high tolerance for inbreeding; DeVries, 1974; Prakash and Singhal, 2003) while *Thinopyrum intermedium*, *Th. junceum* and *Th. elongatum* are cross-pollinated and only slightly to moderately self fertile (relying on wind or sometimes insects in nature: Jensen et al., 1990). Self-sterility in *Thinopyrum* is recessive, so the hybrids are self-fertile, but in the F2 generation (or in the F2 generation following one backcross to wheat) deleterious recessive alleles may recombine so that many of the progeny are homozygous for at least one of these deleterious traits. This results in a drop in yield in the F2 generation, and extensive selection will be necessary to eliminate unfavorable genotypes.

In perennial x annual *Secale* hybrids, inbreeding depression is less of an issue. Annual rye is, itself, mostly an obligate outcrosser (Landes, 1939) and thus the *Secale cereale* x *montanum* hybrid is outcrossing as well. While this protects the hybrid species against inbreeding depression, it also exposes it to pollen limitation. Under conditions of pollen limitation, both annual and perennial rye can become vulnerable to ergot (especially when weather conditions are favourable for the pathogen) as the fungus preferentially colonizes unfertilized spikelets (DeHaan LR, personal communication). The breeding syndrome of perennial rye means that breeders, agronomists and farmers must be cognizant of the vulnerability of this species to ergot attack.

Disease Pressure

A second challenge facing perennial cereals is the increasing buildup of pathogens over a multi-year life cycle. Farmers commonly manage disease through crop rotation: as pathogens are

often specific to a particular species, genus or family, the buildup of a particular pathogen may be avoided by tilling up that crop after harvest and replacing it with an unrelated crop the following season. Crop rotation would not help suppress airborne pathogens such as wheat loose smut, powdery mildew, sugarcane leaf rust or Southern corn leaf blight, which can disperse long distances to colonize new areas (Nagarajan and Singh, 1990). These pathogens are able to spread hundreds or thousands of miles on air currents, and can spread from one area of cultivation to a spatially separated area. However, other pathogens have generally shorter dispersal patterns, and normally overwinter either in the soil or on crop residues. Pathogens which overwinter on residues or in the soil, and which spread through short distance dispersal events (e.g. through water droplets) can be effectively suppressed through crop rotation.

Perennial crops inherently have a multi-year life cycle, and thus rotation cannot be used as a method of disease control. This poses a problem for the control of any pathogen which overwinters in the soil or on crop residues. Shortened rotations and reduced tillage are known to increase the incidence of some fungal diseases, e.g. *Fusarium* head blight (FHB: Xu and Nicholson, 2009), as this pathogen can overwinter on crop residues (Blandino et al., 2009). Thus increased disease prevalence poses a major threat to the viability of perennial cereals. FHB, in particular, could be a critical roadblock to developing perennial wheat, wheatgrass and other perennial cereals for the cold temperate zone.

As cultural control (crop rotation, changing tillage frequency and planting time, removing residues, etc.) cannot be used in a perennial system, options for controlling pests include either biological control, chemical control, or breeding for resistance traits. Biological control would involve bacterial or fungal agents antagonistic to disease-causing agents; chemical control could involve the application of pesticides. Breeding for resistance traits could be an important strategy

in ensuring that perennial cereals do not pay a high disease cost for their perennial life history. If a perennial cereal cultivar is highly resistant to a particular pathogen, then even if it is grown in a single location for multiple years, the pathogen will not be able to colonize.

In Chapter 5 of this dissertation, we will consider a case study particularly relevant to perennial cereals of the cold-temperate zone. Fusarium head blight, caused by the ascomycete fungus *Fusarium graminearum* (teleomorph *Gibberella zae*) is one of the major diseases of wheat and other small grains in north America, as well as an important pest in Europe and China. It not only reduces yields, but also renders the grain toxic to livestock and humans. *F. graminearum* overwinters on crop residues, and thus the presence of over-wintering colonies is considered to be the major source of inoculum under standard field conditions. Biological and chemical efforts to control FHB have achieved partial control, at best, suggesting that if perennial cereals are to become commercially feasible, it is essential to breed for genetic resistance to FHB. For this reason, plant breeders developing perennial cereals have emphasized identifying strongly disease resistant lines, that can maintain a perennial growth habit without becoming a reservoir for disease (e.g. Cox et al., 2005).

Various wild *Thinopyrum* species have been used as sources of resistance to FHB in the past, suggesting that perennial wheat x *Thinopyrum* crosses might show adequate levels of resistance. In Chapter 5, we will attempt to assess FHB resistance in various accessions of three perennial cereals (perennial wheat, perennial rye and intermediate wheatgrass) following inoculation experiments in the greenhouse. We chose this species because of its economic importance in North America, and because it was naturally present to some degree in initial screenings of field-grown perennial wheat plants in 2010.

Some diseases, including important viral pathogens like barley yellow dwarf virus (BYDV) are spread by insect vectors (in this case, aphids, as they move from plant to plant and suck on the leaf sap). In the case of insect-borne viral diseases, the potential of perennial systems to harbor more predatory insects and to experience lower levels of herbivory could potentially help suppress herbivory and thus, the spread of these diseases. While this dissertation does not address BYDV or other insect borne viral diseases, an observational study of three fungal and one viral disease (BYDV, leaf rust, *Septoria* leaf blotch and *Stagonospora* glume blotch) is ongoing at Kellogg Biological Station, and will be completed in 2013: we hope to eventually report on observed susceptibility of selected accessions of perennial rye, perennial wheat and intermediate wheatgrass to these diseases as well. All of these diseases are critical diseases in North American small grain growing region. Although for reasons of space they are not addressed in this dissertation, and although we treat FHB as a case study in disease resistance of perennial cereals, it is important to gather information on these widespread diseases as well, particularly since BYDV in particular affects a broad range of plant taxa and could be applicable to other perennial agroecosystems as well.

Perenniality vs. Productivity Tradeoffs

The third great challenge to the idea of developing perennial grain crops lies in the tradeoff between first year yield and longer-term survival and productivity. Farmers and land managers generally hope for a plant that can give them moderate to high yields beginning as soon as possible, ideally in the first year. Perennial plants are generally less well adapted to rapid growth and yield than annuals- while they may produce more over the course of their lifetimes, this is also not certain. It is often assumed that perennials would have lower production over their

lifespan than a series of annual crops over the same period, but especially in marginal environments, this can be questioned. According to FAO statistics, the yield of bananas and strawberries in Central America (two perennial crops), was an average of 8900 and 2400 kg ha⁻¹ respectively, while the yield of the three major dryland cereals (wheat, sorghum, rice and corn) were 4200, 2600, 3000 and 2100 kg ha⁻¹ respectively (DeHaan *et al.*, 2005).

It is likely that production costs for a perennial crop would be lower (due to reduced tillage, seed expenses, fertilizer expenditure and potentially insecticide as well) and that the ecosystem service benefits would more than outweigh any foregone income: for example, a modeling study in Australia found that in marginal lands, perennial wheat could be economically feasible if it yielded only 60% of annual wheat, with no additional forage gains. It could additionally be economically feasible in the most marginal areas if it yielded 40% of annual wheat yields, with an added 800 kg ha⁻¹ of forage (beyond the forage production of the annual wheat: Bell *et al.*, 2008). While some evidence to date suggests that perennial wheat, for example, can achieve yields up to 70% of good annual wheat cultivars in the first year, more data is needed on how and under what conditions perennial grains are able to achieve these yields, and if they lose other benefits in the process (DeHaan *et al.*, 2005).

The capacity of perennial cereals to achieve yields comparable to annuals depends, in large part, on their capacity to simultaneously allocate resources to vegetative storage organs which ensure multi-year survival, and to seed production (Bazzaz *et al.*, 2000). These can be viewed as alternative “sinks” (resource demanding tissues) that compete for a common resource pool (Reekie and Bazzaz, 1987a). Much of the argument that perennial cereals cannot achieve high seed yields competitive with annuals, depends on the idea that the perennial vegetative sink

will consume so many resources that the capacity of the plant to produce seed is greatly limited. Tradeoffs between perennial life history and seed production have often been assumed in the past, and have been shown in some taxa (Law, 1977; Aragon et al., 2009). However, if such tradeoffs are mild enough that perennial cereals still maintain yields over the acceptable threshold, the existence of somewhat lower yields would not be an insuperable problem.

Resource tradeoffs can be expressed in terms of several limiting resources including carbon, nitrogen (Wheelwright and Logan, 2004; Witkowski and Lamont, 1995), phosphorus (Zotz and Richter, 2006) and others. For example, Zotz and Richter (2006) found that nonstructural carbohydrate concentrations did not decrease following a reproductive event; concentrations of N decreased in some plant tissues but not others, while P, K, and Mg concentrations decreased in all plant tissues. The authors concluded that in this species phosphorus was the factor that limited reproduction, reflecting the fact that up to 60% of total plant phosphorus was concentrated in the reproductive tissues. If nitrogen or phosphorus is the most important limiting factor, then this could help reduce resource costs of perenniality, since perennial storage organs can generally be expected to be fairly carbon-rich and nutrient-poor. If, for example, nitrogen is the limiting resource, then plants could produce large quantities of low-N root tissue without much reducing its ability to invest nitrogen in N-demanding seed. This dissertation will primarily focus on resource tradeoffs as mediated by carbon: it is important, however, to realize that if these plants are in fact limited by other resources (nitrogen, phosphorus, etc.) this could give a conservative estimate of the yields possible in a perennial system. Perennial cereals which are nitrogen or phosphorus limited could potentially invest large amounts of carbon in high carbon, low-nutrient vegetative storage organs, gaining a large advantage in perennial survival and regrowth ability at only a small cost in seed yield.

Carbon is one of the most important currencies in which to assess resource tradeoffs: carbohydrates form the basic energy currency of plants, while fixed carbon is also required for nearly every other biological molecule. Carbohydrates are a versatile energy source that can be moved around the plant and invested in acquiring other resources when they in turn become limiting. For example, under water stress carbohydrates could be invested into key solutes used for osmotic regulation: under nutrient stress they could be invested in root systems or fed to phosphorus-harvesting mycorrhizae; under conditions of predation they could be invested in defence. In addition, many plants are effectively under water stress, and carbon fixation inevitably happens at the expense of water loss through the stomata. Thus for heavily water-limited plants, carbon could be viewed as a limiting resource, as greater rates of carbon fixation at a given level of ambient carbon dioxide would mean that stomata could remain open for fewer hours of the day. For all these reasons, carbon has often been viewed as the most generally relevant currency to assess costs of reproduction (Reekie and Bazzaz, 1987b).

In this dissertation we will consider resource tradeoffs between perennial and annual cereals specifically with respect to their ability to accumulate carbon through photosynthesis, and to allocate fixed carbon to various 'sinks'. This is particularly of value when considering resource costs of perenniality, as perenniating storage organs can generally be expected to be carbon-rich and relatively nitrogen-poor.

The degree of the yield penalty in perennial cereals (amount by which they fall short of annual analogues) will depend on several factors: 1) the extent to which reproductive structures can be self-supporting vs. resource drains on the plant, 2) the actual relative resource costs of perennial survival and of reproduction, 3) the extent to which photosynthetic rates differ in perennial vs. annual cereals, and 4) the extent to which increasing reproductive or vegetative

sink strength stimulates increased compensatory photosynthesis. While 1) and 2) will be briefly dealt with here, Chapters 3 and 4 of this dissertation will consider photosynthetic differences between annual and perennial cereals as well as how these species compensate metabolically for changes in sink strength.

Reproductive Photosynthesis

Tradeoffs between perenniality and seed yield could also be reduced by reproductive photosynthesis, by which reproductive structures themselves support a large portion of their own carbohydrate demands (Obeso, 2002). Consistent evidence shows that in many grass and forb species, reproductive structures can support from about 17% of their own growth in *Ranunculus* to about 65% in *Acer platanoides*, through their own photosynthesis (Galen et al., 1993; Aschan and Pfan, 2003; Bazzaz and Carlson, 1979; Bazzaz et al., 1979) and in some cases can even be a net exporter carbon to damaged root or leaf tissue. Jackson and DeWald found (1994) evidence that in *Tripsacum dactyloides*, reproductive tillers can ‘rescue’ extremely stressed vegetative tillers by exporting carbon. Plants with intact reproductive stalks and vegetative stalks clipped to 5 cm, had about 60% the final biomass, and 75% the reproductive biomass, of intact plants, while plants with defoliated reproductive stalks and clipped vegetative stalks had only about half the final biomass and 40% of the reproductive biomass. This was the case for high-yielding (pistillate) mutants as well as for normal plants.

Salopek-Sondi et al., (2000) and Aschan et al. (2005) found that sepals of *Helleborus* could photosynthesize at 58% to 80% of the rate of leaves despite having much lower levels of chlorophyll, while in *Lilium* the anthers can fix carbon at 73% the rate of leaves (Clement et al., 1997). Hoch (2005) found that in several European trees (beech, hornbeam and linden) fruiting

branches support all of their own carbon demand and do not constitute a sink for the plant. Girdled branches, unable to import carbon and nutrients from the rest of the plant, experienced changes in carbohydrate concentrations but generally did not suffer a yield decrease. He further suggested that this pattern of complete independence of reproductive tillers would probably not hold for commercially valuable fruit trees and herbaceous crops that have been bred for high yield. Nevertheless, this does provide good evidence that at least in some perennial plants, reproductive structures can be self-supporting. However, it can be argued that as branches are specialized for carbon acquisition rather than fruit bearing, they are not good analogues of reproductive tillers on a herbaceous plant.

Annual vs. Perennial Differences in Resource Acquisition

The ability of perennial cereals to allocate resources to both seed yields and perennial, multi-year survival depends ultimately on the combination of resource allocation and resource acquisition patterns. This is because total allocation to reproduction, for example, depends on the product of total resource acquisition, and reproductive allocation. If a species had very high photosynthetic rates, then it could produce large quantities of fruits or seeds even with a low level of reproductive allocation. Likewise, a species with high reproductive allocation, if it had low overall capacity to accumulate resources through photosynthesis, would have low reproductive capacity.

Evolutionary arguments can be made that perennial species should have generally lower photosynthetic rates than closely related annuals (Grime, 1977; Garnier, 1992; Ehrelinger, 1994). Since perennials do not need to complete their entire life cycle in one season, they can devote resources to defence and competition rather than to rapid resource accumulation. Longer lifespan

increases the likelihood of predation, pathogen attack, etc. and thus devoting resources to defence might be a larger priority. This could lead to lower photosynthetic rates, as more durable leaves generally have lower specific leaf area, and as allocation to nitrogenous defence compounds might mean less nitrogen allocation to photosynthetic proteins. Perennials might also generally adopt a more conservative water use strategy, particularly if they must survive harsh periods of cold or drought, or if high conductance rates would put them at high risk for cavitation, and this might also select for the production of leaves with lower maximal photosynthetic capacity (e.g. leaves with lower stomatal conductance).

However, although such theoretical arguments can be made, it is unclear to what extent they are supported empirically. Alternative arguments could be made, that perennial species might in some situations have higher photosynthetic rates than closely related annuals. At least three separate explanations could lead us to hypothesize higher photosynthetic rates in a perennial compared to a closely related annual species.

- *Greater sink strength in perennials.* Perennial species must allocate resources to vegetative storage sinks as well to sexual reproductive sinks, which may create greater sink strength overall. Sink demand for carbohydrates is known to affect photosynthetic rates through feedback inhibition: carbohydrate buildup in chloroplasts inhibits photosynthesis through a variety of mechanisms. If perennials have greater demand overall for carbohydrates, this could promote compensatory changes leading to higher photosynthetic rates (Zhao et al., 2008).
- *Nitrogen tradeoffs.* Annuals generally produce more seed than perennials, and seeds are highly nitrogen-demanding tissues. Perennials, by contrast, must optimize seed production over their life cycle, not within a single season. By

reducing allocation to seeds in one year, they could increase allocation of nitrogen to key photosynthetic proteins in leaves. This might increase the plants' ability to photosynthesize, which could allow them to produce more seed in later years. In support of this claim, perennial *Lesquerella mendocina* is known to have higher leaf N content than its annual relative *L. fendleri* (Ploschuk et al., 2005).

- *Moisture stress.* If perennial species allocate more resources to root growth than closely related annuals, or to feeding associated mycorrhizae, they could accumulate more nitrogen, phosphorus, potassium and micronutrients, as well as maintaining higher water uptake rates, than the annuals. This could allow them to escape moisture stress under dry conditions, and thus to maintain a greater degree of stomatal opening.

Any one of these non-exclusive hypotheses might lead to higher photosynthetic rates in perennial cereals, and at this point little work has been done on how photosynthesis and resource acquisition in perennial cereals compares to annual relatives. It is known that some wild *Oryza* spp. (e.g. *Oryza rufipogon*) maintain photosynthetic rates 25-65% higher than commonly grown rice cultivars (Zhao et al., 2008, 2010). Similarly, higher photosynthetic rates have been found in perennial vs. annual *Lesquerella* (Gonzalez-Paleo and Ravetta, 2011), as well as in a number of other perennial taxa compared to annual relatives (e.g. Taylor et al., 2010). Other experiments with different genera or families have found higher rates in annual species compared to closely related perennials (Ehrelinger, 1994). In order to understand fully the capacity for resource acquisition in perennial cereals like perennial wheat, perennial rye and intermediate wheatgrass, it is necessary to empirically compare photosynthetic rates, as well as to investigate related

parameters that might indicate whether differences in water stress, sink strength or nitrogen allocation account for any observed species differences in photosynthetic rates.

Curves modeling photosynthetic rate (A) as a function of internal carbon dioxide concentration (C_i) are a highly useful way to infer various parameters that effect rates of resource acquisition (Sharkey et al., 2007; Wullschleger, 1993). In Chapter 3 of this dissertation, we shall discuss how the A vs. C_i curves may be used to infer stomatal limitation, carboxylation rates, electron transport and triose phosphate utilization. We will also describe the methods and results of a three year physiological study comparing one- and two-year old perennial cereals to close annual relatives.

Source vs. Sink Limitation of Metabolism

Even if photosynthetic rates are in general similar between perennial cereals and their annual relatives, it is possible that resource flow in both annuals and perennials might be sink limited, such that resource flow (in this case, carbohydrate flow) is controlled by the ability to utilize carbohydrates rather than the ability to produce them through photosynthesis. While sink limitation can refer to other resources, in this section we will restrict ourselves to discussing sink limitation as it pertains to carbon metabolism. Sink limitation occurs when inadequate demand from ‘sinks’ (carbohydrate demanding tissues such as roots, meristems, growing leaf buds, flowers, seeds) leads to buildup of carbohydrates in leaf tissues (‘sources’) and downregulation of photosynthesis. If perennial cereals are source-limited (i.e. limited by the capacity of ‘sources’, green leaves, to photosynthesize) then photosynthesis under current environmental conditions in the field can be expected to be near its maximum, and increased sink demand (i.e. by selecting for greater seed sinks or more allocation to perenniating storage organs) would not

have an effect on photosynthesis and metabolism. Increased allocation to one sink would cause decreased allocation to the other, and there would be close to a one-to-one tradeoff between units of carbohydrate allocated to perenniating storage organs and to seeds.

However, if annual and/or perennial cereals show sink limitation, then they would be able to upregulate or downregulate photosynthetic rates as the ratio of sink tissue to source tissue increases or decreases. The presence of greater sink strength (as a result of more allocation to perennial storage tissues) would promote higher photosynthesis and allow perennial cereal species to accumulate more carbohydrate resources, overall, than annuals. If full sink limitation was seen, perennial cereals would be able to compensate fully for increased allocation to vegetative sinks, by upregulated photosynthesis. If source limitation was seen, by contrast, perennial cereals would already be at their maximal photosynthetic rate, and would not experience photosynthetic regulation. Thus, each additional unit of carbohydrate allocated to perenniating storage organs would mean one less unit allocated to seed. If perennial cereals were co-limited, finally, they would be able to compensate for some but not all of the increase in sink/source ratio. Co-limitation might be indicated by, for example, any of the following:

- 1) A change in photosynthetic rate that was too small to fully compensate for the increased sink demand, e.g. a 20% increase in photosynthetic rate following a 33% increase in sink/source ratio (or alternatively a 25% decrease in source/sink ratio).
- 2) A change in seed size that was too small to fully account for the increased sink demand, e.g. a decrease in seed size of 10% following a 25% increase in sink/source ratio (or alternatively a 20% decrease in source/sink ratio).
- 3) Changes in photosynthetic rate following alteration of source/sink ratio in one direction but not the other. If decreasing sink strength led to decreased photosynthetic rate, but increasing

sink strength did *not* lead to increased photosynthesis, then this would be evidence that photosynthesis was co-limited, i.e. concurrently limited by source strength and sink strength. Under such conditions, photosynthesis would be at the point which balanced source and sink strength, and decreasing either factor would lead to lower photosynthetic rates.

Source limitation, sink limitation and co-limitation of plant carbon metabolism are, of course, only meaningful terms within the context of a particular set of environmental conditions and patterns of allocation. For example, we may consider a plant that is sink-limited at a particular level of seed production and environmental conditions. If this plant increased its level of reproductive allocation, or experienced certain kinds of environmental stresses that decreased photosynthesis, then it would eventually enter a state of source limitation. Likewise, lowering the level of reproductive allocation or growth, increasing light or CO₂ levels, or introducing certain other types of environmental stress (e.g. moderate cold, nitrogen deficiency) would lead a plant that was previously source limited to become sink limited. Generally low temperature, nutrient deficiencies, high CO₂ and high light would all tend to push a plant further towards sink limitation; water stress, shade stress, warm temperatures, and low CO₂ would push a plant more towards source limitation. Plants can shift between source and sink limitation on a day-to-day basis, as has been seen in cherry (Flore and Layne, 1999), or on a medium term basis from one part of the growing season to another.

Thus to study source and sink limitation in a species, and assess the relative importance of these two processes, it is necessary to specify the environmental conditions, level of reproductive allocation, and the degree of source/sink perturbation involved. Ideally, the

responses to source/sink manipulation should be studied at multiple time points during the growing season, as phenology and ambient weather conditions can both profoundly affect the degree of source or sink limitation at a particular time.

There is widespread evidence of sink limitations to photosynthesis and metabolism, in a range of plant taxa. These include many cultivated woody perennials including apple (Zhou & Quebedeaux, 2003), cherry (Flore & Layne, 1999; Layne and Flore, 1993), peach (Gucci et al., 1991), citrus (Iglesias et al., 2002), and coffee (DaMatta et al., 2008), as well in grasses including wheat (Borras et al., 2004) and sugarcane (McCormick et al., 2006). Sink limitation can be inferred from two different lines of evidence. Sink limitation would be evidenced photosynthetic rates responding to changes in sink strength, or conversely by seed size remaining unchanged under conditions of changed sink strength. Alternatively, sink limitation would be evident if photosynthetic rates did not respond to changes in light or CO₂ concentration. Baysdorfer and Bassham (1986) found that alfalfa plants are able to recover from defoliation by upregulating photosynthesis, and ambient CO₂ concentrations are ample for this purpose, indicating sink limitation. Phosphorus limitation may be involved in this case, as these plants were grown without phosphorus, but have the capacity to fix nitrogen; phosphorus is known to limit alfalfa growth on some soils. Joshi *et al.* (2003) carried out experimental manipulation of source size in pearl millet, by removing increasing numbers of leaves on plants. While he was not explicitly looking for evidence of source vs. sink limitation, his data does fit best to a saturating Monod function, which indicates that as carbon availability increases, seed production in pearl millet becomes limited by some aspect of sink strength (whether the limiting factor is nitrogen, phosphorus, available flowering heads, or something else).

Sink limitation has been studied in the context of a variety of broader ecophysiology and population ecology questions. As indicated above, many economically important plant species, both woody fruit trees and annual cereals, have been subjected to source/sink manipulation in order to determine the factors regulating metabolism, which has applied value for horticultural and agronomic purposes. Source vs. sink regulation has also been studied in the context of climate change (Roumet and Roy, 1996) as a means to predict how various plant species will respond to elevated CO₂. Finally, sink regulation of plant metabolism under conditions of low temperature has been studied in the context of elevational limits to plant ranges, and researches have found evidence of sink limitation at high altitudes in many trees.

Hoch and Korner (2005) studied the carbon balance in *Polylepis* trees in Argentina at the upper altitudinal limit of their distribution, and found that nonstructural carbohydrate concentrations actually increased by about 10% at the highest elevations, as did nitrogen concentrations in leaves. In spite of the lower partial pressure of CO₂, these trees were not limited at the highest elevations by a lack of photosynthesis. Rather, they were more likely limited by the inhibitory effect of cold temperature on meristematic growth (sink limitation). In Argentina, *Kageneckia* showed similar evidence of sink limitation with higher TNC concentrations at higher altitudes (Piper *et al.*, 2006). Neither do photosynthesis and metabolism in these plants appear to be limited by nutrients; Korner (1989) found that nitrogen concentrations are higher in plants at the treeline while concentrations of phosphate, K, Mg, N, and Ca are not significantly different. This would tend to indicate that rather being limited by either carbon availability or nutrient availability, resource flow in these plants is limited by inadequate sink strength. The mechanism would be that cold inhibits growth and carbon utilization processes more than it inhibits photosynthesis. Some support for this hypothesis

comes from the fact that in *Ajuga reptans*, cold treatment increases the concentration of total nonstructural carbohydrates by a factor of 10. The most common soluble carbohydrate class, the raffinose family oligosaccharides, are present in highest concentrations in the fall and winter and lowest concentrations in the summer (Bachmann et al., 1994).

One possible mechanism for stimulation of photosynthesis under high sink/source ratio could involve a decrease in total nonstructural carbohydrate including triose phosphate, which could stimulate increased carboxylation. A meta-analysis found that 23 of 109 plant species showed triose phosphate utilization to be the primary limitation of photosynthesis under normal conditions (Wullschleger, 1993). If this is true then increased sink demand should lead to a drawdown of TNC and thus a reduction of feedback inhibition of the carbon reactions of photosynthesis. Thus, a study on grapefruit trees (*Citrus paradisi*) found that shaded leaves had higher asymptotes of the A vs. C_i curve, potentially because of greater sink strength (Jifon and Syvertsen, 2003). Alternatively, sink vs. source regulation could proceed through hormonal mechanisms that result in the downregulation of Rubisco activity or electron transport (Krapp and Stitt, 1995).

Little effort, though, has been devoted to comparing the degree of source vs. sink limitation in perennial species compared to close annual relatives. Perennials might tend, in general, to be more source-limited than annuals, if they are characterized by much larger vegetative sinks and lower photosynthetic capacity. The combination of these two traits would set them closer to their maximal photosynthetic capacity under normal conditions, and they would have less capacity to compensate for increases in sink strength (Arp, 1991). Conversely, the fact that perennials generally allocate less to sexual reproductive sinks than related annual species (i.e. seeds, flowers, etc.), particularly if they maintain high photosynthetic rates, could

mean that they generally maintain a more conservative strategy for resource allocation, and should be more sink-limited (i.e. have greater capacity for compensatory changes in photosynthesis and carbohydrate storage) than annuals (Masnatta and Ravetta, 2011; Gaines et al., 1974).

There are evolutionary reasons why perennials might be expected to maintain a more conservative reproductive strategy. If an annual plant ends the season with excess energy or nutrient reserves, those reserves are 'wasted' since the plant will die; an annual plant should therefore be selected to put the maximum amount of resources into reproduction, so as to optimize short-term productivity. A perennial plant, however, must optimize reproduction over the course of multiple years. In such cases, a conservative reproductive strategy could pay off: it would buffer the plant against environmental stresses during the reproductive period (by maintaining extra reserves) and if unused by the end of the season, those reserves could help support the plant's growth next year. However, as yet few studies have explicitly compared the general degree of source vs. sink limitation in annuals compared to closely related perennials.

One of the few such studies is an experimental study by Roumet and Roy (1996) in which source vs. sink limitation was assessed in a variety of cultivated perennial, wild perennial and wild annual grasses (through measuring responses to elevated CO₂). The study found that wild annuals were more source-limited than wild perennials, but less so than cultivated perennials, suggesting that both selection for high yield and annual vs. perennial life history can profoundly affect the degree of source vs. sink limitation. As perennial cereals are characterized both by perennial life history and by selection for high seed production, it is unclear to what extent they should be expected to show source vs. sink limitation. In addition, no studies have yet, to the best of our knowledge, quantified photosynthetic responses to source vs. sink manipulations in annual

rye, in spite of its agricultural importance. Thus, quantifying source vs. sink limitation in perennial cereals as well as in annual rye is important to predicting the extent to which perennial cereals may be able to support higher yields, as well as to understanding how photosynthetic physiology and plant life history interact.

Various methods exist to manipulate source / sink ratios in plants. Briefly, they include the following. It would be possible to carry out selection experiments, selecting for higher or lower sink strength (e.g. rhizome size, floret number, seed number) or for higher or lower source strength (e.g. leaf mass ratio). It would also be possible to carry out direct experimental manipulations of the source/sink ratio. This might use any of the following techniques.

Source augmentation could be carried out by supplemental lighting (Flore and Layne, 1999), increased concentrations of carbon dioxide (Hunt, 1996; Ainsworth et al., 2003; Reich et al., 2006, etc.), or direct feeding of soluble sugars to be absorbed through roots or leaves (Hiyane, 2010). One commonly used method involves increasing available light to plants. Increasing light intensity could easily lead to photoinhibition (and potentially to photodamage of shade-adapted plants); thus a method sometimes used to increase source strength is to increase daylength in greenhouse plants. In sour cherry (*Prunus cerasus*) continuous lighting led to a 40% decrease in photosynthesis after one week, while 70% defoliation led to a 50% increase, and the combination of both manipulations resulted in an increase comparable to defoliation alone. It would seem that an augmentation of source size does lead to a decline in source activity, but only if there is not a corresponding increase in sink size (Layne and Flore, 1993). Sawada et al. (1989) found similar results in soybeans, where after 1 week at continuous lighting (compared to 10 hours of light under normal conditions) photosynthesis was 54% lower. This decline is presumably attributable to the buildup of soluble carbohydrates and subsequent inhibition.

Similar results were found in *Amaranthus cruentus*; here there was a 50% decline in photosynthesis after 12 days under continuous light, with a 40% increase in sucrose levels and a 200% increase in starch, thus providing a mechanism for the decline in photosynthesis (Sawada et al., 1999). An additional method to increase source size is by physically spraying soluble sugars on leaves (Hiyane et al., 2010).

One of the most common methods of increasing source/sink balance, and most important in light of ongoing climate change, is carbon enrichment. Plants can be grown in the field under a free-air carbon enrichment (FACE) system, or in greenhouse growth chambers; if temperature, nitrogen, phosphorus, and other factors are at appropriate levels, then increased levels of carbon dioxide in the air can allow plants to fix more carbohydrate. For example, McKee and Woodward (1994) found strong evidence of sink limitation in dwarf spring wheat grown in growth chambers. At high (24 / 16 ° C day/night) temperatures, a doubling of atmospheric CO₂ levels produced only a 16% increase in biomass, and biomass actually decreased under elevated CO₂ at colder temperature. The authors attributed this to sink activity being depressed more than source activity, a common observation at low temperatures (e.g. Hoch and Korner, 2005). However, Uprety et al. (2009) found contrasting results when wheat was grown in the field under a FACE system in India. They found that spring wheat showed a 46% increase in biomass and a 22% increase in yield when grown under 550 ppm (under hot conditions ranging from 16/6 ° to 37/20 ° day/night); this was much greater than the increase in diploid or tetraploid wheats (*T. monococcum* or *T. durum*) and led them to conclude that wheat is still highly source-limited. In Arizona, FACE experiments found that wheat plants showed a 28% increase in leaf photosynthetic rate (and a 50% increase under drought conditions), but also found earlier

senescence and little effect on final grain yield, suggesting sink limitation under well watered conditions (Pinter et al., 1996).

Some studies have also been done of perennial C₃ grasses, as well as perennial forbs, and their response to carbon dioxide elevation; since perennial wheat and perennial rye have a multi-year life history, these grasses could be more relevant models. A study of *Lolium*, *Festuca* and *Agrostis* in greenhouse pots found 20% increases in dry mass of all three species when carbon dioxide was raised to 700 ppm (Cotrufo and Gorissen, 1997). However, no effect of CO₂ was found on growth of *Poa pratensis*, a cool season grass, in an open top chamber experiment (Owensby et al., 1993). A study of *Pascopyrum smithii* (formerly *Agropyron*, a relative of intermediate wheatgrass), using pots in a greenhouse, found that photosynthetic rates increased under 700 ppm CO₂, and final biomass increased by 19% (Hunt et al., 1996). Similar experiments with *Pascopyrum* in open-top chambers found that such increases in photosynthesis were driven primarily by increased photosynthetic rates during drought (LeCain et al., 2003). Baxter et al. (1994) found that two montane C₃ grasses, *Poa alpina* and *Agrostis capillaris*, showed increased biomass when grown under elevated CO₂ in open-top chambers, but a *Festuca* species showed a decline.

However, surprisingly, leaf-level differences in photosynthesis in response to elevated CO₂ were not found in the grassland studies either in Switzerland or Minnesota (Reich et al., 2006). While photosynthetic rates are usually higher at elevated CO₂, the A / C_i curve is also shifted left (i.e. the carbon saturated photosynthetic rate is lower), indicating acclimation to elevated CO₂ and subsequent downregulation of photosynthesis. This effect is stronger at low lev-

els of nitrogen, illustrating that nitrogen limitation can contribute to carbon sink limitation and that determining A / C_i curves can be a good way to test for sink limitation (Isopp *et al.*, 2000; Ainsworth and Long, 2005).

Source reduction might involve clipping leaves (Handa *et al.*, 2005; Macedo *et al.*, 2007; Horibata *et al.*, 2007), partially shading leaves (Jifon and Syvertsen, 2003; Araus *et al.*, 1993; Peet and Kramer, 1980; Rosas *et al.*, 1976; Ralphs *et al.*, 1998), or painting leaves with opaque coverings (Niva *et al.*, 2003; Karlsson, 1994). Defoliation is perhaps the most common method of source reduction. A major drawback of this technique, however, is that it could lead to hormonal changes in the plant that could confound the effects of the source size reduction itself. For example, defoliation could promote regrowth, formation of defence compounds, changed allocation to seed, production of volatile chemicals, changes in water status and so forth. An alternative method is to wrap shade cloth sheaths around the selected leaves. McCormick *et al.* (2006) used this technique in sugarcane. It has the advantage that shade cloth sheaths would allow some ventilation, which would reduce the risk of heat and humidity being trapped.

Some researchers have used aluminum foil to shade individual leaves. Aluminum foil has been used in poplar (Davis *et al.*, 1991), cassava (Rosas *et al.*, 1976) and larkspur (Ralphs *et al.*, 1998) among many others. Peet and Kramer (1980) used an adhesive-backed aluminized mylar film applied to the upper surfaces of leaves to shade them. The basic problem with aluminum foil coverings, besides that they can fall off, is that they could trap heat and alter the metabolism of the plant through heat-mediated stress. Though aluminum foil is known to reflect 95% of incident light, the remaining 5%, if converted to heat energy, could do substantial damage to a plant.

A further alternative is painting the leaves to be “shaded” with a nontoxic white paint coating; this was done in *Rhododendron lapponicum* (Karlsson, 1994), and in *Linnea borealis* (Niva et al., 2003), both times using Tipp-Ex correction fluid. The advantage is that white paint is opaque but also highly reflective: it would absorb little radiation, and the leaf would not heat up too much. It is necessary that the paint involved be non-toxic and hopefully also water-soluble so that it does not build up in the soil and is not absorbed into the plant. Tipp-Ex matches these conditions. An advantage of painting leaves, as opposed to designing structures to cover them, is that it is easier to brush small leaves with paint than to delicately design shading treatments to fit them. If they are too large, aluminum foil or shade cloth sheaths could slip off and it would be hard to make them small enough. We conclude that using white paint on individual leaves would seem to be an appropriate method for our perennial grain plants.

Paint treatments, of course, must be applied only to the top surfaces of leaves, so as not to interfere with evaporation or with whatever residual photosynthesis can occur even after the opaque coating (most likely this will be very small). In the dicots that Karlsson (1994) and Niva et al. (2003) studied, this worked well since in most dicots, water loss and light absorption are somewhat spatially separated: most dicot stomata are on the underside (abaxial surface) of leaves. Wheat and wheatgrass are monocots, and thus are amphistomatic, having stomata on both surfaces: in fact, wheat stomata are more common (15-40%) on the upper surface (adaxial) of the flag leaves (Yousufzai et al., 2009), and also in fourth (5-20%) and first node (25%) in first node leaves (Araus et al., 1989). Thus unlike with *Rhododendron*, this method when used in grasses cannot physically perfectly separate the effects of painting on evapotranspiration and on photosynthesis. The situation is also a bit difficult because wheat and grass leaves often are relatively upright and exposed to light from all around. However, since light is absorbed mostly

through the upper surface, and water loss presumably happens roughly equally in proportion to the number of stomata on the adaxial and abaxial surfaces, painting the upper surfaces should allow the leaves to transpire and shed heat while also not absorbing much light.

Sink augmentation can be carried out by altering the photoperiod (Reekie and Bazzaz, 1987a), by increasing hand pollination (Snow and Whigham, 1989; Primack and Hall, 1990) or by extending the flowering period (Laporte and Delph, 2006). Artificially augmented pollination can lead to an increased sink size. For example, hand pollination of pink lady's slipper orchid led to greatly increased seed set, but decreased leaf area (by about 10-13%) and seed set in the following year; these effects were compounded over four years in a multi-year experiment (Primack and Hall, 1990), illustrating a large cost of reproduction. A study in willow, contrariwise, covered calyces to prevent pollination, and studied whether there was an increase in next year's growth and seed set (Fox and Stevens, 1991). Hand pollination has also been used in sticky catchfly (Jennersten, 1991) and in *Tipularia* orchids (Snow and Whigham, 1989); the last mentioned, for example, found that each fruit cost the plant about 2% of its mass. However, pollination manipulation will only allow cost of reproduction or compensatory responses to be estimated if the species under discussion is actually pollen limited (Dudash and Fenster, 1997).

Shading reproductive structures is specifically a way to increase sink size in plant species where there is considerable 'reproductive photosynthesis', i.e. photosynthesis by flowers and reproductive support structures. Some studies have carried out a combination of these manipulations. For example, Gehring and Delph (2006) shaded calyces of *Silene latifolia* and delayed pollination (leading to initiation of more floral buds), both of which effectively increased sink size. This led to more floral abortion and smaller fruit mass, indication that reproductive

output was controlled by carbohydrate availability and not the other way around (i.e. *Silene* is source limited).

Sink reduction involves decreasing the size of reproductive sinks. The most common way of reducing sink size is simply to clip and remove flowering heads or fruits. For example, Jackson and DeWald (1994) removed seed stalks to reduce reproductive sinks in *Tripsacum dactyloides*, and found a small cost of reproduction (although seed stalks were partially self sufficient and could even in extreme circumstances serve as a carbohydrate source). Similar manipulations have been done in *Viscaria* (Jennersten, 1991) among other species. Alternative methods of altering sink size include looking at female vs. male or female vs. hermaphroditic plants (in dioecious or gynodioecious species respectively). Generally, female plants are expected to have higher reproductive allocation. Still another is to alter flowering through altering the photoperiod of the plants (Reekie and Bazzaz, 1987a). This has risks, however, of hormonal alterations on plant metabolism caused by the photoperiod, and is in any case inappropriate for a field experiment where photoperiod cannot be easily manipulated.

Tracing experiments to follow the fate of carbohydrates in the plant can offer supplementary information about the underlying basis of source vs. sink limitation. For example, C^{13} tracing experiments in *Adonis ramosa*, a spring ephemeral, found that current photosynthate fixed by leaves during the flowering period went mostly to belowground storage, and very little went to the fruits: the fruits were to a large extent supported by their own photosynthesis and by long-term stored carbohydrate from the roots. This helped explain why defoliation did not lead to a decrease in fruit production (Horibata et al., 2007). Conversely, in apples (Hansen, 1967) about 90% of photosynthate produced by leaves is transferred to leaves nearby.

In Chapter 4 of this dissertation, we shall describe the results of altering source/sink ratios through source reduction and sink reduction.

Conclusion

Perennial cereals may have the potential to satisfy human needs for food, fuel, and fiber in more environmentally sustainable ways, and provide improved levels of ecosystem services within agroecosystems and their surroundings. However, several challenges must be overcome in order for perennial cereals to become economically feasible to be produced on a significant scale. In the remainder of this dissertation, we will consider various aspects of perennial cereal production from the point of view of agronomy, physiology and disease resistance. The studies detailed in Chapters 2-5 will answer questions about the grain and biomass productivity of perennial cereals over time, the ability of perennial cereals to withstand an important North American cereal disease, the physiological differences in resource acquisition rates between perennial and annual cereals, and the extent to which resource flow in perennial cereals is source vs. sink limited. The goal of this literature review and the following experimental studies are to contribute to the growing literature on perennial cereals, and to address both basic and applied questions regarding a new and highly unusual plant growth form.

REFERENCES

REFERENCES

- Acharya SN, Mir Z, Moyer JR, Orshinsky BR, Thomas JE. 2003. Effect of row spacing and seeding date on forage yield and quality of perennial cereal rye (*Secale cereale* L.). *Canadian Journal of Plant Science* 83: 363-369.
- Acharya SN, Mir Z, Moyer JR. 2004. ACE-1 perennial cereal rye. *Canadian Journal of Plant Science* 84: 819-821.
- Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties, and plant production to rising CO₂. *New Phytologist* 165(2): 351-371.
- Akhtar J, Gorham J, Qureishi RH. Combined effect of salinity and hypoxia in wheat (*Triticum aestivum* L.) and wheat-*Thinopyrum* amphiploids. *Plant and Soil* 166: 47-54.
- Anamthawat-Jonsson K. 1996. Wide-hybrids between wheat and Lyme grass : breeding and agricultural potential. *Buvusindi* 10:101–113.
- Anamthawat-Jonsson K, Bodvarsdottir SK, Bragason BT, Gudmundsson J, Koebner RMD. 1997. Wide hybridization between wheat (*Triticum* L.) and lyme grass (*Leymus* Hochst.). *Euphytica* 93:293–300
- Aragón CF, Méndez M, Escudero A. 2009. Survival costs of reproduction in a perennial plant: live hard, die young. *American Journal of Botany* 96: 904-911.
- Arp WJ. 1991. Effects of source-sink relations on photosynthetic acclimation to elevated CO₂. *Plant, Cell and Environment* 14: 869-875.
- Araus JL, Tapia L, Alegre L. 1989. The effect of changing sowing date on leaf structure and gas exchange characteristics of wheat flag leaves grown under Mediterranean climate conditions. *Journal of Experimental Botany* 40: 639-646.
- Araus JL, Brown HR, Febrero A, Bort J, Serret MD. 1993. Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory CO₂ to differences in grain mass in durum wheat. *Plant, Cell and Environment* 16(4): 383-392.
- Arredondo JT, Jones TA, Johnson DA. Seedling growth of intermountain perennial and weedy annual grasses. *Journal of Range Management* 51(5): 584-589.
- Aschan G, Pfanz H. 2003. Non-foliar photosynthesis- a strategy of additional carbon acquisition. *Flora* 198: 81-97.

- Aschan G, Pfanz H, Vodnik D, Batic F. 2005. Photosynthetic contribution of vegetative and reproductive structures of green hellebore (*Helleborus viridis* L. agg.) *Photosynthetica* 43(1): 55-64.
- Bachmann M, Matile P, Keller F. 1994. Metabolism of the raffinose family oligosaccharides in leaves of *Ajuga reptans* L. (Cold acclimation, translocation, and sink to source transition: discovery of chain elongation enzyme.) *Plant Physiology* 105: 1335-1345.
- Banks PM, Xu SJ, Wang RRC, Larkin PJ. 1993. Varying chromosome composition of 56-chromosome wheat x *Thinopyrum* partial amphiploids. *Genome* 36: 207-215.
- Bausenwein U, Millard P, Raven JA. 2001. Remobilized old-leaf nitrogen predominates for spring growth in two temperate grasses. *New Phytologist* 152(92): 283-290.
- Baxter R, Ashenden TW, Sparks TH, Farrar JF. 1995. Effects of elevated carbon dioxide on three montane grass species: I. Growth and dry matter partitioning. *Journal of Experimental Botany* 45:305-315.
- Baysdorfer C, Bassham JA. 1986. Photosynthate supply and utilization in alfalfa: a developmental shift from a source to a sink limitation of photosynthesis. *Plant Physiology* 77: 313-317.
- Bazzaz, F.A., Ackerly, D.D., and Reekie, E.G. 2000. Reproductive allocation in plants. In: Fenner, M. *Seeds: the ecology of regeneration in plant communities*. Wallingford, UK: CABI Publishing, 2000. pp. 1-30.
- Bazzaz FA, Carlson RW. 1979. Photosynthetic contribution of flowers and seeds to reproductive efforts of an annual colonizer. *New Phytologist* 82(1): 223-232.
- Bazzaz FA, Carlson RW, Harper JL. 1979. Contribution to reproductive effort by photosynthesis of flowers and fruits. *Nature* 279: 554-555
- Bell LW, Byrne F, Ewing MA, Wade LJ. 2008. A preliminary whole-farm economic analysis of perennial wheat in an Australian dryland farming system. *Agricultural Systems* 96: 166-174.
- Berdahl JD, Redfearn JD. 2007. Grasses for arid and semiarid areas. In Barnes, R.F., Nelson, C.J., Moore, K.J., and Collins, M. eds. *Forages: The science of rangeland agriculture*. Blackwell, 2007.
- Bergstrom L. 1987. Nitrate leaching and drainage from annual and perennial crops in tile drained plots and lysimeters. *Journal of Environmental Quality* 16:11-18.
- Blandino M, Reyneri A, Scudellari D. 2009. Crop residues and rain bring together risk of *Fusarium* disease. *Informatore Agrario* 65: 44-46.
- Bommarco R. 1999. Feeding, reproduction and community impact of a predatory carabid in two agricultural habitats. *Oikos* 87(1): 89-96

- Borras L, Slafer GA, Otegui ME. 2004. Seed dry weight response to source-sink manipulations in wheat, maize and soybean: a quantitative reappraisal. *Field Crops Research* 86: 131-146.
- Cai X, Jones SS, Murray TD. 1998. Molecular cytogenetic characterization of *Thinopyrum* and wheat-*Thinopyrum* translocated chromosomes in a wheat-*Thinopyrum* amphiploid. *Chromosome Research* 6: 183-189.
- Cai X, Jones SS, Murray TD, Weber WE. 2001. Molecular cytogenetic characterization of *Thinopyrum* genomes conferring perennial growth in wheat-*Thinopyrum* amphiploids. *Plant Breeding* 1: 20-26.
- Clark MS, Gage SH, Spence JR. 1997. Habitats and management associated with common ground beetles (Coleoptera: Carabidae) in a Michigan agricultural landscape. *Environmental Entomology* 26: 519-527.
- Clement C, Mischler P, Burrus M, Audran JC. 1997. Characteristics of the photosynthetic apparatus and CO₂ fixation in the flower bud of *Lilium*.II. Anther. *International Journal of Plant Sciences* 158(6): 801-810.
- Colmer TD, Flowers TJ, Munns, R. 2006. Use of wild relatives to improve salt tolerance in wheat. *Journal of Experimental Botany* 57: 1059-1078.
- Corley RHV. 1983. Oil palm and other tropical tree crops. In Smith WH, Banta SJ., eds. Symposium on Potential Productivity of Field Crops under Different Environments. pp. 383-399. International Rice Research Institute, Los Baños, Philippines.
- Cotrufo MF, Ineson P, Scott A. 2002. Elevated CO₂ reduces the nitrogen concentration of plant tissues. *Global Change Biology* 4(1): 43-54.
- Cotrufo MF, Gorissen A. 1997. Elevated CO₂ enhances below ground C allocation in three perennial grass species at different levels of N availability. *New Phytologist* 137(3): 421-431.
- Cox TS, Bender M, Picone C, Van Tassel DL, Holland JB, Brummer EC, Zoeller BE, Paterson AH, Jackson W. 2002. Breeding perennial grain crops. *Critical Reviews in Plant Sciences* 21(2): 59-91.
- Cox TS, Glover JD, Van Tassel DL, Cox CM, DeHaan LR. 2006. Prospects for developing perennial grain crops. *BioScience* 56(8): 649-659.
- Cox, C. M., Garrett, K. A., Cox, T. S., Bockus, W. W., and Peters, T. 2005. Reactions of perennial grain accessions to four major cereal pathogens of the Great Plains. *Plant Disease* 89:1235-1240.

Crews TE. 2005. Perennial crops and endogenous nutrient supplies. *Renewable Agriculture and Food Systems* 20:25-37

Crews TE, Peoples MB. 2005. Can the synchrony of nitrogen supply and crop demand be improved in legume- and fertilizer-based agroecosystems? A review. *Nutrient Cycling in Agroecosystems* 72(20): 101-120.

DaMatta FM, Cunha RL, Antunes WC, Martins SCV, Araujo WL, Fernie AR, Moraes GABK. 2008. In field-grown coffee trees source-sink manipulation alters photosynthetic rates, independently of carbon metabolism, via alterations in stomatal function. *New Phytologist* 178: 348-357.

Davis JM, Gordon MP, Smitt BA. 1991. Assimilate movement dictates remote sites of wound-induced gene expression in poplar leaves. *Proceedings of the National Academy of Sciences, USA*. 88: 2393-2396

DeHaan LR, Van Tassel DL, Cox TS. 2005. Perennial grain crops: a synthesis of ecology and plant breeding. *Renewable Agriculture and Food Systems* 20: 5-14.

De Vries AP. 1974. Some aspects of cross-pollination in wheat (*Triticum aestivum* L.). 4. Seed set on male sterile plants as influenced by distance from the pollen source, pollinator: Male sterile ratio and width of the male sterile strip. *Euphytica* 23:601–622.

Dowdell RJ, Webster CP. 1980. A lysimeter study using nitrogen-15 on the uptake of fertilizer nitrogen by perennial ryegrass swards and losses by leaching. *European Journal of Soil Science* 31(1): 65-75.

Dudash MR, Fenster CB. 1997. Multi-year study of pollen limitation and cost of reproduction in the iteroparous *Silene virginica*. *Ecology* 78(2): 484-493.

Dvořák J. 1976. The cytogenetic structure of a 56–chromosome derivative from a cross between *Triticum aestivum* and *Agropyron elongatum* ($2n = 70$). *Canadian Journal of Genetics and Cytology* 18:271–279.

Ehrlén, J., and van Groendendael, J. Storage and the delayed costs of reproduction in the understorey perennial *Lathyrus vernus*. *Journal of Ecology* 89(2): 237-246.

Ehrelinger JR. 1994. Variation in gas exchange characteristics among desert plants. In: Schulze EM, Caldwell MM. 1994. *Ecophysiology of Photosynthesis*. Berlin: Springer Verlag, 1994, pp. 361-392.

Entz MH, Bullied WJ, Forster DA, Gulden R, Vessey JK. 2001. Extraction of subsoil nitrogen by alfalfa, alfalfa-wheat and perennial grass systems. *Agronomy Journal* 93: 495-503.

Evans RA, Eckert RE, Kay BL. 1967. Wheatgrass establishment with paraquat and tillage on downy brome ranges. *Weeds* 159: 50-55.

- Ewel JJ. 1999. Natural systems as models for the design of sustainable systems of land use. *Agroforestry Systems* 45(1-3): 1-21.
- Fielder AK, Landis DA. 2007. Attractiveness of Michigan native plants to arthropod natural enemies and herbivores. *Environmental Entomology* 36(4): 751-765.
- Flore JA, Layne DR. 1999. Photoassimilate production and distribution in cherry. *HortScience* H34: 1015-1019.
- Fox JF, Stevens GC. 1991. Costs of reproduction in a willow: Experimental responses vs. natural variation. *Ecology* 72(3): 1013-1023.
- Füle L, Hódos-Kotvics G, Galli Z, Ács E, Heszky L. 2005. Grain quality and baking qualities of perennial rye (cv. 'Perenne') of interspecific origin (*Secale cereale x montanum*). *Cereal Research Communications* 33: 809-814.
- Gaines MS, Vogt KJ, Hamrick JL, Caldwell J. 1974. Reproductive strategies and growth-patterns in sunflowers (*Helianthus*). *American Naturalist* 108: 889-894.
- Galen C, Dawson TE, Stanton ML. 1993. Carpels as leaves: meeting the carbon cost of reproduction in an alpine buttercup. *Oecologia* 95(2): 187-193.
- Garnier E. 1992. Growth analysis of congeneric annual and perennial grass species. *Journal of Ecology* 80: 665-675.
- Gehring JL, Delph LF. 2006. Effects of reduced source-sink ratio on the cost of reproduction in females of *Silene latifolia*. *International Journal of Plant Sciences* 167(4): 843-851.
- Gloser V. 2005. The consequences of lower nitrogen availability in autumn for internal nitrogen reserves and spring growth of *Calamagrostis epigejos*. *Plant Ecology* 179(1): 119-126.
- González-Paleo L, Ravetta D. 2011. Relationships between reproductive output, morpho-physiological traits, and lifespan in *Lesquerella* (Brassicaceae). *Industrial Crops and Products* 34: 1372-1380.
- Grime JP. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111: 1169-1194.
- Gucci R, Xiloyannis C, Flore JA. 1991. Gas exchange parameters, water relations and carbohydrate partitioning in leaves of field-grown *Prunus domestica* following fruit removal. *Physiologia Plantarum* 83(3): 497-505.
- Gutman M, Noy-Meir I, Pluda D, Seligman NA, Rothman S, Sternberg M. 2001. Biomass partitioning following defoliation of annual and perennial Mediterranean grasses. *Conservation Ecology* 5(2): 1. [online] URL: <http://www.consecol.org/vol5/iss2/art1/>

- Gusewell S. 2005. High nitrogen:phosphorus ratios reduce nutrient retention and second-year growth of wetland sedges. *New Phytologist* 166(2): 537-550.
- Han FP, Fedak G, Benabdelmouna A, Armstrong K, Ouellet T. 2003. Characterization of six wheat x *Thinopyrum intermedium* derivatives by GISH, RFLP, and multicolor GISH. *Genome* 46: 490-495.
- Handa IT, Körner C, Hattenschwiler, S. 2005. A test of the treeline carbon limitation hypothesis by in situ CO₂ enrichment and defoliation. *Ecology* 86(5): 1288-1300.
- Hansen P. 1967. Studies on apple trees: I. The effect of the fruit on the translocation and distribution of photosynthates. *Physiologia Plantarum* 20(2): 382-391.
- Hayes RC, Newell MT, DeHaan LR, Murphy KM, Crane S, Norton MR, Wade LJ, Newberry M, Fahim M, Jones SS, Cox TS, Larkin PJ. 2012. Perennial cereal crops: An initial assessment of wheat derivatives. *Field Crops Research* 133: 68-89.
- Haynes RJ, Francis GS. 1993. Changes in microbial biomass C, soil carbohydrate composition and aggregate stability induced by growth of selected crop and forage species under field conditions. *European Journal of Soil Science* 44(4): 665-675.
- Heckathorn SA, DeLucia EH. Drought-induced nitrogen retranslocation in perennial C₄ grasses of tallgrass prairie. *Ecology* 75(7): 1877-1886.
- Hiyane R, Hiyane S, Tang AC, Boyer JS. 2010. Sucrose feeding reverses shade-induced kernel losses in maize. *Annals of Botany* 106: 395-403.
- Hoch G. 2005. Fruit bearing branchlets are carbon autonomous in mature broad leaved temperate forest trees. *Plant, Cell and Environment* 28(5): 651-659.
- Hoch G, Körner C. 2005. Growth, demography and carbon relations of *Polylepis* trees at the world's highest treeline. *Functional Ecology* 19(6): 941-951.
- Horibata S, Hasegawa SF, Kudo G. 2007. Cost of reproduction in a spring ephemeral species, *Adonis ramosa* (Ranunculaceae): Carbon budget for seed production. *Annals of Botany* 100: 565-571.
- Hunt HW, Elliott ET, Detling JK, Morgan JA, Chen DX. 1996. Response of a C₃ and a C₄ grass to elevated CO₂ and temperature under different water regimes. *Global Change Biology* 2(1): 35-47.
- Iglesias DJ, Lliso I, Tadeo FR, Talon M. 2002. Regulation of photosynthesis through source: sink imbalance in citrus is mediated by carbohydrate content in leaves. *Physiologia Plantarum* 116: 563-572.

- Islam KR, Weil RR. 2000. Land use effects on soil quality in a tropical forest ecosystem of Bangladesh. *Agriculture, Ecosystems and Environment* 79(1): 9-16.
- Isopp H, Frehner M, Long SP, Nosberger J. 2000. Sucrose-phosphate synthase responds differently to source-sink relations and to photosynthetic rates: *Lolium perenne* L. growing at elevated $p\text{CO}_2$ in the field. *Plant, Cell, and Environment* 23: 597-607.
- Jackson L, DeWald C. 1994. Predicting evolutionary consequences of greater reproductive effort in *Tripsacum dactyloides*, a perennial grass. *Ecology* 75(3): 627-641.
- Jauhar PP. 1992. Synthesis and cytological characterization of trigeneric hybrids involving durum wheat, *Thinopyrum bessarabicum*, and *Lophopyrum elongatum*. *Theoretical and Applied Genetics* 84:511-519.
- Jauhar PP. 1995. Meiosis and fertility of F1 hybrids between hexaploid annual wheat and decaploid tall wheatgrass (*Thinopyrum ponticum*). *Theoretical and Applied Genetics* 90: 865-871.
- Jennersten O. 1991. Cost of reproduction in *Viscaria vulgaris* (Caryophyllaceae): A field experiment. *Oikos* 61(2): 197-204.
- Jensen KB, Zhang YF, Dewey DR. 1990. Mode of pollination of perennial species of the Triticeae in relation to genomically defined genera. *Canadian Journal of Plant Science* 70: 215-225.
- Jifon JL, Syvertsen JP. 2003. Moderate shade can increase net gas exchange and reduce photoinhibition in citrus leaves. *Tree Physiology* 23: 119-127.
- Joffre R. 1990. Plants and soil nitrogen dynamics in Mediterranean grasslands: a comparison of annual and perennial grasses. *Oecologia* 85(1): 142-149.
- Jones TA, Zhang XY, Wang RRC. 1991. Genome characterization of MT-2 and OK-906 annual wheat x intermediate wheatgrass hybrids. *Crop Science* 39: 1041-1043.
- Joshi AK, Pandya JN, Buhecha KV, Dave HR, Pethani KV, Dangaria CJ. 2003. Grain yield in pearl millet in relation to source size and proximity to sink. *Photosynthetica* 41(1): 157-159.
- Karlsson PS. 1994. The significance of internal nutrient cycling in branches for growth and reproduction of *Rhododendron lapponicum*. *Oikos* 70(2): 191-200.
- Kemanian AR, Stöckle CO, Huggins DR, Viega LM. 2007. A simple method to estimate harvest index in grain crops. *Field Crops Research* 103: 208-216.
- Knowles RP. 1977. Recurrent mass selection for improved yields in intermediate wheatgrass. *Crop Science* 17: 51-54.

- Körner C. 1989. The nutritional status of plants from high altitudes. *Oecologia* 81(3): 379-391.
- Krapp A, Stitt M. 1995. An evaluation of direct and indirect mechanisms for the "sink regulation" of photosynthesis in spinach: Changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady state transcript levels after cold girdling source leaves. *Planta* 195: 315-323.
- Kubiczek R, Łuczak W, Molski B. 1981. Protein resources of wild *Secale* species. *Kulturpflanze* 29: 159-167.
- Laporte MM, Delph LF. 1996. Sex-specific physiology and source-sink relations in the dioecious plant *Silene latifolia*. *Oecologia* 106(1): 63-72.
- Landes, M. 1939. The causes of self-sterility in rye. *American Journal of Botany* 26(8): 567-571.
- Landis DA, Wratten SD, Gurr GM. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45: 175-201.
- Law R, Bradshaw AD, Putwain PD. 1977. Life history variation in *Poa annua*. *Evolution* 31: 233-246.
- Layne DR, Flore JA. 1993. Physiological responses of *Prunus cerasus* to whole plant source manipulation. Leaf gas exchange, chlorophyll fluorescence, water relations and carbohydrate concentrations. *Physiologia Plantarum* 88: 44-51.
- LeCain DR, Morgan JA, Mosier AR, Nelson JA. 2003. Soil and plant water relations determine photosynthetic responses of C₃ and C₄ grasses in a semi-arid ecosystem under elevated CO₂. *Annals of Botany* 92:41-52.
- Lammer D, Cai XW, Arterburn M, Chatelain J, Murray S, Jones S. 2004. A single chromosome addition from *Thinopyrum elongatum* confers a polycarpic, perennial habit to annual wheat. *Journal of Experimental Botany* 55: 1715-1720.
- Li MJ, Wu SY. 2006. Assessment to adult plant resistance to stripe rust of for quality improvement of wheat germplasm parent materials in Yunnan. *Journal of Triticeae Crops* 26(1): 113- 116 (in Chinese with an English abstract).
- Li YS, Redmann RE, van Kessel C. 1992. Nitrogen budget and ¹⁵N translocation in a perennial wheatgrass. *Functional Ecology* 6(2): 221-225.
- Li Z, Li B, Tong Y. The contribution of distant hybridization with decaploid *Agropyron elongatum* to wheat improvement in China. *Journal of Genetics and Genomics* 35: 451-456.
- Liebig MA, Schmer MR, Vogel KP, Mitchell RB. 2008. Soil carbon storage by switchgrass grown as bioenergy. *Bioenergy Research* 1(3-4): 215-222.

- Lym RG, Tober DA. 1997. Competitive grasses for leafy spurge (*Euphorbia esula*) reduction. *Weed Technology* 11(4): 787-792.
- Macedo TB, Peterson RKD, Weaver K. 2006. Photosynthetic responses of wheat, *Triticum aestivum* L. plants to simulated insect defoliation during vegetative growth and at grain fill. *Environmental Entomology* 35(6): 1702-1709.
- Malinowski DP, Hopkins A A, Pinchak WE, Sij JW, Ansley RJ. 2003. Productivity and survival of defoliated wheatgrasses in the rolling plains of Texas. *Agronomy Journal* 95: 614-626
- Marais GF. 1992. The modification of a common wheat-*Thinopyrum distichum* translocated chromosome with a locus homoallelic to Lr19. *Theoretical and Applied Genetics* 85: 73-78.
- Masnatta WJ, Ravetta DA. 2011. Seed-yield and yield components response to source sink ratio in annual and perennial species of *Lesquerella* (Brassicaceae). *Industrial Crops and Products* 34: 1393-1398.
- McCormick AJ, Cramer MD, Watt DA. 2006. Sink strength regulates photosynthesis in sugarcane. *New Phytologist* 171: 759-770.
- McCormick AJ, Cramer MD, Watt DA. 2008. Changes in photosynthetic rates and gene expression of leaves during a source-sink perturbation in sugarcane. *Annals of Botany* 101: 89-102
- McKee IF, Woodward FI. 1994. CO₂ enrichment responses of wheat: Interactions with temperature, nitrate and phosphate. *New Phytologist* 127(3): 447-453.
- Milne RM, Haynes RJ. 2004. Soil organic matter, microbial properties, and aggregate stability under annual and perennial pastures. *Biology and Fertility of Soils*. 39:172-178.
- Miyamoto S, Martinez I, Padilla M., Portillo A. 2004. Landscape plant lists for salt tolerance assessment. Texas A&M University Agricultural Research and Extension Center at El Paso, Texas Agricultural Experiment Station.
- Moore KJ, Vogel KP, Klopfenstein TJ, Masters RA, Anderson BE. 1995. Evaluation of four intermediate wheatgrass populations under grazing. *Agronomy Journal* 87: 744-747
- Murphy KM, Lyon SR, Balow KA, Jones SS. 2010. Post-sexual cycle regrowth and grain yield in *Thinopyrum elongatum* x *Triticum aestivum* amphiploid. *Plant Breeding* 129: 480-483.
- Nagarajan S, Singh DV. 1990. Long distance dispersal of rust pathogens. *Annual Review of Phytopathology* 28: 139-153.

- Nelson JDJ, Schoenau JJ, Malhi S. 2008. Soil organic carbon changes and distribution in cultivated and restored grassland soils in Saskatchewan. *Nutrient Cycling in Agroecosystems* 82(2): 137-148.
- Nippert JB, Knapp AK. 2007. Soil water partitioning contributes to species coexistence in tallgrass prairie. *Oikos* 116(6): 1017-1029.
- Niva M, Svensson BM, Karlsson PS. 2003. Nutrient resorption from senescing leaves of the clonal plant *Linnea borealis* in relation to reproductive state and resource availability. *Functional Ecology* 17(4): 438-444.
- Obeso JR. 2002. The costs of reproduction in plants. *New Phytologist* 155(3): 321-348.
- Oram RN. 1996. *Secale montanum*: a wider role in Australasia? *New Zealand Journal of Agricultural Research* 39:629-633.
- Owensby CE, Coyne PI, Ham JM, Auen LM, Knapp AK. 1993. Biomass production in a tallgrass prairie ecosystem exposed to ambient and elevated CO₂. *Ecological Applications* 3(4): 644-653.
- Peet MM, Kramer PJ. 1980. Effects of decreasing source/sink ratio in soybeans on photosynthesis, photorespiration, transpiration and yield. *Plant, Cell and Environment* 3(3): 201-206.
- Pinter PJ, Kimball BA, Garcia RL, Wall GW, Hunsaker DJ, LaMorte RL. 1996. Free air CO₂ enrichment: responses of cotton and wheat crops. In Koch GW, Mooney HA. eds. *Carbon Dioxide and Terrestrial Ecosystems*. Physical Ecology Series. San Diego, CA: Academic Press, 1996. pp. 215-250.
- Piper FI, Cavieres LA, Reyes-Diaz M, Corcuera LJ. 2006. Carbon sink limitation and frost tolerance control performance of the tree *Kageneckia angustifolia* D. Don (Rosaceae) at the treeline in central Chile. *Plant Ecology* 185(1): 29-39.
- Ploschuk EL, Slafer GA, Ravetta DA. 2005. Reproductive allocation of biomass and nitrogen in annual and perennial *Lesquerella* crops. *Annals of Botany* 96: 127-135.
- Prakash S, Singhal NC. 2003. Natural cross-pollination in bread wheat (*Triticum aestivum* L.). *Seed Research* 31: 22-26.
- Primack RB, Hall P. 1990. Costs of reproduction in the pink lady's slipper orchid: a four year experimental study. *American Naturalist* 136(5): 638-656.
- Pritts MP, Hancock JF. 1985. Lifetime biomass partitioning and yield component relationships in the highbush blueberry, *Vaccinium corymbosum*. *American Journal of Botany* 72: 446-452.

Ralphs MH, Manners GD, Gardner DR. 1998. Influence of light and photosynthesis on alkaloid concentration in larkspur. *Journal of Chemical Ecology* 24(1): 167-182.

Reekie EG, Bazzaz FA. 1987a. Reproductive effort in plants. 1. Carbon allocation to reproduction *American Naturalist* 129: 876-896.

Reekie EG, Bazzaz FA. 1987b. Reproductive effort in plants. 2. Does carbon reflect the allocation of other resources? *American Naturalist* 129: 897-906.

Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JMH, Naeem S, Trost, J. 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO₂. *Nature* 440: 922-925.

Ridley AM, Simpson RJ, White RE. Nitrate leaching under phalaris, cocksfoot, and annual ryegrass pastures and implications for soil acidification. *Australian Journal of Agricultural Research* 50(1): 55-64.

Ridley AM, White RE, Helyar KR, Morrison GR, Heng LK, Fisher R. 2001. Nitrate leaching loss under annual and perennial pastures with and without lime on a duplex (texture contrast) soil in humid southeastern Australia. *European Journal of Soil Science* 52(2): 237-252.

Reimann-Philipp, R. 1986. Perennial spring rye as a crop alternative. *Journal of Agronomy and Crop Science* 157: 281-285.

Reimann-Philipp R. 1995. Breeding perennial rye. *Plant Breeding Reviews* 13:265–292.

Robertson GP, Paul EA, Harwood RR. Greenhouse gases in intensive agriculture: contributions of individual gases to the radiative forcing of the atmosphere. *Science* 289(5486): 1922-1925.

Rosas C, Cock JH, Sandoval G. 1976. Leaf fall in cassava. *Experimental Agriculture* 12: 395-400.

Roumet C, Roy J. 1996. Prediction of the growth response to elevated CO₂: A search for physiological criteria in closely related grass species. *New Phytologist* 134: 615-621.

Roumet C, Urcelay, C, Díaz, S. 2006. Suites of root traits differ between annual and perennial species growing in the field. *New Phytologist* 170(2): 357-368.

Salopek-Sondi B, Kovac M, Ljubescic N, Volker M. 2000. Fruit initiation in *Helleborus niger* L. triggers chloroplast formation and photosynthesis in the perianth. *Journal of Plant Physiology* 157(4): 357-364.

Sawada S, Hasegawa Y, Kasai M, Sasaki M. 1989. Photosynthetic electron transport and carbon metabolism during altered source/sink balance in single rooted soybean leaves. *Plant and Cell Physiology* 30: 691-698.

- Sawada S, Arakawa A, Muraki I, Echigo H, Miyashita M, Iwafune M, Kasai M, Usuda I. 1999. Photosynthesis with single-rooted *Amaranthus* leaves. I. Changes in the activities of RuBP-1,5-bisphosphate carboxylase and phosphoenolpyruvate carboxylase and the amounts of intermediates in photosynthetic metabolism in response to changes in the source-sink balance. *Plant and Cell Physiology* 40: 1143-1151.
- Scheinost P, Lammer D, Cai X, Murray TD, Jones SS. 2001. Perennial wheat: a sustainable cropping system for the Pacific Northwest. *American Journal of Alternative Agriculture* 16:147–151.
- Schulz-Schaeffer J, Haller SE. 1987. Registration of Montana-2 perennial x *Agrotriticum* intermediodurum Khizhnyak. *Crop Science* 27: 822-823.
- Schulz-Schaeffer J, Haller SE. 1988. Alien chromosome addition in wheat: Advanced progeny. *Genome* 30: 303-306.
- Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL. 2007. Fitting photosynthetic carbon dioxide response curves for C₃ leaves. *Plant, Cell and Environment* 30: 1035-1040.
- Shi C, Tian C, Li J, Zhang Y. 2011. Prospect of perennial wheat in agroecological systems. *Procedia Environmental Sciences* 11: 1574-1579.
- Smoliak S. 1981. Hay and pasture crops: Kinds of forage crops. 7-37. In Alberta Forage Crops Advisory Committee, Alberta Forage Manual, Alberta Agriculture, Edmonton.
- Snow AA, Whigham DF. 1989. Costs of flower and fruit production in *Tipularia discolor* (Orchidaceae). *Ecology* 70(5): 1286-1293.
- Tang ZX, Ross K, Ren ZL, Yang ZJ, Zhang HY, Chikmawati T, Gustafson JP. 2011. Secale. Wild Crop Relatives: Genomic and Breeding Resources. Chittaranjan K. ed. Berlin: Springer Verlag, 2011. pp. 367-396.
- Taylor SH, Hulme SP, Rees M, Ripley BS, Woodward FI, Osborne CP. 2010. Ecophysiological traits in C₃ and C₄ grasses: a phylogenetically controlled screening experiment. *New Phytologist* 185: 780-791.
- Thomas MB, Wratten SD, Sotherton NW. 1992. Creation of island habitats in farmland to manipulate populations of beneficial arthropods: Predator densities and species composition. *Journal of Applied Ecology* 29(2): 524-531.
- Tscharntke T., Greiler HJ. Insect communities, grasses and grasslands. *Annual Review of Entomology* 40: 535-558.
- Tsitsin NV. 1965. Remote hybridisation as a method of creating new species and varieties of plants. *Euphytica* 14:326–330.

- Tuell JK, Fiedler AK, Landis D, Isaacs R. 2008. Visits by wild and managed bees (Hymenoptera: Apoidea) to eastern U.S. native plants for use in conservation programs. *Environmental Entomology* 37(3): 707-718.
- Upreti DC, Dwivedi N, Raj A, Jaiswal S, Paswan G, Jain V, Maini HK. 2009. Study on the response of diploid, tetraploid and hexaploid wheat to elevated CO₂. *Physiology and Molecular Biology of Plants* 15: 161-168.
- Van Tassel DL, DeHaan LR, Cox TS. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications* 3: 434-452.
- Vazquez de Aldana BR, Geerts RHEM, Berendse, F. 1996. Nitrogen losses from perennial grass species. *Oecologia* 106: 137-143.
- Villalobos FJ, Testi L, Hidalgo J, Pastor M, Orgaz F. 2006. Modelling potential growth and yield of olive (*Olea europaea* L.) canopies. *European Journal of Agronomy* 24: 296-303.
- Wagoner P. 1995. Intermediate wheatgrass (*Thinopyrum intermedium*): development of a perennial grain crop. In: Williams JT ed(s). *Cereals and pseudocereals. (Underutilized crop series; 2)*. London: Chapman and Hall, pp.247-259.
- Wagoner P, Schaeffer JR. 1990. Perennial grain development: past efforts and potential for the future. *Critical Reviews in Plant Sciences* 9(5): 381-408.
- Wagoner P, Schauer A. 1990. Intermediate wheatgrass as a perennial grain crop. In Janick, J. and Simon, J.E. eds. *Advances in new crops : proceedings of the First National Symposium NEW CROPS, Research, Development, Economics, Indianapolis, Indiana, Oct 23-26, 1988*. Portland: Timber Press, 1990.
- Wang RRC, Larson SR, Jensen KB. 2010. Analyses of *Thinopyrum bessarabicum*, *T. elongatum* and *T. junceum* chromosomes using EST-SSR markers. *Genome* 53: 1083-1089.
- Wang RRC, Li X-M, Hu Z-M, Zhang J-Y, Larson SR, Zhang X-Y, Grieve CM, Shannon MC. 2003. Development of salinity tolerant wheat recombinant lines from a wheat disomic addition carrying a *Thinopyrum junceum* chromosome. *International Journal of Plant Sciences* 164: 25-33.
- Weik L, Kaul H-P, Kubler E, Aufhammer W. 2002. Grain yields of perennial grain crops in pure and mixed stands. *Journal of Agronomy and Crop Science* 188(5): 342-349.
- West TO, Post WM. Soil organic carbon sequestration rates by tillage and crop rotation: a global data analysis. *Soil Science Society of America Journal* 66: 1930-1946.
- Wheelwright NT, Logan BA. 2004. Previous year reproduction reduces photosynthetic capacity and slows lifetime growth in females of a neotropical tree. *Proceedings of the National Academy of Sciences* 101: 8051-8055.

Witkowski ETF, Lamont BB. 1995. Disproportionate allocation of mineral nutrients and carbon between vegetative and reproductive structures in *Banksia hookeriana*. *Oecologia* 105: 38-42.

Wullschlegel SD. 1993. Biochemical limitations to carbon assimilation in C₃ plants: a retrospective analysis of the A / C_i curves from 109 species. *Journal of Experimental Botany* 44: 907-920.

Xia G, Cheng G, Lakso A, Goffinet M. 2009. Effects of nitrogen supply on source-sink balance and fruit size of 'Gala' apple trees. *Journal of the American Society for Horticultural Science* 134: 126-133.

Xu X, Nicholson P. 2009. Community ecology of fungal pathogens causing wheat head blight. *Annual Review of Phytopathology* 47: 83-103.

Yousufzai M-N, Siddiqui KA, Soomro AQ. 2009. Flag leaf stomatal frequency and the interrelationship with yield and yield components in wheat (*Triticum aestivum*). *Pakistani Journal of Botany* 41(2): 663-666.

Zan CS, Fyles JW, Girouard P, Samson RA. 2001. Carbon sequestration in perennial bioenergy, annual corn and uncultivated systems in southern Quebec. *Agriculture, Ecosystems and Environment* 86(2): 135-144.

Zhao M, Botwright Acuña TL, Lafitte HR, Dimayuga G, Sacks E. 2008. Perennial hybrids of *Oryza sativa* x *O. rufipogon*: Carbon exchange and assimilate partitioning. *Field Crops Research* 106: 214-223.

Zhao M, Ding Z, Lafitte R, Sacks E, Dimayuga G, Holt D. 2010. Photosynthetic characteristics in *Oryza* species. *Photosynthetica* 48: 234-240.

Zhou R, Quebedeaux B. 2003. Changes in photosynthesis and carbohydrate metabolism in mature apple leaves in response to whole plant source-sink manipulation. *Journal of the American Society for Horticultural Science* 128: 113-119.

Zotz G, Richter A. 2006. Changes in carbohydrate and nutrient contents throughout a reproductive cycle indicate that phosphorus is a limiting nutrient in the epiphytic bromeliad, *Werauhia sanguinolenta*. *Annals of Botany* 97: 745-754.

CHAPTER 2. AGRONOMY OF PERENNIAL WHEAT AND RYE

Introduction

In recent years, increasing attention has been paid to developing perennial alternatives to annual food crops (Cox et al., 2006; Sacks et al., 2005; Piper and Kulakow, 1994; DeHaan et al., 2005; Jackson and DeWald, 1994). Perennial cereals are of interest due to potential for improved environmental services (e.g. Ridley et al., 2001; Crews, 2005) and lower production costs. Since the majority of cropping systems worldwide are based on staple cereals, developing perennial cereals will be critical to large-scale perennialization of agriculture. Two promising perennial cereal candidates for cold-temperate regions, developed by hybridizing annual cereals with perennial wild relatives, include perennial wheat (*Triticum aestivum* x *Thinopyrum* spp.) and perennial rye (*Secale cereale* x *S. montanum*). The current scarcity of plant life-forms combining herbaceous growth form, perennial life history, and very high reproductive allocation (Van Tassel et al., 2010) makes these two cereals interesting ecologically as well as agronomically.

It is thought that grain yield penalties may occur in perennials due to resource tradeoffs between reproduction on the one hand, and winter survival and regrowth on the other. A modeling study (Bell et al., 2008) concluded that perennial wheat would be economically viable in Australia if it achieved 40% of annual wheat grain yields, combined with at least 800 kg ha⁻¹ of forage per year (or alternatively if it could achieve at least 60% of annual wheat yield with no additional forage production). This prompts the question: can perennial cereals, at their current stage of development, achieve the threshold level of 40-60% yield level relative to annuals? Forage production, particularly early in the season, may be an important additional contribution of perennial cereals, which makes it critical to measure biomass production as well as grain yield. Finally, an agronomic assessment of perennial cereals will need to include an

understanding of their phenology. Differences in phenology between perennial cereals and their annual relatives could affect early season grazing potential, susceptibility to pathogens (Emrich et al., 2008), and vulnerability to extreme weather, but little information is available on the phenology of emerging perennial cereal species.

To understand the agronomic potential of perennial cereals, they must be studied over multiple years, as grain production may change with stand age. Perennial cereals might display delayed reproductive investment, similarly to many woody perennials: in this case, plants would show low reproductive investment the first year (the establishment year), but in future years the established root and crown reserves would allow plants to begin growth earlier, grow larger, and show marked increases in seed production (Jackson and Jackson, 1999). Alternatively, yield decreases might be observed due to the buildup of soil pathogens, short plant life span, or the proliferation of weak and unproductive tillers. This is a pattern that is observed in grasses such as *Bromus inermis*, *Festuca arundinacea*, and *Poa pratensis* (Loch et al., 1999; Fulkerson, 1980; Fairey and Lefkovitch, 2001), although a number of other cool season forage grasses show stability in seed yields over two to six seasons (Chastain et al., 1997; Canode and Law, 1978; Mueller-Warrant and Rosato, 2002; Fulkerson, 1980) or even increases as demonstrated for *Agropyron cristatum* and *Festuca rubra* (Canode, 1968). In order to test the hypothesis that yields will decrease over time, it is necessary to study perennial cereals over at least two years.

Perennial wheat and rye are still in the process of development, and there are relatively few studies of grain yield under agronomic conditions. Early work on perennial wheat was done in the former Soviet Union, but little of this data has been easily accessible to researchers in other countries. More recently, first year grain yield was measured in a study of 31 perennial wheat genotypes in eastern Washington (Murphy et al., 2010), finding yields of up to 93% of

annual wheat in the highest yielding accession. Grain yield was studied in approximately 90 perennial wheat derivatives over two years in Australia (Hayes et al., 2012) and with some earlier cultivars of perennial rye over two- or three-year periods: “Permontra” (Reimann-Philipp, 1986; Weik et al., 2002) and the newly developed ACE-1 (Acharya et al., 2003; Acharya et al., 2004). These studies generally found perennial rye yielded approximately 55-60% of annual rye, and that grain yield of perennial wheat lines were highly variable, ranging between 2% and 135% of annual wheat among those lines that showed appreciable regrowth. However, these multi-year studies did not explicitly separate the effects of calendar year (reflecting year to year weather fluctuations) from plant age (reflecting possible effects of senescence, metabolic tradeoffs, changes in allocation, changing energy and nutrient status of the plants, and other age-related phenomena). Our study makes a novel contribution to the growing literature on perennial cereals by observing one year-old plants in two different years (achieving replication in time), in a new and different environment, and by comparing one and two year-old plants within a single year (thus separating the effects of plant age and calendar year). We were thus able to consider how genotype / cultivar, calendar year, and plant age each affected plant yield, biomass and phenology.

Between 2008 and 2010, we assessed the agronomic potential of perennial wheat and perennial rye in southwest Michigan using a multi-year field experiment. We measured parameters relating to biomass, yield, and phenology to document how these plants compare, over time, to their annual relatives. Our guiding hypotheses were the following: 1) One year-old perennial wheat and rye will yield lower than their annual analogues, 2) Two year-old perennial wheat and rye will produce lower biomass and lower seed yields, compared to first-year plants, and 3) Two year-old perennial wheat and rye will show more early season growth, and earlier

flowering, than one year-old perennial wheat and rye. We also hoped to establish whether current accessions of perennial wheat and rye could achieve the threshold for economic feasibility set by modeling studies (Bell et al., 2008).

Materials and Methods

Site Characteristics

We conducted this study at the W. K. Kellogg Biological Station (KBS) located in southwest Michigan, USA, 50 km east of Lake Michigan (42° 24' N, 85° 24' W, elevation 288 m) on soils developed from glacial outwash deposited 12000 years ago. Soils are mainly of the Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs), Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs) and Miami (fine-loamy, mixed mesic Typic Hapludalfs) series, which all occur on our site (Crum and Collins, 1995). The area receives *ca.* 897 mm of precipitation annually (based on a 24-year average), with approximately half as snow, and the mean annual temperature is 9.7° C. The study was laid out in a field approximately 20 m wide by 73 m long, oriented roughly north-south on level ground. Further site and soils descriptions for KBS are available at http://lter.kbs.msu.edu/Data/LTER_Metadata.jsp/Dataset/KBS042.

Accession Selection

Eight accessions (either breeding lines or named varieties) were involved in the study. The annual winter wheat (*Triticum aestivum*) ‘checks’ included Frankenmuth (PVP 8000165), and Pioneer 25R37 (PVP 0020232: Pioneer Hi-Bred Ltd.). Frankenmuth is an older cultivar

which was released in 1971 from MSU. It was commonly grown in the 1980s and 1990s (e.g., Huebner et al., 1999) and has been used as a benchmark for yield and quality in breeding studies at Michigan State University. It lacks the semi-dwarfing genes common in modern cultivars, and has somewhat greater biomass and lower grain yield. In a 2007 comparison of grain yields across 29 varieties and six sites, Frankenmuth yielded approximately 13% lower than the mean, and 11% lower than Pioneer 25R37. Pioneer 25R37 is a newer, high yielding variety that was widely grown in the early to mid-2000s and considered well adapted for Michigan. In the aforementioned study from 2007, Pioneer 25R37 yielded the same as the mean across all 29 lines. Pioneer 25R37 is a soft red variety while Frankenmuth is a soft white.

Four perennial wheat accessions were included (*Triticum aestivum* cv. ‘Chinese Spring’ x *Thinopyrum elongatum* // *T. aestivum* cv. ‘Madsen’), all of which were drawn from a larger group of accessions contributed by Washington State University in 2005, and had been involved in previous studies in Washington (Murphy et al., 2009; Murphy et al. 2010). We selected four accessions out of the 20 based on observed differences in harvest index and morphology displayed in a 2008 pilot observational trial at Kellogg Biological Station. P3 showed a relatively low harvest index (as well as more ‘grass-like’ spikelet morphology) while P11 had the highest harvest index combined with spikelets resembling annual wheat: P15 and P19 had intermediate harvest index and morphology. Also included was a cover annual rye cultivar (*Secale cereale* cv. ‘Wheeler’), and a perennial hybrid rye cultivar (*Secale cereale* x *montanum* cv. ‘Rival’).

Experimental Design

This study was laid out as a split plot RCBD with five blocks, using ‘planting year’ as the

whole-plot factor and ‘accession’ as the split-plot factor. ‘Planting year’ was an indication of when a particular plot was first planted (2008 or 2009). When comparing one year-old plants in 2009 to one year-old plants in 2010, ‘planting year’ thus reflected year-to-year weather variation (and will be referred to in the analysis as ‘year’); when comparing older vs. younger perennial plants in 2010, ‘planting year’ reflected the age of the plants, and will be referred to as ‘age’ (since one year-old plants had been planted in 2009, and two year-old plants had been planted in 2008). Thus we were able to consider separately the effects of both year-to-year weather variation, as well as plant age (Shefferson and Roach, 2010). A very similar approach (comparing a single cohort across years, and multiple age cohorts within years) has been used to study a close relative of an agricultural plant, *Beta maritima*, in the greenhouse (Van Dijk, 2009). In the analysis, tables, and graphs, the term ‘2009’ will be taken to refer to the entire Oct. 2008 - Sept. 2009 growing season, and ‘2010’ to the entire Oct. 2009 – Sept. 2010 growing season.

One full set of plants, with each of the eight accessions each replicated five times, was first planted in fall 2008. Following harvest in Aug. 2009, perennial accessions were allowed to regrow into a second season, while the annual plots were tilled under and replanted with the same annual cultivars in Nov. 2009. Also during Nov. 2009, a full second set of perennial plants was planted. Thus in 2010 the study included two year-old perennial plants, one year-old perennial plants, and one year-old annual plants. The subplots were each approximately 5.47 m long, and included six rows of plants with 19 cm between rows; there were 57 cm alleys between subplots and 90 cm between whole plots (Table 1).

Management

Dates of field operations are given in Table 2. Plants were seeded at 1.5 million

seeds ha^{-1} about 1.9 cm deep, using an Almaco small plot planter. The field was fertilized in fall 2008 with composted dairy manure (at the rate of 90 kg ha^{-1} N), while in fall 2009 we fertilized with approximately 102 kg ha^{-1} N in the form of pig blood meal (13% N). Plots were irrigated once in summer 2009, applying approximately 3.2 cm of water. In Oct. 2010, regrowth was extremely low and variable, with no accession showing more than 10% regrowth, and over half the plots showing zero regrowth. This might have been due to the warm spring coupled with hot and dry weather in the late summer, and intense weed pressure. Because of very poor regrowth, the experiment was ended in Oct. 2010. During each season, weeding took place four times and was carried out by hand.

Measurements

On 20 March 2009 and 16 March 2010, about one week after snowmelt, the number of growing plant stems per meter in each plot were counted (which estimated spring emergence for annuals and one year-old perennials, and regrowth for the two year-old perennials). Plant height was estimated by sampling six plants from each plot, at the same time that we counted plant stems. To estimate flowering date, plants were observed once every four days from 20 May to 23 July 2009 or from 1 May to 6 July 2010. We estimated the percent having flowered at each date and calculated a 50% flowering date through linear interpolation. In the analysis, “first-year plants” denotes plants during their first growing season. “Second-year plants” denotes plants during their second full growing season.

Harvest during the 2009 and 2010 growing seasons took place six to seven weeks following flowering for each accession. Harvest dates in 2009 were 23 July for perennial rye,

annual rye and annual wheat, and 13 August for perennial wheat. Harvest dates in 2010 were 10 July for annual wheat, 17 July for annual and perennial rye, and 9 August for perennial wheat. Hand harvests were taken for yield estimation on 4 m lengths for each of the two central rows: seed heads, leaves, and stems were dried at 65^o C for four days (until weight did not change) and re-weighed. Because a pilot study had found that perennial wheat had low threshability in standard equipment, we used a customized tabletop seed cleaner at Michigan State University to estimate the grain percentage of the total seed head mass. These estimates were based on the grain recovery from 70 g subsamples of unthreshed seed heads for each accession. Our calculated grain mass / seed head mass ratios obtained from the tabletop seed cleaner were multiplied by the measured total seed head mass for each plot, to give an estimate of threshed seed yield. At the time of harvest, we counted reproductive tillers on the harvested rows: following seed cleaning we measured the thousand-kernel weight on bulked seed samples of each accession and divided by 1000 to obtain kernel mass. In Oct. of each year, four 20 cm² microplots in each plot were sampled for regrowth. Regrowth was estimated as (number of visibly regrowing green leaves) x (number of reproductive tillers at harvest)⁻¹ x 100.

Data Analysis

To determine the effect of year-to-year variation on each parameter of interest, we compared first-year plants in 2009 to first-year plants in 2010 (including annual wheat and rye checks). Our model was a split plot RCBD using calendar 'year' as the main plot factor, 'accession' as the subplot factor, calendar 'year x accession', and block. Following the overall analysis, we then held year constant to determine the effect of accession differences within each

year. To determine the effect of plant age on each parameter, we compared first-year plants (perennial accession only) in 2010 to second-year plants within the same year. Here our model was a split plot RCBD using plant ‘age’ as the main plot factor, ‘accession’ as the subplot factor, ‘age x accession’, and block. To determine differences between accession within each age class, we held age constant and considered the effect of accession. All analyses were done using the MIXED procedure in SAS 9.2 (SAS Institute, 2008) followed by planned contrasts using protected LSD.

Analysis of kernel mass did not include a block effect since these were measured after bulking seed from all blocks; we used five replicates for each variety. Mass-related parameters were log-transformed to improve normality, threshability and harvest index were logit-transformed to meet assumptions of ANOVA, and other variables were untransformed.

The overall model for one of the response variables (threshed yield) was, e.g.:

$$\ln(\text{yield}) = \text{block} + \text{year} + \text{block} \times \text{year} + \text{accession} + \text{year} \times \text{accession}.$$

Results

Environment

The pattern of precipitation in the fall of 2008 and 2009 was similar, with a dry November (50% below the 24 year average), but otherwise well distributed precipitation (Figure 1). Winter temperatures may have affected survival, as evidenced by the lower springtime emergence in 2010 following a very cold January). Early spring precipitation was wet in 2009 (54% above the 24-year average) and dry in 2010 (25% below the 24-year average). However,

there was no difference between the years in terms of early spring growth of perennial cereals, as indicated by March plant height measurements (Table 6). The later spring (May and June) was much wetter in 2010 than in 2009, with 120% higher precipitation; overall, the spring was consistently warmer in 2010 than in 2009, with an average difference of 1.8^o C over the March-June period. Thus the 2010 spring was warmer, drier early on but much wetter in the later spring, as compared to 2009.

Yield of First-year Perennials and Annuals

Threshed grain yield of first-year plants was similar between 2009 and 2010, with no overall main effect of year (Table 3). The yield of the annual cereals was low: averaging across both years, grain yields were 2.41 Mg ha⁻¹ for Pioneer 25R37, 2.94 Mg ha⁻¹ for Frankenmuth and 1.83 Mg ha⁻¹ for Wheeler rye. These yields are consistent with those achieved at our site in the past (Smith et al., 2008) reflecting the sandy soils and the fact that our southwest Michigan location is at the fringes of the wheat belt. Two accessions (P19 and P15) yielded 40% higher in 2010 compared to 2009, while all other accessions did not differ between the two years. Perennial wheat and perennial rye plants consistently yielded lower than their annual counterparts (Table 3). The first-year perennial wheat plants, averaging across accession, yielded 45% of annual wheat in 2009 ($t = 10.16, p < 0.001$), and 54% of annual wheat in 2010 ($t = 8.86, p < 0.0001$; Figure 2). Similarly, perennial rye, achieving 76% of annual rye yields in 2009 and 69% of annual rye in 2010 ($t = 3.06, p = 0.003$; Figure 3). All four perennial wheat accessions performed the same in 2009, but in 2010 the first-year P19 and P15 plants achieved approximately 81% higher yields than the lowest yielding line P3.

The lower yields in perennial cereals as opposed to their annual relatives reflected lower ratio of grain to chaff, lower kernel mass, lower harvest index, and a lower density of reproductive tillers (Table 3): they did not, by contrast, appear to reflect lower biomass production or lower number of seeds per tiller. The percentage of grain in unthreshed seed heads was lower in perennial cereals than in annuals: when using the tabletop seed cleaner, our seed recovery ranged from 66%-67% (in the case of P3 and perennial rye) to 78% (in the case of P19): seed recovery was 84-86% for the three annual lines. Kernel mass also explained much of the perennial vs. annual yield differences. Kernels of first year perennial wheat plants (averaging across accessions) were 31% smaller than annual wheat in 2009 ($t = 10.92$, $p < 0.0001$), and 56% smaller in 2010 ($t = 8.53$, $p < 0.0001$). Similarly, kernel mass of first year perennial rye was 24% lower than annual rye in 2009, and 20% lower in 2010 ($p < 0.0001$).

Lower number of reproductive tillers per meter contributed to lower seed yield in perennial wheat, but not in perennial rye. Perennial rye had 50% more reproductive tillers than annual rye in 2009 ($t = 2.87$, $p = 0.006$), and equivalent number of reproductive tillers in 2010, illustrating that lower grain yields in perennial rye were not explainable by a lower number of reproductive tillers. Harvest index differed among accessions: first year perennial wheat had a harvest index 43% lower than annual wheat, while first year perennial rye had a harvest index 23% lower than annual rye. Total biomass, by contrast, was actually higher in perennial rye compared to annual rye in 2010, while perennial wheat biomass was equivalent to annual wheat that year. This suggests that differences in biomass were not an important contributor to the lower grain yields in perennials: rather, annuals appear to allocate a greater fraction of biomass to seed reproduction and thus had a higher harvest index than perennials (Table 3).

Similarly, lower grain yields in perennials do not seem to reflect lower number of seeds per reproductive tiller. Perennial rye, in both years, had equivalent number of seeds per reproductive tiller as annual rye. Perennial wheat did not have a consistently lower number of seeds per tiller, and in fact in 2010 three of the four lines had equivalent number of seeds per reproductive tiller as Frankenmuth annual wheat, and 60-89% more than Pioneer 25R37. Thus lower grain yields in the perennial species are not explained by fewer grains per tiller. This is, importantly, not equivalent to saying that they have equal fertility to annual wheat and rye, since we did not count the number of sterile spikelets: if perennial wheat and rye have many more spikelets than their annual analogues, it is possible that lower fertility might coexist with a comparable or higher number of seeds per tiller. Further work is needed to determine whether perennials in fact display lower fertility, in the sense of a lower ratio of mature seeds to total spikelets, as some preliminary observations suggest (DeHaan, L. R., personal communication).

Effects of Plant Age on Yield and Yield Components

Overall, first-year and second-year perennial plants in 2010 showed consistent yields, with no effect of plant age or age x accession interaction observed on grain yield (Table 4). Second-year perennial wheat lines yielded 53% of annual wheat ($t = 5.08$, $p < 0.0001$), equivalent to the first-year plants. Grain yield of second-year perennial rye plants, at approximately 1.44 Mg ha^{-1} , was not significantly different from either annual rye ($p = 0.45$), or first year perennial rye plants ($p = 0.10$). First-year perennial wheat plants showed accession-related differences in yield, with P19 and P15 producing 81% higher yields than P3; in the second year, however, P19 and P15 yielded only 24% higher than P3, suggesting a decline in their relative yield advantage.

The overall consistent grain yields reflected the lack of strong age-related trends in yield components, although some individual accessions did show changes in yield components between first and second year plants (Table 4). Kernel mass, for example, showed a strong accession effect ($F = 12.00$, $p < 0.0001$), an accession x age interaction ($F = 4.91$, $p = 0.03$), reflecting the fact that some lines did show a decrease in kernel mass with age. Second-year perennial rye and P19 plants had 15% smaller kernels than first year plants, while the other three perennial wheat lines showed no differences (Figure 4). Similarly, there were no consistent age-related trends in terms of biomass or harvest index. Comparing across generations in 2010, we found that biomass was significantly affected by accession, but not by age, nor was there an age x accession interaction observed (Table 4). Harvest index, similarly, showed strong age x accession interactions. Specifically, older plants of P11 and perennial rye had roughly a 16% lower harvest index than younger plants, while P3, P15 and P19 had a 16 – 33% higher harvest indices. Thus there appeared to be few overall clear trends in yield components between the two years, which helped to explain the overall lack of change in grain yield.

Plant Growth, PSCR and Phenology

Interestingly, a plant age effect was observed for early season growth: plant height achieved by mid-March differed between first and second year perennials, with the older plants being on average 110% taller (Table 6). In mid-March 2010, first-year perennial wheat was 22% shorter than annual wheat and two year-old perennial wheat was 59% taller. A similar pattern was observed in perennial rye, where one year-old perennial plants were 27% shorter than annual rye and two year-old plants were 75% taller.). Early season height differences were, to some extent, maintained later in the season: two year-old perennials were overall 10% taller at anthesis

than one year-old perennials, with a 26% difference in the case of perennial rye (Table 6). Second-year perennial wheat and rye were 21% and 11% taller than annual wheat and rye in 2010, respectively. Height at flowering showed effects of calendar year as well as plant age, with first-year perennial wheat and rye both being shorter in 2010 compared to 2009.

The number of plant stems present in March 2010 was similar for both one year-old and two year-old perennial plants (Table 6) and there was no main effect of age nor age x accession interaction. This suggests that each perennial stand, as a whole, was able to fully replenish itself through either regrowth or re-seeding. Regrowth in the fall of 2009 (Table 5) was vigorous, with perennial rye and P19 perennial wheat both showing over 100% regrowth, and with the most poorly regrowing line, P3, showing over 50% regrowth. In the fall of 2010, however, regrowth was extremely poor: no accession achieved over 10% regrowth, and 55% of plots showed zero detectable regrowth. Regrowth in the fall of 2010 was equally poor when one and two year-old plots were compared.

Flowering dates of one year-old plants also showed strong effects of accession (with perennials generally showing later flowering) as well as effects of calendar year, and the interaction of calendar year x accession (Table 5). The one year-old perennial wheat generally flowered on 31 June in 2009 (26 days after annual wheat, $p < 0.0001$) and 19 June in 2010 (15 days after annual wheat, $p < 0.0001$), while the average flowering date was 11 June for perennial rye in both years (11 – 12 days after annual rye). The tendency of plants to flower earlier in 2010, due to the difference in weather, was one of the strongest and most distinct trends between the years, and was much more marked in the perennial species than in the annual species.

Flowering date in 2010 showed strong differences between first- and second- year perennials (Table 6). When comparing the one and two year-old perennial plants in 2010, we

found that flowering date was significantly affected by accession and by age, but not by the interaction of the two; thus all second-year perennials showed a similar shift in flowering date relative to first-year plants (approximately 7 – 11 days earlier). Two year old perennial wheat plants flowered on 13 June , nine days after annual wheat ($t = 7.75, p < 0.0001$), while two year-old perennial rye plants flowered at approximately the same time as annual rye, on 3 June. Perennial wheat accessions did not significantly differ in terms of flowering date. To sum up, the strong age effects on early-season plant height and flowering date reflect an overall “shift” in phenology towards earlier in the year. It is interesting that plant age and perennial vs. annual life history both seem to have effects of similar magnitude on flowering date (shifting them by one to two weeks in either direction).

Discussion

To the best of our knowledge, this is one of the first reports outside the former Soviet Union on a two-year test of yield potential for perennial wheat and rye that separates effects of plant age from effects of year-to-year weather variation. This allowed testing of our hypotheses that first-year grain yields in perennial cereals would be lower than annuals, and that perennial grain yields would decline with increasing plant age.

Consistent with our first hypothesis, first-year yield in perennial wheat and perennial rye were consistently lower than their annual analogues: perennial rye yielded 72% of annual rye, while perennial wheat yielded 50% of annual wheat. These results are somewhat higher than the normal range found for perennial wheat lines tested in Australia or in the Pacific Northwest, but

comparable to previous results on these particular lines. A previous study of 31 perennial wheat lines in Washington (Murphy et al., 2010) included all four of the lines included in this study, and reported yields ranging from 28% of annual wheat (in the case of P15) to 51% of annual wheat (in the case of P19). A more recent study (Hayes et al., 2012) compared over 90 perennial cereal lines at a plot scale and an additional 86 in single rows. Among the 40 lines which showed evidence of post-sexual cycle regrowth, grain yield was generally 26 – 42% of annual wheat at the 95% confidence interval; among the pool of Washington State University (WSU) breeding lines from which our four lines were selected, grain yield was 23 – 43% of annual wheat, but in absolute terms higher than achieved in our study (possibly reflecting adverse environmental and soil conditions at our site). P19, for example, produced approximately 2.2 t ha^{-1} grain yield, which was about 50% of ‘Wedgetail’ annual wheat at the site. Additionally, there was no correlation between vigor of regrowth and grain yield. Annual wheat yields in our study were higher than in Washington but lower than in Australia, reflecting the marginal nature of our site.

Our estimates of perennial rye yield were 1.3 to 1.4 Mg ha^{-1} in first year plants, about 60% of previously reported values (e.g., 2.2 to 2.4 Mg ha^{-1} in central Europe; Reimann-Philipp, 1986; Weik et al., 2002). However, perennial rye yields relative to annual rye were actually higher in our study than in previous studies, indicating that in this relatively low-yielding environment perennial rye suffers less of a yield reduction than annual rye. For example, studies of the perennial rye ACE-1 in western Canada found yields of 2.5 Mg ha^{-1} , averaging over 1 – 3 year old plants, approximately 55% of annual rye yield at that site (Acharya et al., 2004).

The lower yields in perennial cereals reflected lower reproductive allocation and kernel mass. This is consistent with previous studies of perennial sorghum, which showed 35% lower

kernel mass and 16% lower reproductive allocation than annual sorghum (Piper and Kulakow, 1994) as well as ACE-1 perennial rye which had lower harvest index but greater biomass than annual rye (Acharya et al., 2004). Lower grain yields in perennials may also have reflected lower initial plant population density. In both years the perennial lines generally had fewer growing plant stems in the early spring, as well as (in perennial wheat) fewer reproductive tillers. Interestingly, the shorter period for pre-winter growth in the fall of 2009, does not appear to have reduced yields in 2010 relative to 2009. Growing conditions prior to vernalization can have a very strong effect on subsequent year yields in cool season perennial grasses (Chastain and Young, 1998), but such effects were not seen in our study.

We did not find support for our second hypothesis: grain yield in perennial wheat and rye showed no effect of plant age. This is consistent with the results of Hayes et al. (2012) who found that second-year perennial wheat grain yield was highly variable between the perenniating lines, ranging from about 1% to over 1000% of first-year yield (depending on the accession). The data from this previously published experiment suggests that some perennial wheat accessions do show a strong pattern of delayed reproductive investment, while others are close to being fully annual with very little second year seed production. The four lines in our study were in the middle of their range of observations, with approximately equal first- and second-year yields, as well as slightly higher yields overall. We found that perennial rye also maintained equivalent yields in the first and second years: this is consistent with an early study of ‘Permontra’ (Reimann-Philipp, 1986) but conflicts with a later study that found a 73 – 88% decline in yield (Weik et al., 2002). However, yield declines in the 2002 study were attributed to heavy weed pressure and possibly nitrogen limitation as weed cover increased 2500% in the second year. In particular, our study highlights the potential that perennial rye might have under high-nutrient,

intensively managed conditions. The lack of yield declines in our two perennial species is encouraging.

Previous studies of perennial forage grasses supplement the sparse literature on perennial wheat and rye, and provide conflicting evidence concerning the potential for second-year seed yield. A study of *Pennisetum polystachyon* and *Andropogon gayanus*, involving spaced plants, found increases in seed yield between years 1 and 2, followed by decrease (Mishra and Chatterjee, 1968). Seed yields doubled from the first to the second year in desert wheatgrass (*Agropyron desertorum*: Canode and Law, 1978) and also increased in red fescue (*Festuca rubra*: Canode, 1968). In contrast, yields of Kentucky bluegrass (*Poa pratensis*: Chastain et al., 1997), orchardgrass (*Dactylis glomerata*: Fulkerson, 1980) and perennial ryegrass (*Lolium perenne*: Mueller-Warrant and Caprice Rosato, 2002) stay generally stable over the first few years. Still other grasses show yield declines starting from the first year, e.g. Russian wildrye (*Elymus junceus*: Lawrence and Ashford, 1964) and timothy (*Phleum pratense*; Fulkerson, 1980). Thus changes in yield are highly dependent on the species, and our study provides important new information about two individual species of interest.

As yet, little is known about the biomass production potential of perennial cereals, in spite of the fact that increased forage production could be an important supplementary benefit of these species. In our study we found that biomass was equivalent or higher in perennial wheat and rye, compared to their annual analogues, while perennials allocate a lower fraction of biomass to reproduction. No significant differences in biomass were seen between one and two-year-old perennial plants, indicating no decline or increase as plants age (Table 5). This contrasts with a field study which found that perennial rye biomass yields declined from around 14 to around 7.5 tons ha⁻¹ between their first- and second-years (Acharya et al., 2003). Because

perennial rye biomass was harvested twice in their experiment, these results are not directly comparable to our own. It is suggestive, however, that in the western Canada environment the authors found substantially higher first-year biomass yields of perennial rye than we found in our study. Results from studies of forage grasses over multiple years vary between species. Grasses often produce increasing biomass yields during the first few years of their life: for example, *Miscanthus* biomass production tripled between the first and second year (Clifton-Brown and Lewandowski, 2002) and similar increases in yield over the first three years have been found in *Arundo donax* (Mantineo et al., 2009) and in switchgrass (Schmer et al., 2010). However, a different study of newly planted forage grasses, including close wheat relatives, found strong declines in biomass yields over five years of study (White, 1985). We found that reproductive allocation, reflected by harvest index, remained constant between one year-old and two year-old plants. This is consistent with the hypothesis that age has little effect on seed production or reproductive allocation in these perennial cereals. Perennial forage grasses may show increases in harvest index between the first and second years (e.g. *Festuca arundinacea*) or decreases (*Poa pratensis*), depending on the species (Fairey and Lefkovitz, 2001) while woody agricultural perennials tend to show increases.

The high rate of regrowth in fall 2009 is encouraging, given that previous work on these lines in Washington had found only 40% PSCR in the fall (Murphy et al., 2010). Hayes et al. (2012) found that only a minority of lines showed strong regrowth, and specifically that of the four perennial wheat lines involved in our study, two showed no regrowth and two showed very poor regrowth (generally scoring between 0 – 1.9, compared to 7.2 for perennial rye). Similarly a field study of *Oryza sativa x rufipogon* found that perenniality could quickly be lost following backcrossing to the annual parent (Sacks et al., 2006). However, in keeping with the findings of

these previous studies, we did find strong year-to-year variation in the ability of these perennial plants for PSCR. In 2010 we found very low regrowth in all perennial lines, possibly due to the combination of weed pressure, a hot spring, and very dry weather in late summer. The extreme variability of regrowth in perennial wheat and rye indicates that further breeding efforts should prioritize vigorous regrowth and reliable perenniality. As yet perenniality in these lines is not reliable enough for them to be used as economically viable perennial crops.

Our findings regarding phenology were consistent with the general trend that perennial grasses tend to have delayed phenology compared to closely related annuals (Garnier et al., 1997). Interestingly, older perennial plants flowered substantially earlier than younger ones in our study. There is contrasting evidence from the literature regarding the effect of plant age on phenology, which differs across species. For example, a study of *Beta maritima*, a close relative of the agriculturally important common beet, found that with each increase of one year in age, plants flowered approximately 1.3 days later (Van Dijk, 2009). Conversely, older plants flower earlier in perennial *Lupinus* populations (Bishop and Schemske, 1998) as well as in other species (Torimaru and Tomaru, 2006). Weik and colleagues (2002) found that two year-old perennial rye plants flowered 2 – 7 days later than one year-old plants, in contrast to our results where older plants flowered earlier. However, in the Weik (2002) experiment there was no explicit separation of calendar year and plant age effects. Our study suggests that calendar year, perennial growth habit, and plant age can all exert effects of similar magnitude on flowering date (*ca.* 1 – 2 weeks) which makes separating age and year effects important for future studies. Flowering date is an important characteristic of perennial cereal accessions as it could affect harvest time as well as vulnerability to weather stresses and pathogen attack.

Perennial cereal plants appear to grow more slowly than annuals in their first spring, as

illustrated by the lower height achieved early in the season, although we found that plants in their second spring are larger than annual wheat and rye. This could reflect the ability to draw on stored root and crown reserves early in the spring, and under certain environmental conditions may potentially contribute to perennials growing larger than annuals and compensating for lower reproductive allocation, although this was not observed in our study. While Ward et al. (2011) found no difference in root or shoot growth over the first month in potted annual and perennial wheat plants, this does not appear to reflect performance of the plants in the following spring under natural conditions. Increased growth early in the spring found for second-year perennials may be one factor contributing to earlier flowering when compared to first-year plants.

Conclusions

Yield potential of perennial cereals did not decline over multiple years, and perennial wheat and rye consistently produced approximately 50% and 75% of their respective annual counterparts. This exceeds the 50% minimum threshold of economic feasibility proposed for perennial cereals to succeed as a single-use grain crop in the Australian context. For perennial wheat to be economically feasible in places other than extremely marginal lands, however, these modest seed yields will probably need to be coupled with increased biomass production for forage, and our experiment found little evidence that perennial cereals can as yet produce higher biomass than annuals. This may be partly due to insufficient planting density, and future studies should experiment with higher planting densities. Clearly, North American ecological and economic situations are different than those of the Australian wheat belt, and analogous

longitudinal studies in diverse ecological and socioeconomic contexts are needed. Despite this, our results are promising as they provide some of the first field-based evidence that growth and yield potential of novel perennial cereal accessions can be substantial relative to annual counterparts. Importantly, our results were consistent over multiple years, indicating that first- and second-year plants can provide earlier biomass growth, equivalent or higher biomass, and approximately 50 – 75% of annual cereal yield potential. Improved nutrient content (Murphy et al., 2009; Fule et al., 2005) may also compensate for some of the reduced yield of perennial cereals.

Perennial wheat and rye thus appear to be nearing the threshold of being viable crops. Further breeding should focus on increasing biomass as well as grain yield, and selecting for larger kernel size will likely be an important aspect of improving grain yields. The consistency of yield and yield components over multiple ages and years indicates that perennial wheat and rye are reaching the point of reliable perenniality and that perennial rye, in particular, could be grown as a grain crop in marginal areas.

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APPENDIX

Figures and Tables for Chapter 2

Figure 1. Precipitation recorded in 2009 and 2010 growing seasons. Data were taken at Kellogg Biological Station, Hickory Corners, MI. 2009 covers the Oct 2008-Sept 2009 growing season and 2010 covers Oct 2009-Sept 2010.

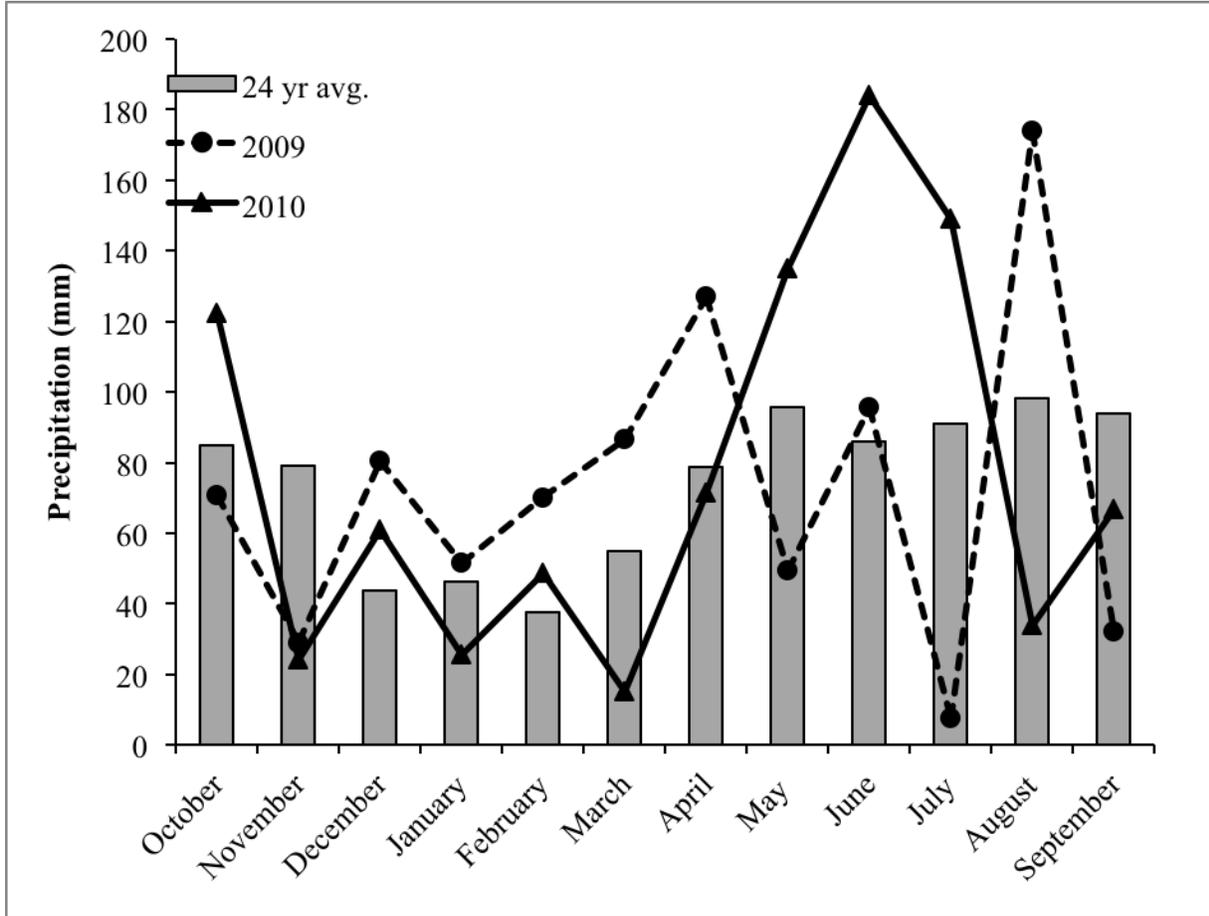


Figure 2. Seed yield for *Triticum* spp. in a two year agronomy study. Accessions in the study include two annual checks and four perennial test lines. Data were taken from a 2009-2010 field study at Kellogg Biological Station, Hickory Corners, MI. Means and standard errors for each accession in 2009 and 2010 are shown separately.

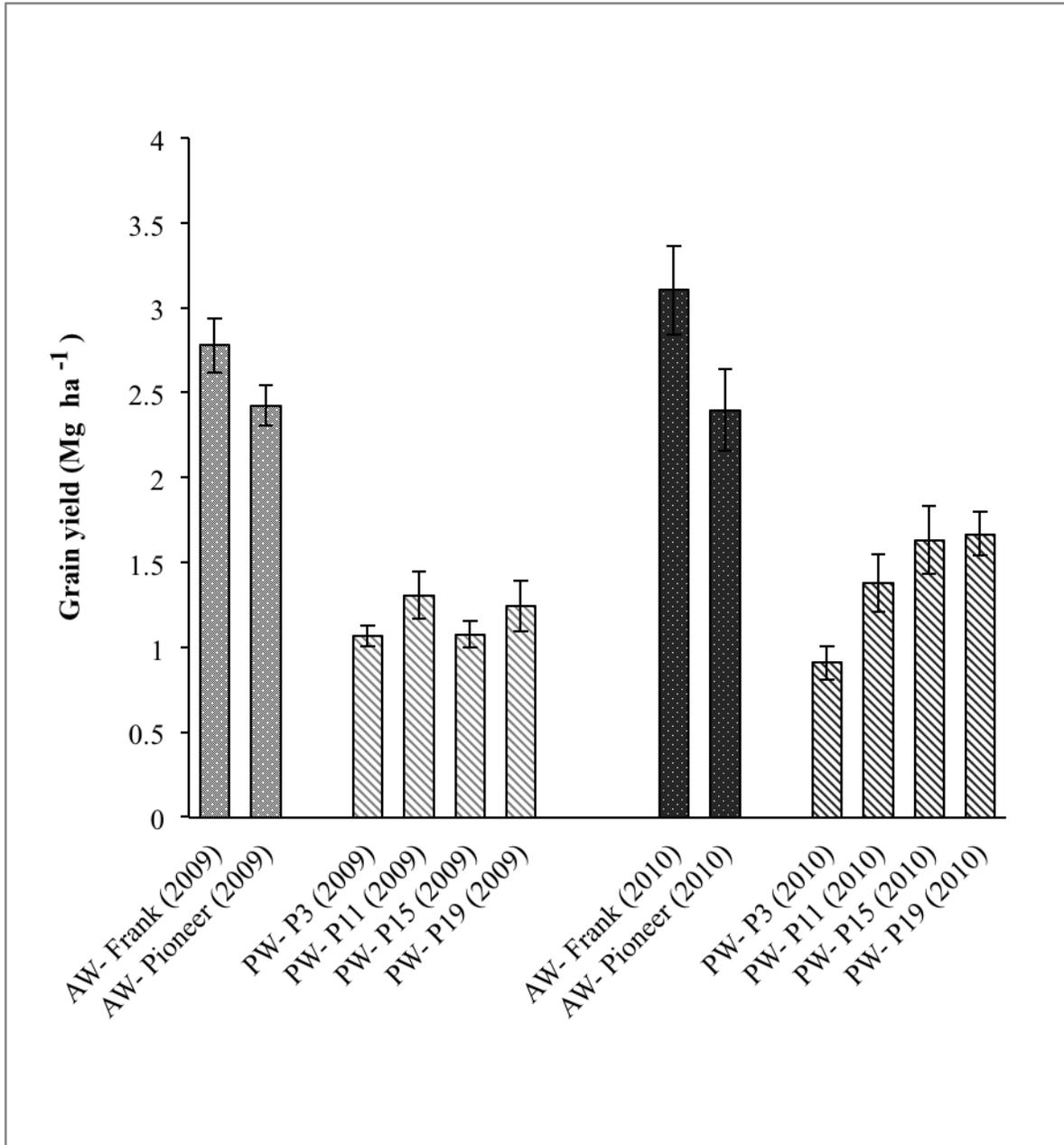


Figure 3. Seed yield for *Secale* spp. in a two year agronomy study. Accessions in the study include one annual check and one perennial test line. Data were taken from a 2009-2010 field study at Kellogg Biological Station, Hickory Corners, MI. Means and standard errors for each accession in 2009 and 2010 are shown separately.

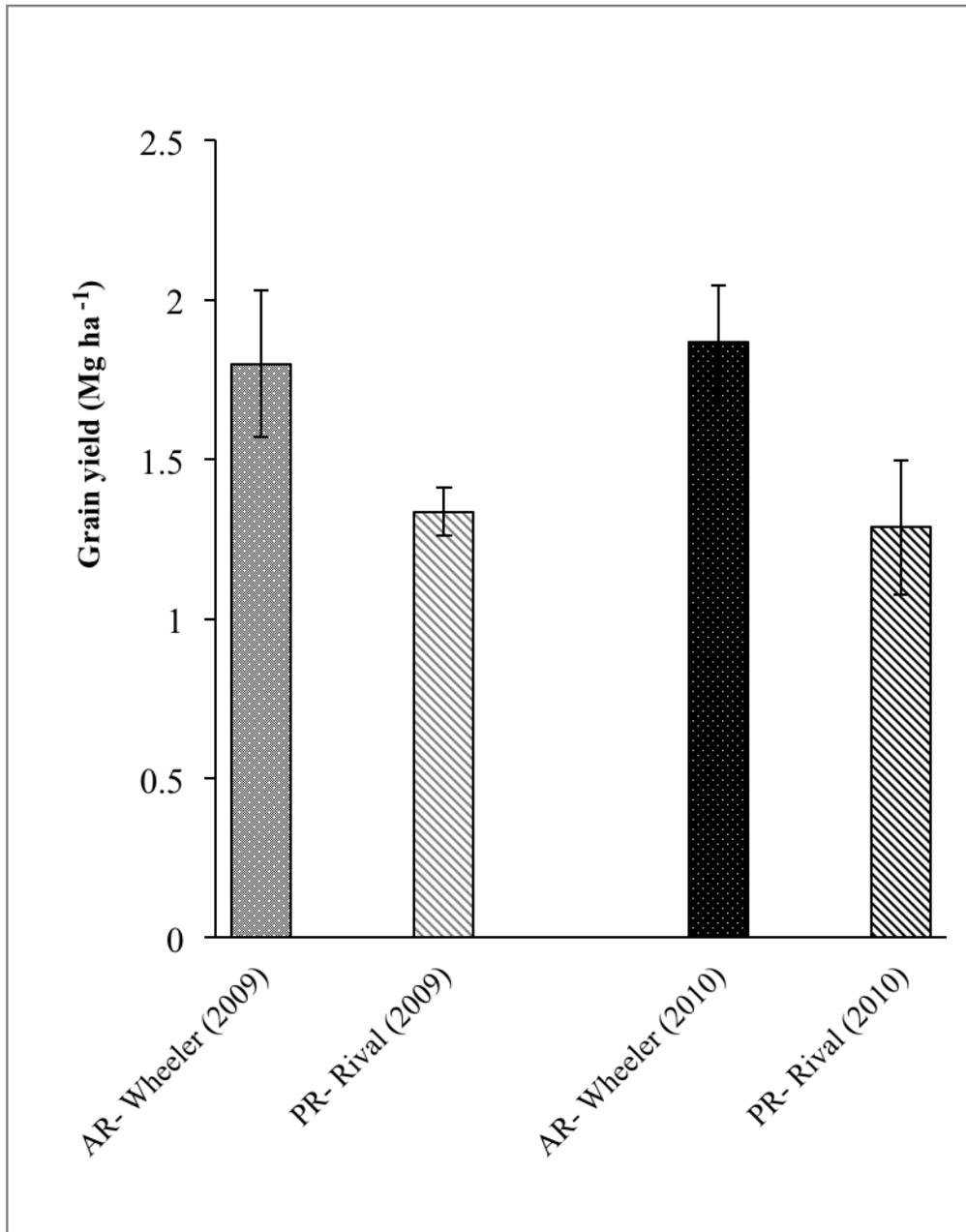


Figure 4. Kernel mass for one and two year old perennial wheat and perennial rye. Data include four perennial wheat lines (*Triticum aestivum* x *Thinopyrum elongatum*) and one perennial rye (*Secale cereale* x *montanum*), including one- and two- year old plants, in a 2010 field study at Kellogg Biological Station, Hickory Corners, MI. Means and standard errors for one-year old (1 y) and two- year old plants (2 y) are shown separately.

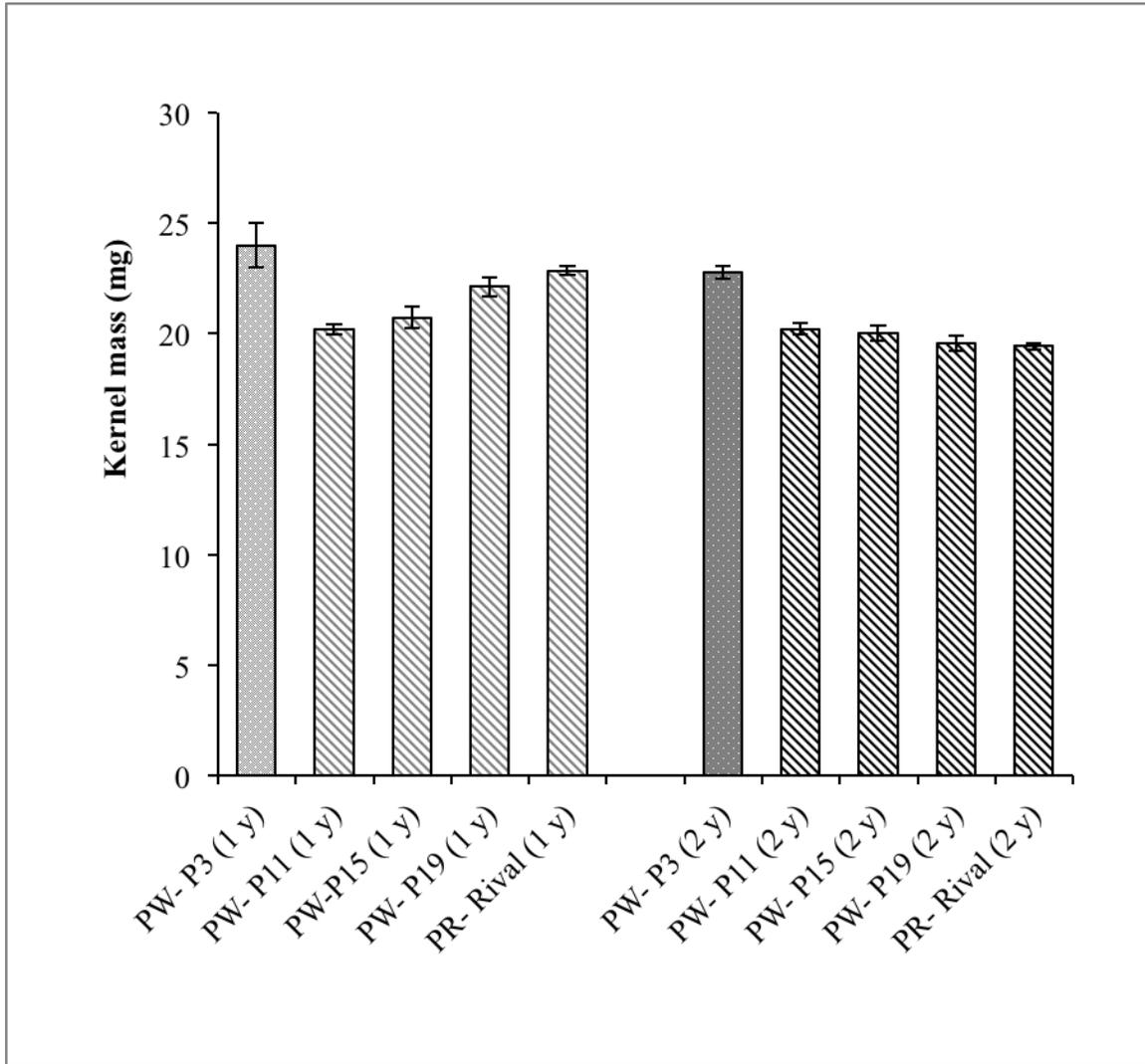


Table 1. Accessions and planting year / age in 2008-2010 agronomy study. Perennial accessions are indicated with italics.

Planting year	Measurement year	Species	Accession	Plant age at measurement
2009	2009	Annual wheat	Frankenmuth	1
	2009	Annual wheat	Pioneer 25R37	1
	2009	<i>Perennial wheat</i>	<i>P3</i>	1
	2009	<i>Perennial wheat</i>	<i>P11</i>	1
	2009	<i>Perennial wheat</i>	<i>P15</i>	1
	2009	<i>Perennial wheat</i>	<i>P19</i>	1
	2009	Annual rye	Wheeler	1
	2009	<i>Perennial rye</i>	<i>Rival</i>	1
	2010	<i>Perennial wheat</i>	<i>P3</i>	2
	2010	<i>Perennial wheat</i>	<i>P11</i>	2
	2010	<i>Perennial wheat</i>	<i>P15</i>	2
	2010	<i>Perennial wheat</i>	<i>P19</i>	2
	2010	<i>Perennial rye</i>	<i>Rival</i>	2
	2010	2010	Annual wheat	Frankenmuth
2010		Annual wheat	Pioneer 25R37	1
2010		<i>Perennial wheat</i>	<i>P3</i>	1
2010		<i>Perennial wheat</i>	<i>P11</i>	1
2010		<i>Perennial wheat</i>	<i>P15</i>	1
2010		<i>Perennial wheat</i>	<i>P19</i>	1
2010		Annual rye	Wheeler	1
2010		<i>Perennial rye</i>	<i>Rival</i>	1

Table 2. Dates for field operations in 2008-2010 agronomy study. Field operations carried out at Kellogg Biological Station, southwest Michigan, USA.

Field operation	2009	2010
Tilled	13 Oct. 2008	7 Oct. 2009
Fertilized	22 Oct. 2008	20 Oct. 2009
Soil finished	24 Oct. 2008	19 Oct. 2009
Planted	25 Oct. 2008	9 Nov. 2009
Irrigation	3 Aug. 2009	5 Sept. 2010
Weeding 1	15 – 20 May 2009	1 – 5 May 2010
Weeding 2	7 – 14 June 2009	1 – 7 June 2010
Weeding 3	29 June – 7 July 2009	25 June – 2 July 2010
Weeding 4	30 Aug – 5 Sept. 2009	28 Aug – 1 Sept. 2010
Post-harvest tillage	7 Oct 2009	23 Oct 2010

Table 3. Means, Fisher's LSD values and *F*-values for yield and yield components, averaged over 2009 and 2010. Means in the same column followed by a different letter differ significantly according to LSD post-hoc tests. Perennial accessions are indicated with italics. Threshed yield and biomass are expressed in Mg ha⁻¹, kernels per tiller are unitless, kernel mass is expressed in mg, threshability and harvest index in %, and tiller number in tillers m⁻².

Accession	Threshed yield	Threshability	Tiller number	Kernels/tiller	Kernel mass	Biomass	Harvest index
Pioneer 25R37 wheat	2.41a	83.8a	60.1a	21.4a	36.7a	4.43ad	54.4a
Frankenmuth wheat	2.94b	85.6b	57.4a	30.5b	32.8b	6.07b	48.5b
<i>P3 wheat</i>	0.99c	67.0c	43.8b	18.9a	24.6c	3.94c	26.6c
<i>P11 wheat</i>	1.34d	71.7d	42.1b	26.5b	23.3cd	3.44c	39.0d
<i>P15 wheat</i>	1.36d	69.2e	40.9b	30.1b	22.1d	4.40acd	16.8ef
<i>P19 wheat</i>	1.46d	75.4f	44.4b	29.6b	22.3cd	4.29d	18.4ef
Wheeler rye	1.83e	85.2g	40.7b	21.8a	34.0b	5.17d	18.7e
<i>Rival rye</i>	1.33d	68.1h	51.8a	27.3b	26.4e	5.02ad	14.3f
LSD	0.28	0.1	11.4	4.8	2.1	0.87	4.3
ANOVA results+							
Accession	31.12***	15.19***	3.88**	5.72***	113.6***	6.99***	41.89**
Year	1.65	-----	2.08	18.62*	111.5***	6.98	10.33*
Accession x year	2.25*	-----	3.15**	2.38*	18.09***	3.30**	1.85

+*F*-values are presented for each independent variable and interaction term

* Denotes that treatment effects are significant at $P \leq 0.05$

** Denotes that treatment effects are significant at $P \leq 0.01$

Table 4. Means, Fisher's LSD values, and *F*-values for yield and yield components in one year old (1 y) and two year old (2 y) perennial cereal plants, in 2010. Means in the same column followed by a different letter differ significantly according to LSD post-hoc tests. Threshed yield and biomass are expressed in Mg ha⁻¹, kernels per tiller are unitless, threshability and harvest index are expressed in %, and tiller number in tillers m⁻².

Accession	Threshed yield		Tiller number		Kernels/tiller		Biomass		Harvest index	
	1 y	2 y	1 y	2 y	1 y	2 y	1 y	2 y	1 y	2 y
<i>P3 wheat</i>	0.91c	1.29	32.4b	43.4ab	22.8b	26.8a	4.29a	4.33ab	0.30c	0.23a
<i>P11 wheat</i>	1.38d	1.35	41.4bc	36.6a	31.1ac	34.6b	4.29a	3.70a	0.31c	0.37b
<i>P15 wheat</i>	1.63d	1.44	42.2bc	35.8a	36.5a	37.3b	4.40a	5.75b	0.33cd	0.28ab
<i>P19 wheat</i>	1.67d	1.76	44.8c	41.6a	32.4ac	41.7b	4.88a	5.31ab	0.36d	0.32ab
<i>Rival rye</i>	1.29de	1.44	37.6b	58.4b	28.0c	24.3a	6.33b	4.76ab	0.23e	0.27ab
LSD	0.36	0.52	10.8	15.3	6.5	7.7	1.20	1.83	0.04	0.09
ANOVA results+										
Accession		5.08**	1.82		12.00***		3.08*		13.07***	
Plant age		0.23	0.50		3.40		0.01		1.58	
Plant age x accession		1.66	3.66*		2.01		2.14		6.42***	

+*F*-values are presented for each independent variable and interaction term

* Denotes that treatment effects are significant at $P \leq 0.05$

** Denotes that treatment effects are significant at $P \leq 0.01$

*** Denotes that treatment effects are significant at $P \leq 0.001$.

Table 5. Means, Fisher's LSD values, and *F*-values for plant growth and phenology data, sampled from one year old annual and perennial cereal plants in 2009 and 2010. Means in the same column followed by a different letter differ significantly according to LSD post-hoc tests. Perennial accessions are indicated with italics. Emergence is expressed in stems per meter, flowering date in terms of day of year, early season and flowering heights in cm, and regrowth as a %.

Accession	Emergence		Flowering date		Early season height		Flowering height		Regrowth	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Pioneer	59.1a	40.5a	157.6a	157.9a	7.9	8.0a	49.5a	67.3a	---	---
25R37 wheat										
Frankenmuth wheat	76.0b	56.5b	153.4b	151.2b	6.7	7.5ab	66.9b	86.9b	---	---
<i>P3 wheat</i>	31.2c	12.6c	179.8c	169.2cd	7.8	6.3bc	80.7c	93.3b	53.6	0.0
<i>P11 wheat</i>	38.0ac	13.9c	180.2c	171.2c	6.9	6.5bc	72.3bc	84.9b	84.7	1.4
<i>P15 wheat</i>	31.9c	11.4c	182.0d	170.6cd	7.7	6.2bc	71.9bc	91.1b	80.9	4.0
<i>P19 wheat</i>	34.5c	15.1c	184.4e	168.4cb	7.0	5.5c	73.5bc	90.4b	104.8	0.9
Wheeler rye	33.8c	32.0a	150.4f	151.8b	7.5	10.1d	143.5e	163.5c	---	---
<i>Rival rye</i>	56.4a	9.4c	162.2g	162.4e	5.9	7.4ab	142.0e	142.9d	116.1	0.9
LSD	11.2	15.3	2.2	2.4	2.3	1.4	9.1	8.85	23.4	11.7
ANOVA results+										
Accession	23.95***		254.28***		3.06**		230.68***		2.05	
Year	65.39**		143.77***		0.01		103.51***		80.65***	
Accession x year	1.30		22.54***		2.71*		2.47*		2.26	

+*F*-values are presented for each independent variable and interaction term

* Denotes that treatment effects are significant at $P \leq 0.05$

** Denotes that treatment effects are significant at $P \leq 0.01$

*** Denotes that treatment effects are significant at $P \leq 0.001$.

Table 6. Means, Fisher's LSD values, and *F*-values for plant growth and phenology data in one (1 y) and two year-old (2 y) plants of perennial accessions in 2010. Means in the same column followed by a different letter differ significantly according to LSD post-hoc tests. Emergence is expressed in stems per meter, flowering date in terms of day of year, early season and flowering heights in cm, and regrowth as a %.

Accession	Emergence		Flowering date		Early season height		Flowering height		Regrowth	
	1 y	2 y	1 y	2 y	1 y	2 y	1y	2y	1y	2y
<i>P3 wheat</i>	12.6	12.9ab	169.2a	179.8a	6.3	11.9a	93.3a	103.1a	0.0a	0.7
<i>P11 wheat</i>	13.9	14.3ab	171.2a	180.2a	6.5	13.3a	84.9a	80.1b	1.4a	1.2
<i>P15 wheat</i>	11.4	12.9ab	170.6a	182.0a	6.2	12.07a	91.1a	91.8c	4.0b	1.0
<i>P19 wheat</i>	15.1	11.7a	168.4a	184.4a	5.5	12.07a	90.4a	97.0c	0.9a	1.1
<i>Rival rye</i>	11.4	16.3b	162.4b	162.0b	7.4	18.1b	142.9b	179.7d	0.9a	0.8
LSD	15.3	4.1	4.2	4.0	2.3	3.1	8.9	11.7	7.7	11.5
ANOVA results+										
Accession	3.08**		14.69***		6.65**		151.00***		1.46	
Age	0.86		33.62**		61.57***		18.01*		0.01	
Age x accession	1.46		1.79		2.61‡		9.66***		0.97	

+*F*-values are presented for each independent variable and interaction term

* Denotes that treatment effects are significant at $P \leq 0.05$

** Denotes that treatment effects are significant at $P \leq 0.01$

*** Denotes that treatment effects are significant at $P \leq 0.001$

‡ Denotes that treatment effects are marginally significant at $P \leq 0.06$.

REFERENCES

REFERENCES

- Acharya SN, Mir Z, Moyer JR, Orshinsky BR, Thomas JE. 2003. Effect of row spacing and seeding date on forage yield and quality of perennial cereal rye (*Secale cereale* L.). *Canadian Journal of Plant Science* 83: 363-369.
- Acharya SN, Mir Z, Moyer JR. 2004. ACE-1 perennial cereal rye. *Canadian Journal of Plant Science* 84: 819-821.
- Bell LW, Byrne F, Ewing MA, Wade LJ. 2008. A preliminary whole-farm economic analysis of perennial wheat in an Australian dryland farming system. *Agricultural Systems* 96: 166-174.
- Bishop JG., Schemske DW. 1998. Variation in flowering phenology and its consequences for lupines colonizing Mount St. Helens. *Ecology* 79: 534-546.
- Canode CL. 1968. Influence of row space and nitrogen fertilization on grass seed production. *Agronomy Journal* 60: 263-267.
- Canode CL, Law AG. 1978. Influence of fertilization and residue management on grass seed production. *Agronomy Journal* 70:543-546.
- Chastain TG, Kiemnec GL, Cook GH, Garbacik CJ, Quebbeman BM, Crowe FJ. 1997. Residue management strategies for Kentucky bluegrass seed production. *Crop Science* 37:1836-1840.
- Chastain TG, Young WG. 1998. Vegetative plant development and seed production in cool season perennial grasses. *Seed Science Research* 8: 295-301.
- Clifton-Brown JC, Lewandowski I. 2002. Screening *Miscanthus* genotypes in field trials to optimize biomass yield and quality in southern Germany. *European Journal of Agronomy* 16: 97-110.
- Cox TS, Glover JD, Van Tassel DL, Cox CM, DeHaan LR. 2006. Prospects for developing perennial grain crops. *BioScience* 56: 649-659.
- Crews TE. 2005. Perennial crops and endogenous nutrient supplies. *Renewable Agriculture and Food Systems* 20: 25-37.
- Crum JR, Collins HP. 1995. KBS soils. [Online]. Available at www.lter.kbs.msu.edu/soil/characterization (verified 21 July 2006). W. K. Kellogg Biol. Stn., Long-Term Ecological Research Project, Michigan State University, Hickory Corners, MI.
- DeHaan LR, Van Tassel DL, Cox TS. 2005. Perennial grain crops: a synthesis of ecology and plant breeding. *Renewable Agriculture and Food Systems* 20: 5-14.

- Emrich K, Wilde F, Miedaner T, Piepho HP. 2008. REML approach for adjusting the Fusarium head blight rating to a phenological date in inoculated selection experiments of wheat. *Theoretical Applied Genetics* 117: 65-73.
- Fairey NA, Lefkovitch LP. 2001. Effect of post harvest management on seed production of creeping red fescue, tall fescue and Kentucky bluegrass in the Peace River region of north-western Canada. *Canadian Journal of Plant Science* 81: 693-701.
- Füle L, Hódos-Kotvics G, Galli Z, Ács E, Heszky L. 2005. Grain quality and baking qualities of perennial rye (cv. 'Perenne') of interspecific origin (*Secale cereale* x *montanum*). *Cereal Research Communications* 33: 809-814.
- Fulkerson RA. 1980. Seed yield responses of three grasses to post harvest stubble removal. *Canadian Journal of Plant Science* 60: 841-846.
- Garnier E, Cordonnier P, Guillermin J-L, Sonié, L. 1997. Specific leaf area and leaf nitrogen concentration in annual and perennial grass species growing in Mediterranean old fields. *Oecologia* 111: 490-498.
- Hayes RC, Newell MT, DeHaan LR, Murphy KM, Crane S, Norton MR, Wade LJ, Newberry M, Fahim M, Jones SS, Cox TS, Larkin PJ. 2012. Perennial cereal crops: An initial assessment of wheat derivatives. *Field Crops Research* 133: 68-89.
- Huebner FR, Bietz JA, Nelsen T, Bains GS, Finney PL. 1999. Soft wheat quality as related to protein composition. *Cereal Chemistry* 76: 650-655.
- Jackson LL, Dewald CL. 1994. Predicting evolutionary consequences of greater reproductive effort in *Tripsacum dactyloides*, a perennial grass. *Ecology* 75: 627-641.
- Jackson W, Jackson LL. 1999. Developing high seed yielding perennial polycultures as a mimic of mid-grass prairie. In: Agriculture as a Mimic of Natural Systems pp. 1-37. Lefroy EC, Hobbs RJ, O'Connor MH, Pate JS. Eds., Kluwer Academic Publishers, Dordrecht, Netherlands.
- Lawrence T, Ashford R. 1964. Seed yield and morphological development of Russian wild ryegrass as influenced by grazing, *Canadian Journal of Plant Science* 44: 311-320.
- Loch DS, Ramírez Áviles L, Harvey GL. 1999. Crop management: Grasses. In Loch DS, Ferguson JE. (eds). *Forage Seed Production Vol. 2: Tropical and Subtropical Species*. 159-176. (CAB International: Oxon, UK).
- Mantineo M, D'Agosta GM, Copani V, Patané C, Cosentino SL. 2009. Biomass yield and energy balance of three perennial crops for use in the semi-arid Mediterranean environment. *Field Crops Research* 114: 204-213.
- Mishra ML, Chatterjee BN. 1968. Seed production in the forage grasses *Pennisetum polystachyon* and *Andropogon gayanus* in the Indian tropics. *Tropical Grasslands* 2: 51-56.

- Mueller-Warrant GW, Caprice Rosato S. 2002. Weed control for stand duration of perennial ryegrass seed production. II: Residue Retained. *Agronomy Journal* 94: 1192-1203.
- Murphy KM, Hoagland LA, Reeves PG, Baik B-K, Jones SS. 2009. Nutritional and quality characteristics expressed in 31 perennial wheat breeding lines. *Renewable Agriculture and Food Systems* 24: 285-292.
- Murphy KM, Lyon SR, Balow KA, Jones SS. 2010. Post-sexual cycle regrowth and grain yield in *Thinopyrum elongatum* x *Triticum aestivum* amphiploids. *Plant Breeding* 129: 480-483.
- Piper JK, Kulakow PA. 1994. Seed yield and biomass allocation in *Sorghum bicolor* and F1 and backcross generations of *S. bicolor* x *S. halepense* hybrids. *Canadian Journal of Botany* 72: 468-474.
- Reimann-Philipp, R. 1986. Perennial spring rye as a crop alternative. *Journal of Agronomy and Crop Science* 157: 281-285.
- Ridley AM, White RE, Helyar KR, Morrison GR, Heng LK, Fisher R. 2001. Nitrate leaching loss under annual and perennial pastures with and without lime on a duplex (texture contrast) soil in humid southeastern Australia. *European Journal of Soil Science* 52: 237-252.
- Sacks EJ, Dhanapala MP, Tao DY, Sta Cruz MT, Sallan R. 2005. Breeding for perennial growth and fertility in an *Oryza sativa* x *longistaminata* population. *Field Crops Research* 95: 39-48.
- Sacks EJ, Dhanapala MP, Sta Cruz MT, Sallan R. 2006. Clonal performance of perennial *Oryza sativa* / *O. rufipogon* selections and their combining ability with *O. sativa* for survival, stolon production and yield. *Field Crops Research* 100: 155-167.
- SAS Institute. 2008. The SAS System for Windows. v. 9.2. SAS Institute, Cary, NC.
- Schmer MR, Mitchell RB, Vogel KP, Schacht WH, Marx DB. 2010. Spatial and temporal effects on switchgrass stands and yield in the Great Plains. *Bioenergy Research* 3: 159-171.
- Shefferson RP, Roach DA. 2010. Longitudinal analysis of *Plantago*: adaptive benefits of iteroparity in a short-lived, herbaceous perennial. *Ecology* 91: 441-447.
- Smith RG, Gross KL, Robertson GP. 2008. Effects of crop diversity on agroecosystem function: Crop yield response. *Ecosystems* 11: 355-366.
- Torimaru T, Tomaru N. 2006. Relationships between flowering phenology, plant size, and female reproductive output in a dioecious shrub, *Ilex leucoclada* (Aquifoliaceae). *Canadian Journal of Botany* 84: 1860-1869.
- Van Dijk, H. 2009. Ageing affects in an iteroparous plant species with a variable life span. *Annals of Botany* 104: 115-124.

Van Tassel DL, DeHaan LR, Cox TS. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications* 3: 434-452.

Ward PR, JA Palta, Waddell HA. 2011. Root and shoot growth by seedlings of annual and perennial medic, and annual and perennial wheat. *Crop Pasture Science* 62: 367–373.

Weik L, Kaul H-P, Kübler E, Aufhammer W. 2002. Grain yields of perennial grain crops in pure and mixed stands. *Journal of Agronomy and Crop Science* 188: 342-349.

White LM. 1985. Stand age, precipitation, and temperature effects on forage yield. *Journal of Range Management* 38: 39-43.

CHAPTER 3.

RESOURCE ACQUISITION TRAITS IN PERENNIAL AND ANNUAL CEREALS

Introduction

Annual monocarpy and perennial polycarpy represent conflicting plant life history strategies, which are commonly seen across the plant kingdom and which require radically different patterns of resource allocation. In one, plants completely sacrifice long-term survival to maximize current reproduction: in the other, plants must balance allocation to current reproduction and future survival (Aragon et al., 2009; Reekie and Bazzaz, 1987a). Because plants face tradeoffs between allocation to various life history functions (seed size vs. seed number, reproduction vs. survival, growth vs. defence) perennials are generally expected to show lower levels of reproductive allocation than annuals, which can afford to spend as much as possible of the resources accumulated by mid-season, on reproduction. While some woody perennials do show very high levels of reproductive allocation in a given year, these are usually compensated for by lower allocation during the establishment phase.

An ongoing focus of research, however, is the extent to which perennials and annuals differ not only in patterns of resource *allocation*, but resource *acquisition* as well (Van Noordwijk and de Jong, 1986). As reproduction depends on the product of resource acquisition and reproductive allocation (Houle, 1991), differences between annuals and perennials in terms of reproductive output as well as growth and survival could be critically affected by changes in the ability to accumulate resources. It is unclear how much of the decreased seed production and increased investment in storage in perennials is explainable by simple shifts in allocation from one function to another, as opposed to differences in resource acquisition ability. For example, if shifting to perennial life history involved a decline in resource acquisition, then costs of perennality in terms of reduced reproduction would be greater than strict one-to-one tradeoffs

predicted. Conversely, if the shift to a perennial life history was associated with a decline in photosynthetic rates and resource acquisition capability, the cost of perenniality would be bigger than might be predicted based on one-to-one tradeoffs in carbohydrate allocation would predict. In order to more accurately understand costs of reproduction and costs of perenniality, it would be ideal to determine how photosynthesis and resource acquisition traits vary as a function of life history.

Newly developed perennial cereals (DeHaan et al., 2005) provide an ideal novel system to address these questions. Perennial cereals developed for cold temperate environments include perennial wheat (*Triticum aestivum* x *Thinopyrum* spp.), perennial rye (*Secale cereale* x *montanum*) and intermediate wheatgrass (*Thinopyrum intermedium*). All three have been developed as potential alternative food crops for the cold temperate zone. The first two are hybrids of annual wheat and rye with related perennial grasses, while the third is a steppe grass closely related to wheat. These species, unusually, combine herbaceous growth form, perenniality and relatively high reproductive allocation (Culman S. et al., unpublished data; Jaikumar et al., 2012; Murphy et al., 2010), a trait combination rare in nature or agroecosystems (Van Tassel et al., 2010). While some woody perennials, once established, (e.g. apple) can achieve reproductive allocation levels of 80%, exceeding annual crops like wheat or barley (Xia et al., 2009; Kemanian et al., 2007), no herbaceous perennial currently achieves these levels of reproductive allocation. By comparing resource acquisition traits of these species to close annual relatives (annual wheat and annual rye) we can better understand how shifts between annual and perennial life history affect resource acquisition traits.

Costs of reproduction and perenniality can be expressed in terms of various limiting resources: carbon, nitrogen (Wheelwright and Logan, 2004), phosphorus (Zotz and Richter,

2006), and others. Reekie and Bazzaz (1987b) argued that carbon is the most broadly relevant currency to assess costs of reproduction, since plants can increase access to other nutrients by investing carbon in roots. In this paper, we consider how perennial and annual cereals differ with respect to their ability to accumulate carbon through photosynthesis.

Perennials might be expected to have lower photosynthetic rates (A_{\max}) on the basis of evolutionary theory: plants with longer lifespans should adopt more conservative strategies, trading off rapid resource accumulation and high seed yield for increased lifespan and stress tolerance (Grime, 1977; Garnier, 1992). While this is a plausible argument it may not be always true: empirical comparisons of closely related perennials and annuals indicate photosynthetic rates in perennials can be higher (Zhao et al., 2010; Gonzalez-Paleo and Ravetta, 2011b; Taylor et al., 2010) or lower (Sobrado, 2011; Van Auken and Bush, 2011) than annual relatives.

Perennial cereals might achieve higher A_{\max} than annual relatives if they allocated more nitrogen to photosynthetic proteins at the expense of seeds (the ‘nitrogen allocation’ hypothesis), if their deeper root systems allowed greater access to moisture (the ‘moisture stress’ hypothesis), or if the presence of larger vegetative sinks removed feedback inhibition of photosynthesis (the ‘sink strength’ hypothesis: Zhao et al., 2008). As source/sink balance, nitrogen availability and rooting depth may change with plant age, it is necessary to study different age classes of perennial cereals to understand the effects of both species identity and age on photosynthesis and associated traits. Furthermore, it would be desirable to measure other physiological traits which might elucidate which of these three non-exclusive hypotheses (more nitrogen allocation to leaves, less moisture stress, higher sink capacity) might explain higher photosynthetic rates in perennials, if such trends are observed.

Greater nitrogen allocation to leaves, and higher sink capacity, would affect

photosynthetic rates primarily by affecting biochemical traits that affect photosynthesis. In other words, they would contribute to increased photosynthetic rates through increased enzymatic content or activity. This might be reflected in higher ribulose biphosphate carboxylation capacity (V_C), higher electron transport capacity (J) or higher triose phosphate utilization capacity (TPU). These three processes are all biochemical processes which limit photosynthesis at a particular level of internal carbon dioxide (C_i) and therefore, would contribute to increasing or decreasing photosynthesis at a given C_i . In contrast, lower moisture stress would contribute to higher photosynthetic rates through increased stomatal opening, which would effect the degree of stomatal limitation, and the ratio of intercellular to ambient CO_2 . These factors would contribute to increased C_i within the leaf, rather than increasing photosynthesis at a given C_i .

In a three year study in southwest Michigan, we compared first- and second-year plants of three perennial cereals (perennial wheat, intermediate wheatgrass, and perennial rye) to close annual relatives (annual wheat in the first two cases, annual rye in the third). Mid-season A_{max} , and various biochemical and hydraulic traits related to photosynthesis, were measured. We hypothesized: **1)** Perennial cereals will show higher mid-season photosynthetic rates than their annual analogues. **2)** Photosynthetic rates will be similar between one- and two-year old perennial cereal plants. **3)** The higher photosynthetic rates in perennial cereals are explained by differences in biochemical traits rather than hydraulic traits. associated with by higher stomatal conductance (g_s) and transpiration (E) rates, less stomatal limitation (L_s), higher ratio of intercellular to ambient carbon dioxide (C_i / C_a), higher capacity for ribulose biphosphate carboxylation (V_C) and electron transport (J), and greater sink strength (as measured by triose

phosphate utilization, *TPU*).

Thus this study should help elucidate some plant traits that might be associated with any photosynthetic differences that we observe, and help test various explanations proposed above (lower moisture stress, greater sink strength or more nitrogen allocation to leaves) for why perennial and annual species might differ. If perennials achieve higher photosynthetic rates because deeper root systems allow them to avoid water stress (the water stress hypothesis) then we would expect perennial cereals to show higher g_s , higher E , higher C_i / C_a and lower L_s than annual relatives. If perennial cereals achieve higher photosynthetic rates than annuals because of greater allocation of nitrogen to photosynthetic machinery as opposed to seeds (the nitrogen tradeoff hypothesis), or because of higher sink strength (the sink strength hypothesis) we should see higher V_C , J , and *TPU* in perennial cereals compared to annual relatives. If the nitrogen tradeoff hypothesis is correct, we would also expect higher leaf protein and Rubisco content in perennials, and lower total N allocation to seeds, but these parameters were not tested in our study.

Materials and Methods

Site and Goals

We conducted this study at W. K. Kellogg Biological Station, located in southwest Michigan, USA, 50 km east of Lake Michigan (42° 24' N, 85° 24' W, elevation 288 m) on soils developed from glacial outwash deposited 12000 years ago. Soils are mainly fine to coarse loamy,

mixed mesic Typic Hapludalfs. The area receives *ca.* 890 mm of precipitation annually (based on a 24-year average), about half as snow, and the mean annual temperature is 9.7° C. Further site and soils descriptions for KBS are available at http://lter.kbs.msu.edu/Data/LTER_Metadata.jsp/Dataset/KBS042.

Our study included four field experiments, to identify differences between perennial and annual cereals, as well as between first- and second-year perennials. The first study (Experiment 1), in 2008-2010, considered two perennial species, and measured only one parameter, early- to mid-season A_{\max} . A second study (Experiment 2), in 2010-2012, replicated the results from Experiment 1, added a third perennial species, and measured more parameters. We measured early- to mid-season A_{\max} as well as other parameters linked to photosynthesis: conductance (g_s), ratio of intercellular to ambient carbon dioxide (C_i/C_a ratio), leaf mass per area (LMA), diurnal variation in photosynthesis (ΔA) and leaf mass ratio (LMR). We also used gas-exchange data to infer carboxylation capacity (V_C), electron transport rate (J), triose phosphate utilization (TPU) and degree of stomatal limitation (L_S). Species was the main factor of interest, with plant age nested within species.

Three smaller, supplementary studies were included to give extra information about photosynthetic rates in intermediate wheatgrass and perennial wheat. Experiment 3 sampled photosynthetic rates, conductance and C_i/C_a across a population of intermediate wheatgrass genotypes, to determine how the wheatgrass accession in Experiment 2 compared to the *Thinopyrum intermedium* population as a whole. In Experiment 4, we compared second-year

wheatgrass photosynthetic rates to a second annual wheat cultivar (cv. ‘Caledonia’). The purpose of this experiment was to determine whether the trend towards higher photosynthesis in wheatgrass was maintained when it was compared to a different wheat variety. Finally, the purpose of Experiment 5 was to determine whether the association between perennial life history and higher photosynthetic rate held across a range of wheat x *Thinopyrum* amphiploid crosses. It is possible that any differences between our perennial and annual wheat lines could be a result of having chosen an unusual perennial cultivar. To rule out this possibility and ensure that any association between perenniality and photosynthetic rate that we found was real, we attempted to test whether interspecific crosses which displayed perennial regrowth differed from those which did not, in terms of key resource acquisition traits.

Selection of Accessions

Experiment 1 included four species: perennial rye (PR-Rival), perennial wheat (PW-P19), annual rye (AR-Wheeler), and annual wheat (AW-Frank). Experiment 2 added another species, intermediate wheatgrass (IWG-TLC1). The perennial rye (*Secale cereale* x *montanum* cv. ‘Rival’: Peters Seed Company, OR) had demonstrated perenniality in Michigan (Jaikumar et al., 2012). The annual rye (*Secale cereale* L. ‘Wheeler’: Hesel and Thomas, 1987), was a forage cultivar developed in southern Michigan. The perennial wheat accession, ‘P-0019’, obtained from Washington State University, represented the F5 generation of interspecific hybrids backcrossed once to annual wheat (*Thinopyrum elongatum* x *Triticum aestivum* ‘Chinese Spring’ // *T. aestivum* ‘Madsen’: Murphy et al., 2010). It was chosen because it had displayed moderately high yields, and reasonable ability to perennialize, in both Michigan and Washington (Murphy et al., 2010; Jaikumar et al., 2012). The annual wheat accession (*Triticum aestivum* cv.

‘Frankenmuth’, PVP 8000165: Huebner et al., 1999), is used as a benchmark for yield and quality in breeding studies at Michigan State University. Both wheatgrass and perennial wheat were compared against annual wheat. The wheatgrass accession, TLI-C1, was a breeding population that had undergone two cycles of selection for higher grain yield and larger seed size at The Land Institute (Salina, KS).

The two supplementary experiments considered a broader range of accessions, with the goal of gaining more information about variability within our perennial cereal species. Experiment 3 included a different annual wheat variety, cv. ‘Caledonia’. This is a highly productive variety, well adapted to Michigan, which was considered the variety ‘to beat’ for breeding efforts in the mid 2000s (Lewis, Janet M., personal communication), and we hoped to determine how intermediate wheatgrass photosynthetic rates compared to a more modern wheat line as well as to the older line ‘Frankenmuth’. Finally, in Experiment 4 we considered a range of 28 breeding lines of *Thinopyrum intermedium*, currently under selection at the Land Institute for various desirable agronomic traits including larger seeds and greater palatability, to determine the normal range of mid-season photosynthetic rates for this species.

Design and Layout

Experiment 1 was laid out as a nested RCBD ($n = 5$ blocks), each including one 8.30 m^2 plot for each species and age-class. Each species was planted on Oct 25, 2008 at 1.5 million seeds ha^{-1} about 2.5 cm deep. Following the harvest, perennial plants regrew into a second season, while a second set of plots of each species was planted on November 7, 2009 at 1.5 million seeds ha^{-1} . The field was fertilized in Oct 2008 with 90 kg ha^{-1} N (2% dairy manure) and

in Oct 2009 with 102 ha^{-1} N (13% blood meal). Thus in 2010 the study included annuals, second-year perennials and first-year perennials. Weeding was done by hand, four times during the season.

Experiment 2 was laid out as a nested RCBD, ($n = 6$ blocks) each including one 2.30 m^2 plot for each species and age-class. Each species was planted in Oct 2010 at 1.75 million seeds ha^{-1} approximately 2.5 cm deep. This change in planting density was made because we had experienced weed pressures in Experiment 1, indicating that plant density might be inadequate. Following the harvest perennial plants regrew into a second season, while annual plots were tilled under and a second set of plots of each species (annuals and perennials) was planted at the same density in November 2011. The field was fertilized in Oct 2010 and Oct 2011 with 91 kg ha^{-1} N (4% poultry manure). Thus in 2012 the study included annuals, second-year perennials, and first-year perennials. In both studies perennial wheat and wheatgrass (if present) were compared against annual wheat, while perennial rye was compared against annual rye: first- and second-year plants of each perennial species were also compared with each other. Weeding was done by hand, four times during each season. Pictures of Experiment 2 are shown in Figures 20-21 (Appendix 2 to Chapter 4).

Experiment 3 sampled photosynthetic and hydraulic traits across a broad population of *Thinopyrum intermedium* genotypes. As only one wheatgrass accession (TLI-C1) was used in this experiment, we wished to gain a sense for how this accession compared to the broader population of *Th. intermedium* accessions, as this could help strengthen or qualify our conclusions about intermediate wheatgrass as compared to wheat. In September 2010, individual first-year plants of 28 *Thinopyrum intermedium* accessions were obtained from the Land Institute

(Salina, KS): these accessions represented a range of genetic variation and had been selected for seed size, resistance to shattering, and other agronomic traits. Each of these accessions, including 1-3 plants, was transplanted in an unreplicated variety trial at Kellogg Biological Station, and fertilized with 3.4 liters of 24:8:16 water soluble fertilizer solution over six weeks: the following spring they were fertilized again with 150 kg ha^{-1} N (urea). Weeding was carried out by hand, three times during the season. A picture of Experiment 3 is shown in Figure 22 (Appendix 2 to Chapter 4).

Experiment 4 was laid out as a RCBD split plot design with $n = 4$ blocks arranged east to west. Two genotypes were used: ‘Caledonia’ annual wheat, and TLI-C1 intermediate wheatgrass (the same population used in Experiments 1 and 2). Each species was planted on Nov 12, 2009 at the rate of 1.25 million seeds ha^{-1} about 2.5 cm deep. The field was fertilized in Oct 2009 with about 90 kg ha^{-1} N in the form of poultry manure (4% N), as well as with 200 kg ha^{-1} potash (K_2O) and 2240 kg ha^{-1} lime (CaMgO_3): in October 2010 90 kg ha^{-1} N was applied again, but no further potash or lime were added. Following the first year’s harvest, annual wheat plots were tilled under, fertilized again, and replanted, while wheatgrass plots were allowed to grow into a second season. Thus in 2011 the experiment included annual wheat and second-year intermediate wheatgrass.

In Experiment 5, to supplement our study of photosynthetic rates in perennial cereals, a broad range of perennial cereal genotypes was sampled in a correlational study to determine whether there was an association between life history and photosynthetic rate. In October 2010, 50 different amphiploid lines (obtained by various crosses between annual wheat, *T. aestivum* and perennial *Thinopyrum elongatum*, *Th. intermedium* or *Th. junceum*) were obtained from The

Land Institute (Salina, KS). These were planted in unreplicated rows at Kellogg Biological Station, following tillage and fertilization with 91 kg ha^{-1} N in the form of poultry manure.

These 50 amphiploid lines were derived from 20 distinct *Triticum* x *Thinopyrum* crosses, and thus formed 20 independent observations. Weeding was done by hand, three times during the season.

Mid Season Photosynthetic Rate

Early to mid season A_{max} was measured, in Experiment 1, on May 10 – 12 (first- and second-year plants). In Experiment 2 measurements were taken on April 28 – 30, 2012 (first- and second-year plants). At these points all four species were in late vegetative or mid-elongation phase, with annual wheat 25 days before anthesis in 2010 and 30 days before anthesis in 2012. In addition, A_{max} measurements were taken on annual wheat and wheatgrass on April 20, 2012 (40 days before annual wheat anthesis). As wheatgrass is the most vigorously perennial of the three perennial cereals, and was not represented in Experiment 1, we wished to have additional data for this annual vs. perennial species comparison.

Photosynthesis was measured on flag leaves of three visibly green, apparently healthy plants per plot, using a Licor LI-6400, over a period between 8:30 and 12:00 a.m., sequentially by block. We measured photosynthetic rates in the morning as this is expected to be the time of greatest photosynthetic activity. Measurement took place at ambient CO_2 (394 ppm), ambient air temperature, and saturating PAR ($1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$).

To determine whether photosynthetic differences were maintained throughout the late spring, all plants were sampled again (only one subsample per plot) during spring 2012.

Measurements were taken when annual wheat was 16 days before anthesis, on May 12 – 15.

Diurnal Variation in Photosynthesis

In spring 2011, additional measurements were taken in both morning and afternoon, on plants of all five species, to determine whether the species showed a similar degree of diurnal variation in photosynthesis. Morning measurements were taken between 8:30 a.m. and 12 noon on May 6-9, 2011; afternoon measurements were taken over the same time period, between 2:30 and 4:00 in the afternoon. If perennial species showed less of a decline over the course of the day, this could be suggestive of either less moisture stress, or less feedback inhibition due to greater sink strength. Temperature, irradiance and humidity settings were as specified above.

Conductance, Transpiration and C_i / C_a

At the May 12 – 15 sampling (16 days before annual wheat anthesis) we also measured intercellular carbon dioxide concentration (C_i) using the LI-6400 XT, and calculated the ratio of ambient and intercellular carbon dioxide (C_i / C_a). Stomatal conductance and transpiration were measured on each sampled plant, on May 22-24, with the LI-6400 XT. The C_i / C_a ratio is a measure of the degree of CO_2 depletion within the leaf, and is associated with water use efficiency, with lower values of the ratio corresponding to greater water use efficiency (Ehrlinger and Cerling, 1995).

Triose Phosphate Utilization, Carboxylation, Electron Transport and Stomatal Limitation

To gain more information about biochemical and hydraulic limitations of photosynthesis,

A / C_i curves (i.e. relationships showing how light saturated photosynthetic rate, A , changes with intercellular CO_2 concentration, C_i) were generated for each species and age class through intensive field measurements. From these curves, five parameters were estimated: TPU (triose phosphate utilization, an estimate of CO_2 – saturated photosynthesis), initial ribulose bisphosphate carboxylation rate (V_C , indicative of the maximal capacity of Rubisco to catalyze carboxylation), electron transport rate (J , indicative of the ability to regenerate ribulose bisphosphate), carbon compensation point (x_0) and degree of stomatal limitation (L_S).

TPU capacity sets the upper limit of photosynthesis, and measures the rate at which carbohydrates can be phosphorylated and exported from chloroplasts. It is affected by temperature and phosphate availability, but also by sink strength, as the strength of carbohydrate sinks throughout the plant affects rates of carbohydrate export from leaves. In our study, it is unlikely that phosphate availability was involved, since test analyses of the soil at our site indicated ample soil phosphorus. V_C depends on both the quantity and activation state of the key carboxylation enzyme Rubisco (ribulose 1,5-bisphosphate carboxylase-oxygenase), as well as on Γ^* , its relative affinity for CO_2 relative to O_2 (though the relative affinity generally varies little across species). V_C is approximated by the initial slope of the A / C_i curve at low values of C_i , and can alternatively be inferred by curve-fitting and optimization of sums of squares. Inference of V_C from the A / C_i curve has been found to closely correlate with estimates of V_C from chlorophyll fluorescence (Manter and Kerrigan, 2004). J is the electron transport rate, which controls the rate at which reductant (NADPH) can be formed and the substrate for carboxylation

(RuBP, ribulose 1,5-bisphosphate) can be regenerated. All three parameters are temperature dependent and must be standardized to a single temperature to allow comparison across species and age-classes.

To estimate these parameters, A at a range of 12 to 14 C_a concentrations from 0-1600 ppm was measured between 8:30 a.m. and 12:30 p.m. on May 10 – 20, 2012, using the LI-6400 XT under temperature, humidity and irradiance conditions specified above. C_a concentrations used for this experiment were 0, 50, 100, 150, 200, 250, 300, 400 (twice), 600, 800, 1200, 1400, and 1600 ppm. TPU, V_C and J were calculated using the curve-fitting method of Sharkey et al. (2007), which models photosynthesis as a piecewise function sequentially limited by ribulose bisphosphate (RuBP) carboxylation, RuBP regeneration controlled by electron transport (both represented as saturating Monod functions) and triose phosphate utilization (represented as a horizontal asymptote). Formulas for the three portions of the A / C_i curve, as well as a more detailed explanation of the procedure, is given in **Appendix 3** following this chapter. All three biochemical parameters are reported below. Stomatal limitation was calculated as follows:

$$L_s = 1 - A_o / A_i$$

where A_o corresponds to A at $C_a = 400$, and A_i corresponds to A at $C_i = 400$, determined from the A / C_i curve through linear interpolation (Farquhar and Sharkey, 1982). Degree of stomatal limitation indicates how much of the limitation of A is due to resistance between the air and the cell interior, r_L . While r_L technically represents leaf resistance as a whole (comprising both boundary-layer and stomatal resistance), it can be used as a rough proxy for stomatal resistance, which is expected to be much greater than boundary layer resistance. If perennial cereals show

less stomatal limitation than annuals, this could indicate that differences in photosynthetic rates are due to differences in access to soil water and subsequent stomatal closure.

Carbon compensation point (x_0) was defined as the level of C_i for which $A = 0$ (i.e. the level at which respiration + photorespiration cancelled out photosynthesis) and was estimated by linear interpolation.

LMR and LMA

LMR (leaf dry mass / total dry mass) was measured by clipping four plants of each species at anthesis (in 2011), drying at 60° C to constant weight, separating into heads, leaves, and stems, and then weighing each tissue. It is a useful parameter in this study since it resource acquisition rates depend not merely on the rate of photosynthesis per unit leaf area, but also on the total size of the leaf mass pool, i.e. how much of the total plant tissue is allocated to leaves. LMA (leaf mass per area) was measured by clipping four flag leaves of each species (on June 4, 2011), scanning them through a Licor LI-3100 leaf area scanner, drying at 60° C to constant weight, and then weighing the leaves.

Experiment 3: Characterizing the Intermediate Wheatgrass Population

On May 12 – 14, 2011, between 8:30 – 11:30 a.m. we measured A_{\max} on flag leaves of each plant in the variety trial. To better understand how TLC-1 compared to the broader intermediate wheatgrass gene pool, we also measured A_{\max} on the first year wheatgrass plants growing in Experiment 2 ($n = 6$). On May 17 – 18 2011, we measured g_s , E and C_i / C_a on 13

randomly selected genotypes from the variety trial and used these values to characterize the population.

Experiments 4 and 5

In Experiment 4, A_{\max} was measured on May 11, 2011, following the above procedure. In Experiment 5, on May 12 – 14, 2011, approximately at mid-boot stage (Zadoks rating = 45), A_{\max} was measured on each line following the above specified procedure. In September 2011, following harvest, post sexual cycle regrowth (PSCR) was estimated on each line by visual inspection. Eight families showed no regrowth in any of the constituent lines, and were considered non-perennial. Eight other families included at least one line with more than 50% plants regrowing: we selected only the strongest regrowing lines within these eight families (with more than 50% regrowth), and averaged A_{\max} for the strongly regrowing lines within each family. A_{\max} was then compared between the strongly perennial and non-perennial families.

Statistical Analysis

The model for all parameters in Experiments 1 and 2 was a nested mixed-model ANOVA with species as a fixed factor, block as a random factor, and plant age as a nested, fixed factor within the perennial species:

$$A_{\max} = \mu + \text{Block} + \text{Species} + \text{Age} (\text{Species}).$$

If an age effect existed, individual comparisons of first- and second-year plants were made using paired *t*-tests to determine which of the three species showed an age effect. Where no age effect was found, measurements from first- and second-year plants were averaged. For parameters

measured in 2011, all plants were in their first year, so age was not a factor. Analyses were run using PROC MIXED in SAS 9.2 (SAS Institute: Cary, NC). Species comparisons (e.g. first-year wheatgrass vs. annual wheat or second-year wheatgrass vs. annual wheat) were made by paired t -tests following the Bonferroni correction for k multiple comparisons. Since our experiments included two or three perennial species and two age classes, we used $k = 4$ in Experiment 1 and $k = 6$ in Experiment 2. For purposes of fitting the photosynthetic model of Sharkey et al. (2007), to determine TPU , V_C and J , mesophyll conductance (g_m) was constrained between 0 and 10, day respiration (R_d) was constrained between 0 and $-3.00 \mu\text{mol m}^{-2} \text{s}^{-1}$, and the value of each parameter was standardized to 25°C , based on the measured temperature on that day and existing literature on temperature response curves. Parameters were estimated so as to minimize the total sum of squares for the model, and the single data point closest to $C_i = 250$ ppm was omitted from the model (as it was assumed to be the zone of transition between Rubisco and electron transport limitation).

A retrospective power analysis for Experiment 2 was made, using the following assumptions: 1) observations for each species and age class were normally distributed with a coefficient of variation of 8% (based on average coefficients of variation across the photosynthetic and biochemical parameters); 2) half this variation could be removed through blocking, which was suggested by inspection of the data; 3) comparisons were made using the Bonferroni procedure with six comparisons. The power analysis depicted in Figure 5 indicates that a 10% difference between treatments would be detectable with only 7% probability, but a 17.5% difference with 50% probability, a 21.2% difference with 80% probability, and a 32% difference with 99% probability.

In Experiment 4, intermediate wheatgrass was compared to ‘Caledonia’ annual wheat through a paired t -test. In Experiment 5, photosynthetic rate was compared between annual and perennial families, using an unpaired t -test.

Results

Photosynthetic Rates (Experiment 1)

Mid-season A_{\max} was elevated in perennial wheat and rye compared to annual relatives ($F = 25.06$, $p < 0.0001$, $df = 24$), but did not differ between first- and second-year plants of the same species ($F = 0.18$, $p = 0.83$): there was no block effect ($F = 0.74$, $p = 0.23$; Figure 6).

Perennial wheat had 18% higher A_{\max} than annual wheat ($t = 5.68$, $p = 0.02$, $n = 5$). Perennial rye had 16% higher A_{\max} than annual rye ($t = 5.02$, $p = 0.029$, $n = 5$).

Photosynthetic Rates (Experiment 2)

In April, perennial wheat and perennial rye showed higher photosynthetic rate (A_{\max}) than annual wheat and rye respectively, while first- and second-year individuals of these species did not differ (Table 7). In late April, perennial wheat had 21% higher A_{\max} than annual wheat ($t = 8.35$, $p = 0.0024$, $n = 6$) and perennial rye had 11% higher A_{\max} than annual rye ($t = 4.90$, $p = 0.027$, $n = 6$). Similar trends were maintained in mid-May: 37% higher in perennial wheat than in annual wheat ($t = 5.47$, $p = 0.017$, $n = 6$) and photosynthetic rates were 47% higher in perennial

rye compared to annual rye ($t = 4.56, p = 0.036, n = 6$).

Wheatgrass, unlike perennial wheat or perennial rye, showed pronounced differences in photosynthetic rate between first- and second-year plants, maintained across all three sampling dates, although both age classes still exceeded annual wheat (Table 7, Figure 7). First-year wheatgrass showed A_{\max} rates 29% higher than annual wheat in mid-April ($t = 4.48, p = 0.039, n = 6$), 61% higher in late April ($t = 6.11, p = 0.011, n = 6$), and 47% higher in mid-May ($t = 6.23, p = 0.01, n = 6$). By contrast, second-year wheatgrass showed A_{\max} rates only 11% higher than annual wheat in mid-April ($t = 4.68, p = 0.032, n = 6$), 28% higher in late April ($t = 7.23, p = 0.05, n = 6$) and 33% higher in mid-May ($t = 5.29, p = 0.019, n = 6$). In mid-May, wheatgrass A_{\max} was $25 - 28 \mu\text{mol m}^{-2} \text{s}^{-1}$ for first-year plants and $22 - 26 \mu\text{mol m}^{-2} \text{s}^{-1}$ for second-year plants, at the 95% confidence interval.

The carbon compensation point (x_0) did not vary between species or age-classes, and was generally reached at a C_i between between 41 and 46 ppm (Table 8).

Biochemical Traits

Representative A vs. C_i curves for individual leaves are depicted in Figures 8-10 below. Figure 8 depicts A / C_i curves for annual wheat, and one- and two-year old wheatgrass; Figure 9 depicts annual rye and one- and two-year old perennial rye; Figure 10 depicts annual wheat, and one- and two-year old perennial wheat. Overall, TPU capacity was higher in the perennial cereals than in the annuals (Table 9) but showed no effect of plant age. TPU was 40% higher in wheatgrass ($t = 4.46, p = 0.04, n = 6$), and 29% higher in first-year perennial wheat than in

annual wheat ($t = 4.43$, $p = 0.041$, $n = 6$). In perennial rye, TPU was 35% higher than in annual rye ($t = 4.66$, $p = 0.033$, $n = 6$). TPU capacity was approximately $14 - 30 \mu\text{mol m}^{-2} \text{s}^{-1}$ for wheatgrass, approximately $16 - 28 \mu\text{mol m}^{-2} \text{s}^{-1}$ for first-year perennial wheat, and approximately $22 - 28 \mu\text{mol m}^{-2} \text{s}^{-1}$ for perennial rye.

Initial carboxylation rate (V_C) was affected by both species and, in wheatgrass, by plant age (Table 9). V_C was 31% higher in first year wheatgrass ($t = 7.71$, $p = 0.004$, $n = 6$), 17% higher in second-year wheatgrass ($t = 5.26$, $p = 0.02$, $n = 6$), and 24% higher in perennial wheat than in annual wheat ($t = 5.82$, $p = 0.013$, $n = 6$): however, V_C did not differ between annual and perennial rye ($t = 2.32$, $p = 0.41$, $n = 6$). Electron transport rate (J) was higher in perennial cereals than annuals, and also showed age effects (Table 9). J was 53% higher in first year wheatgrass ($t = 4.84$, $p = 0.028$, $n = 6$) and 24% higher in second-year wheatgrass ($t = 5.11$, $p = 0.022$, $n = 6$) compared to annual wheat, while J was 37% higher in perennial rye compared to annual rye ($t = 4.44$, $p = 0.04$, $n = 6$). While first year perennial wheat showed 41% higher J than annual wheat ($t = 5.36$, $p = 0.018$, $n = 6$), second-year perennial wheat did not differ from annual wheat.

Hydraulic Traits

Stomatal conductance (g_s) did not differ between annual and perennial rye, or between annual and perennial wheat (Table 8). However, while second-year wheatgrass did not differ from annual wheat, first-year wheatgrass had 110% higher stomatal conductance ($t = 4.47$, $p = 0.04$, $n = 6$). Transpiration rates were 42% higher in first year wheatgrass than in annual wheat (t

= 4.34, $p = 0.044$, $n = 6$), while second-year wheatgrass was no higher than annual wheat: perennial and annual wheat did not differ, nor did perennial and annual rye. Conductance values on May 20 ranged from approximately $300 \text{ mmol m}^{-2} \text{ s}^{-1}$ for perennial rye, to approximately $850 \text{ mmol m}^{-2} \text{ s}^{-1}$ for first-year wheatgrass. The ratio of intercellular to ambient $[\text{CO}_2]$, C_i / C_a , did not vary between species or age classes, and C_i was generally in the range of 70-85% of C_a . Stomatal limitation (L_S) did not vary between species and was generally between 5% and 20%.

Diurnal Variation in Photosynthesis

In 2011, diurnal variation in photosynthetic rate (change between morning and mid-afternoon) varied between species, but showed no effect of perennial vs. annual life history. All species showed a 13 – 30% decline in photosynthetic rates in the afternoon relative to morning. Decline in photosynthetic rate was greater in annual and perennial rye than in annual wheat, perennial wheat or wheatgrass: there was no difference between annual and perennial species (Table 10).

Morphological Traits

LMR did not vary between the species, and generally was between 25% and 40% (Table 10). LMA varied between species: wheatgrass and perennial wheat had 26% higher and 28% higher LMA than annual wheat, respectively. Perennial and annual rye did not differ in terms of LMA.

Experiment 3: Sampling the Intermediate Wheatgrass Population

Our sampling of the broader intermediate wheatgrass gene pool in spring 2011 indicated that A_{\max} for the population ranged between 19 – 32 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while C_i / C_a was between 74-78%. TLI-C1 was within an average range (65th and 63rd percentile, respectively) for the wheatgrass population in terms of A_{\max} and C_i / C_a , but above average for conductance and transpiration (Table 11). This suggests that our data for TLI-C1 can be taken as indicative of the broader population rather than an unusually productive outlier.

Experiment 4: Replicating Wheat vs. Wheatgrass Comparisons

The same trend, of higher A_{\max} in wheatgrass compared to annual wheat, was observed when first- and second- year intermediate wheatgrass were separately compared to a different wheat cultivar, *Triticum aestivum* cv. ‘Caledonia’. Second-year intermediate wheatgrass had 31% higher photosynthetic rates than ‘Caledonia’ annual wheat ($t = 9.90$, $p = 0.004$, $df = 3$: Figure 11).

Experiment 5: Post-Sexual Cycle Regrowth and Photosynthetic Rate

Among the sixteen wheat x *Thinopyrum* amphiploid families sampled in Experiment 3, strongly perennial families showed 13% higher A_{\max} at mid-boot stage than non-perennial families ($t = 3.32$, $p = 0.004$, $n = 19$; Figure 11.). Among perennial families, A_{\max} was moderately correlated with the degree of post-sexual cycle regrowth ($r^2 = 0.53$, $p = 0.004$, $n = 8$: Figure 12.). Average A_{\max} among strongly regrowing lines was 22.7 – 27.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$ compared to 20 – 24 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for non-perennial lines.

Discussion

Our study found substantially higher A_{\max} at mid-season in all three perennial cereals than their annual analogues. In perennial wheat and perennial rye these differences (17 – 20%) were consistent between first- and second- year plants, and across calendar years. By contrast, in wheatgrass, A_{\max} was lower in second-year as compared to first-year plants at certain time points, with first-year wheatgrass showing the highest A_{\max} in our study. These trends appeared to be fairly robust, holding true when intermediate wheatgrass was compared to a second annual wheat cultivar, and when wheat x *Thinopyrum* amphiploids that showed perennial regrowth were compared with those which did not. The wheatgrass line used in our study and compared against annual wheat, proved to be fairly typical of the broader wheatgrass population.

These results, while surprising, are consistent with studies that have found higher A_{\max} in some perennial plants compared to annual relatives. The perennial rice relative *Oryza rufipogon*, for example, shows A_{\max} 25% – 57% higher than annual rice checks (Zhao et al., 2010). Perennial *Lesquerella* species maintained 42% higher A_{\max} than annual *Lesquerella* spp. (Brassicaceae: Gonzalez-Paleo and Ravetta, 2011b) while perennial *Panicum* and *Aristida* spp. have 36 – 44% higher A_{\max} than annual *P. miliaceum* and *A. adscensionis* respectively (Poaceae: Taylor et al., 2010). Higher A_{\max} is also found in perennial compared to annual races of *Machaeranthera gracilis* (Asteraceae: Monson and Szarek, 1981). By contrast, some

phylogenetically controlled annual vs. perennial contrasts show higher rates in annuals, e.g. in *Polygonum* (Zangerl and Bazzaz, 1983), Malvaceae (Van Auken and Bush, 2011), and *Zea* (Sobrado, 2011). Our results suggest that these three perennial species match trends found in perennial *Oryza* and *Lesquerella*, in which higher allocation to seed in annuals may come at the expense of lower photosynthetic rates.

At certain time points, older wheatgrass plants had lower A_{\max} and g_s than younger ones, while older perennial wheat plants had lower TPU than younger plants. Woody perennials sometimes show higher A_{\max} during the seedling establishment phase compared to older plants. For example, current-year seedlings of giant sequoia had 150% higher maximum A_{\max} , and higher g_s , than two-year-old saplings (Grulke and Miller, 1994): similar results were also found in black cherry (*Prunus serotina*: Fredricksen et al., 1996), although many deciduous trees show opposite trends (Rijkers et al., 2000; Steppe et al., 2011). To the best of our knowledge, changes in physiology with whole-plant aging have rarely been studied in grasses or other herbaceous perennials, although *Urtica dioica* shows declines in some photosynthetic parameters between 4-month and 12-month plants (Oñate and Munné-Bosch, 2009) and sugarcane (Amaya et al., 1995) also shows photosynthetic declines with age. Our study provides evidence that such age-related effects exist, at certain time points, in a perennial grass (intermediate wheatgrass). Some authors have suggested evolutionary explanations for the frequently observed higher A_{\max} in seedlings. Very young plants face hazardous environments and lower probabilities of survival: therefore, a more risk-taking strategy, associated with morphological differences in leaf traits resulting in higher A_{\max} , could be selectively advantageous (Bond, 2000). Further research should

investigate whether changes in leaf composition and morphology, consistent with this hypothesis, explain age-related physiological changes in wheatgrass.

Numerous hypotheses might explain the observed elevated A_{\max} in our perennial species. First, perennial cereals might, for example, invest more in roots than in annuals, allowing them greater access to water, thereby leading to less stomatal closure. Secondly, perennial cereals might maintain higher A_{\max} due to greater vegetative sink strength (Zhao et al., 2008). Buildup of carbohydrates in leaf tissue can lead to reductions in photosynthetic rate (Flore and Layne, 1999; McCormick et al., 2006) and greater sink capacity may remove such feedback inhibition. In support of this claim, selection for high yield led to lowered A_{\max} during the vegetative period in annual but not in perennial *Lesquerella*, which could reflect the effect of lower vegetative sink strength (Gonzalez-Paleo and Ravetta, 2011a). Perennial cereals may, finally, allocate more nitrogen to leaves as opposed to seeds, resulting in higher concentration of key photosynthetic leaf proteins; for example, perennial *Lesquerella mendocina* has higher N allocation to leaves than the annual *L. fendleri* (Ploschuk et al., 2005). This would be an adaptation which might optimize seed production over the course of a multi-year lifespan, but result in lower seed production on an annual basis. If this is true, higher A_{\max} in these perennial cereals would reflect underlying reproduction vs. survival tradeoffs based around allocation of nitrogen, rather than carbon (McGinley and Charnov, 1988).

Our study failed to find annual vs. perennial differences in stomatal limitation, or C_i / C_a , indicating that differences in access to soil moisture is unlikely to explain the higher A_{\max} in these perennial species. Stomatal conductance and transpiration were much higher in the younger

wheatgrass plants than in annual wheat, but similar trends were not found in two-year old wheatgrass or in either of the other perennial species. Thus while increased access to soil water, and subsequent increases in g_s may explain part of the observed differences in photosynthesis in young wheatgrass plants, other factors are most likely involved. In particular, the lack of species differences in C_i / C_a suggests that rather than maintaining higher C_i through avoiding stomatal closure, these three perennials may be more efficient photosynthesizers than their annual relatives at a given C_i . The low values for stomatal limitation indicate that 80 – 95% of the limitation on photosynthetic rate is due to biochemical factors rather than to stomatal resistance, and is an indication that these plants, under the conditions of our study, were not water stressed. This is a further indication that the photosynthetic increases in perennial cereals are not primarily due to improved water relations.

We did find evidence that coordinated increase in all three biochemical determinants of photosynthesis contribute to elevated photosynthetic rates in the perennials. In particular, increases in A_{max} are at a similar scale to increases in TPU, indicating that total carbon-saturated photosynthetic capacity, and photosynthetic rate under ambient conditions in these species, are fairly closely coupled. It is unclear, however, whether the nitrogen tradeoff hypothesis or the sink strength hypothesis (or both, as these are non exclusive) might explain the coordinated increase in biochemical determinants of photosynthesis in our perennial species.

Concurrent study of photosynthetic responses to source-sink manipulation (see Chapter 5 of this dissertation) shows that perennial wheat and rye do appear to show some degree of sink regulation of photosynthesis. Furthermore, ongoing research at Kellogg Biological Station (Sprunger, C. unpublished data) indicates that wheatgrass shows much more root growth and

more total biomass production on a yearly basis than annual wheat, indicating that they have substantially larger vegetative sinks. It is possible, therefore, that increased sink strength could be a factor. Since these species, under ambient [CO₂], do not appear to be experiencing TPU limitation, it is likely that increased sink strength contributes to higher A_{\max} indirectly through changes in Rubisco activation, electron transport rate or stomatal opening (Pieters et al., 2001). It is possible, in theory, that deeper root systems might allow the perennials increased access to soil phosphorus, which could overcome TPU limitation due to inadequate internal phosphate pools.

We did not, in this study, explicitly test the third hypothesis explaining higher photosynthetic rates in perennials (i.e. greater allocation to key photosynthetic enzymes and proteins). We did find evidence for higher activity of key enzymatic processes that limit photosynthesis. Greater capacity for ribulose biphosphate carboxylation and electron transport was found in the perennial species, with these trends more strongly pronounced in younger wheatgrass plants. This is consistent with the idea that they allocate more nitrogen to photosynthetic machinery and less to seeds. In support of this idea, ongoing research at KBS (Sprunger, C. unpublished data) has found that intermediate wheatgrass shows much less nitrogen leaching than annual wheat, which is consistent with the wheatgrass plants taking up nitrogen more efficiently than annual wheat. Perennial wheat appears to have approximately 50% more protein per unit seed mass than annual wheat (Lewis, J. A. unpublished data; Murphy et al., 2009) while intermediate wheatgrass has approximately 75% more protein (Becker et al., 2007). At the same time, first- and second-year perennial wheat (of the same cultivar in this study) achieves only around 50% of annual wheat grain yields (see Chapter 1 of this study) while intermediate wheatgrass, in its second year, has only demonstrated an ability to produce approximately 33% of annual wheat yields (Culman et al., unpublished data). Thus both

perennial wheat and intermediate wheatgrass appear to be absorbing large quantities of nitrogen but allocating less nitrogen to seed production than annual wheat (40% less in the case of wheatgrass, 25% less in the case of perennial wheat). This suggests that perennial wheat and intermediate wheatgrass may, in fact, be shifting their nitrogen budgets to allocate more nitrogen to leaf proteins, as the nitrogen tradeoff hypothesis would predict (and as observed in *Lesquerella*).

However, as yet we have no direct evidence for the hypothesis of shifting nitrogen allocation to leaves. The observed higher carboxylation and electron transport rates are also consistent with the sink strength hypothesis, as higher sink strength is known to contribute to increases in Rubisco activity and electron transport. Increases in carboxylation and electron transport are not necessarily linked to higher concentrations of key photosynthetic proteins, as they may reflect changes in the activation state of photosynthetic enzymes such as Rubisco, rather than in their amount.

We thus find no support for the water-stress hypothesis, and indications that higher photosynthetic rates in perennial cereals are due to biochemical rather than hydraulic factors. Rather than increased access to soil moisture, perennial cereals maintain higher photosynthetic rates through coordinated increases in carboxylation rates, electron transport and triose phosphate utilization. As yet, it is unclear whether higher sink capacity, increased access to soil nitrogen and phosphorus, shifting allocation of nitrogen budgets, or all three factors are involved in explaining higher photosynthetic rates in perennial cereals. Further research is needed to determine whether perennial and annual cereals show differences in photosynthetic protein levels, as well as in total nitrogen allocation to seeds and leaves, in order to test the nitrogen tradeoff hypothesis.

Conclusion

Our study provides new information about an unusual plant growth form, and suggests that high-yielding herbaceous perennials may balance multi-year survival with high reproductive effort through maintaining higher resource acquisition rates. Elevated A_{\max} appears to be associated with higher stomatal conductance (in one species), and with a coordinated increase in all three biochemical determinants of photosynthetic rate. Perennials show higher carboxylation capacity and electron transport rates, and with higher TPU capacity, possibly reflecting higher sink strength as well as greater allocation of nitrogen to leaf proteins at the expense of seeds. This could be part of a strategy to optimize seed production over the long term, through increasing the ability to accumulate carbohydrate reserves, at the cost of lower seed production in the first season. Further research is necessary to identify factors contributing to elevated A_{\max} in our three perennial cereal species, as well as in perennial *Oryza*, *Lesquerella*, *Aristida* and other species which maintain higher A_{\max} than close annual relatives, and to understand in more detail the role that aging plays in photosynthetic physiology of herbaceous plants.

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and advice; to Wayne Loescher for equipment; and to Deborah Roach, Jeannine Cavender-Bares and Christian Körner for helpful comments.

APPENDICES

APPENDIX A. Figures and Tables for Chapter 3

Figure 5. Statistical power ($1 - \beta$) as a function of underlying effect size for photosynthetic parameters in 2012 study. Power calculations make the assumptions that the coefficient of variation for each species / age combination is approximately 8%, that half of this variation can be removed through blocking, that observations are normally distributed and that comparisons are made using the Bonferroni procedure for six comparisons. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

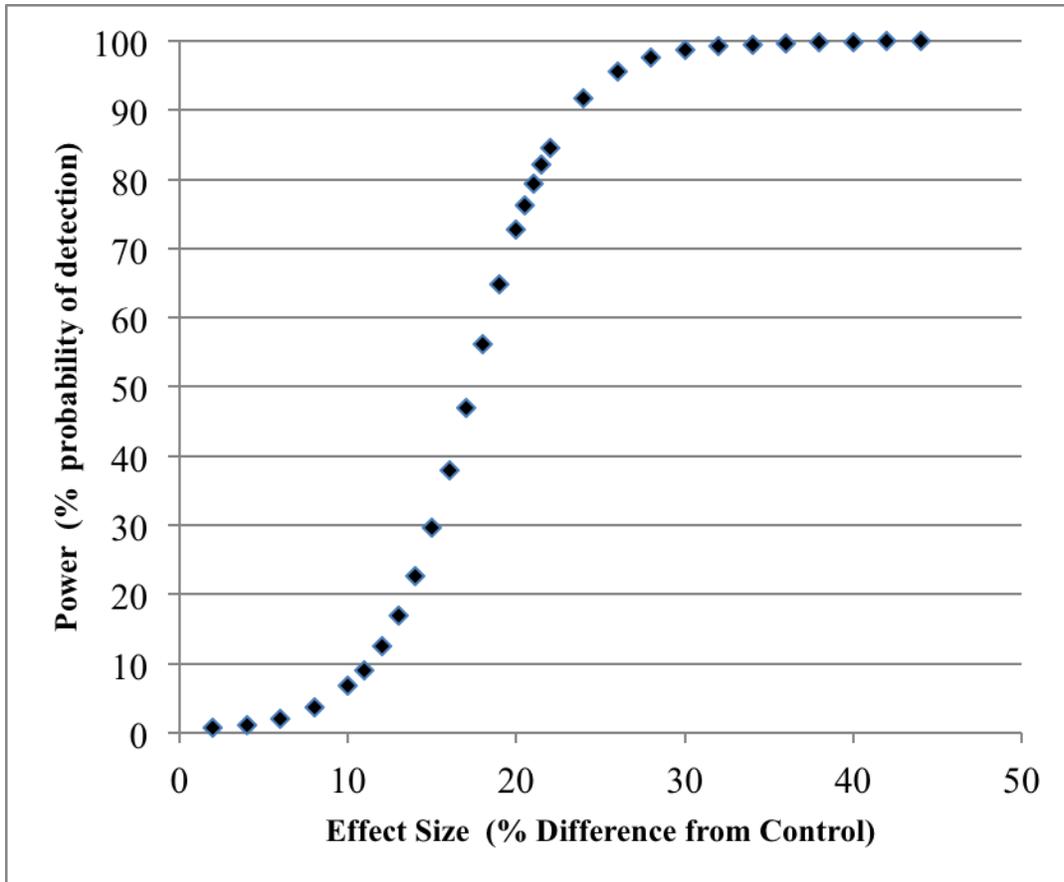


Figure 6. Photosynthetic rates (A_{\max}) for first year and second year perennial wheat and perennial rye plants, compared to annual relatives (wheat and rye) in 2010. Data were taken from a 2008-2010 study of perennial cereal physiology at Kellogg Biological Station (Hickory Corners, MI). The symbol ‘*’ represents significant differences ($p < 0.05$) following the Bonferroni correction for 4 comparisons. Red represents perennial rye, pink annual rye, dark green perennial wheat, light green annual wheat.

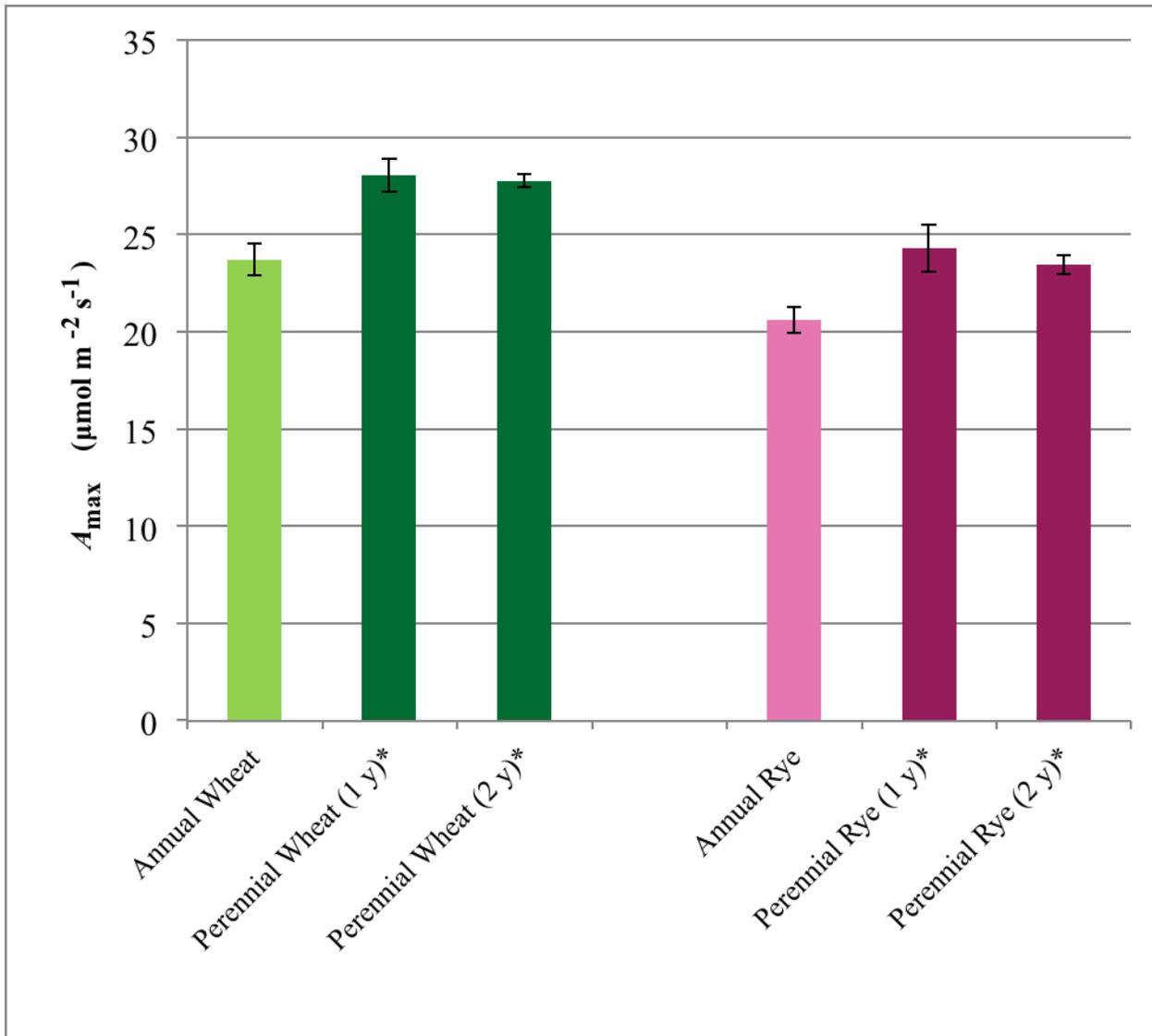


Figure 7. Photosynthetic rates (A_{\max}) measured at three time points in spring 2012, on annual wheat, one year old wheatgrass (IWG-1), and two year old wheatgrass (IWG-2). Data were taken from a 2010-2012 study of perennial cereal physiology at Kellogg Biological Station (Hickory Corners, MI). The symbols ‘*’ and ‘**’ represent significant differences ($p < 0.05$ and $p < 0.01$ respectively) following the Bonferroni correction for 6 comparisons. Green represents annual wheat, light blue represents first-year wheatgrass, and dark blue second-year wheatgrass.

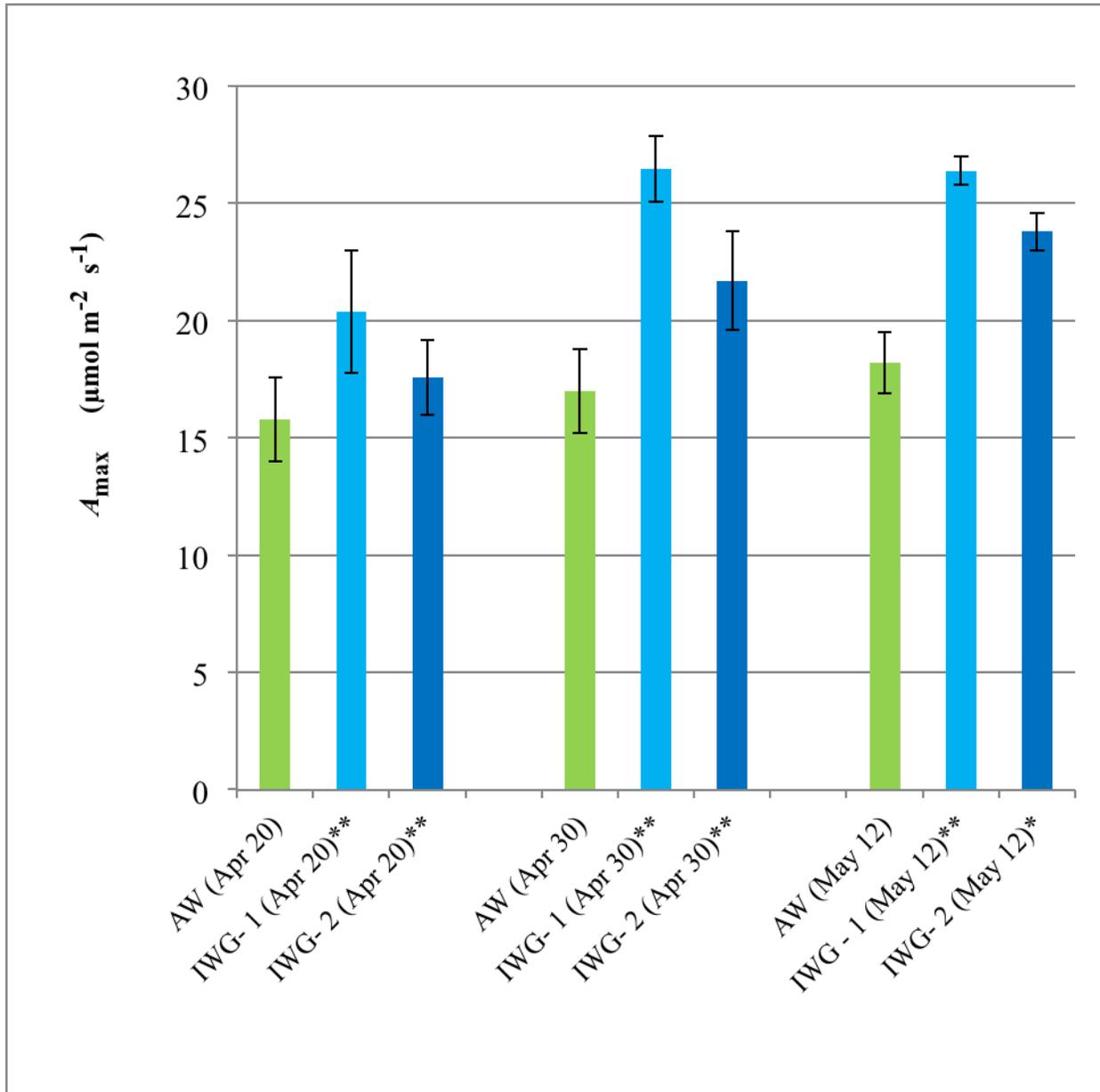


Figure 8. Photosynthetic rate (A) as a function of intercellular CO_2 (C_i) in annual wheat, one year old wheatgrass (*Thinopyrum intermedium*: IWG – 1 y) and two year old wheatgrass (IWG – 2 y). Data are taken from a 2012 study of ecophysiological differences in annual and perennial cereals at Kellogg Biological Station (Hickory Corners, MI). Each of the three curves is based on measurements from a single leaf, all within one block of the experiment.

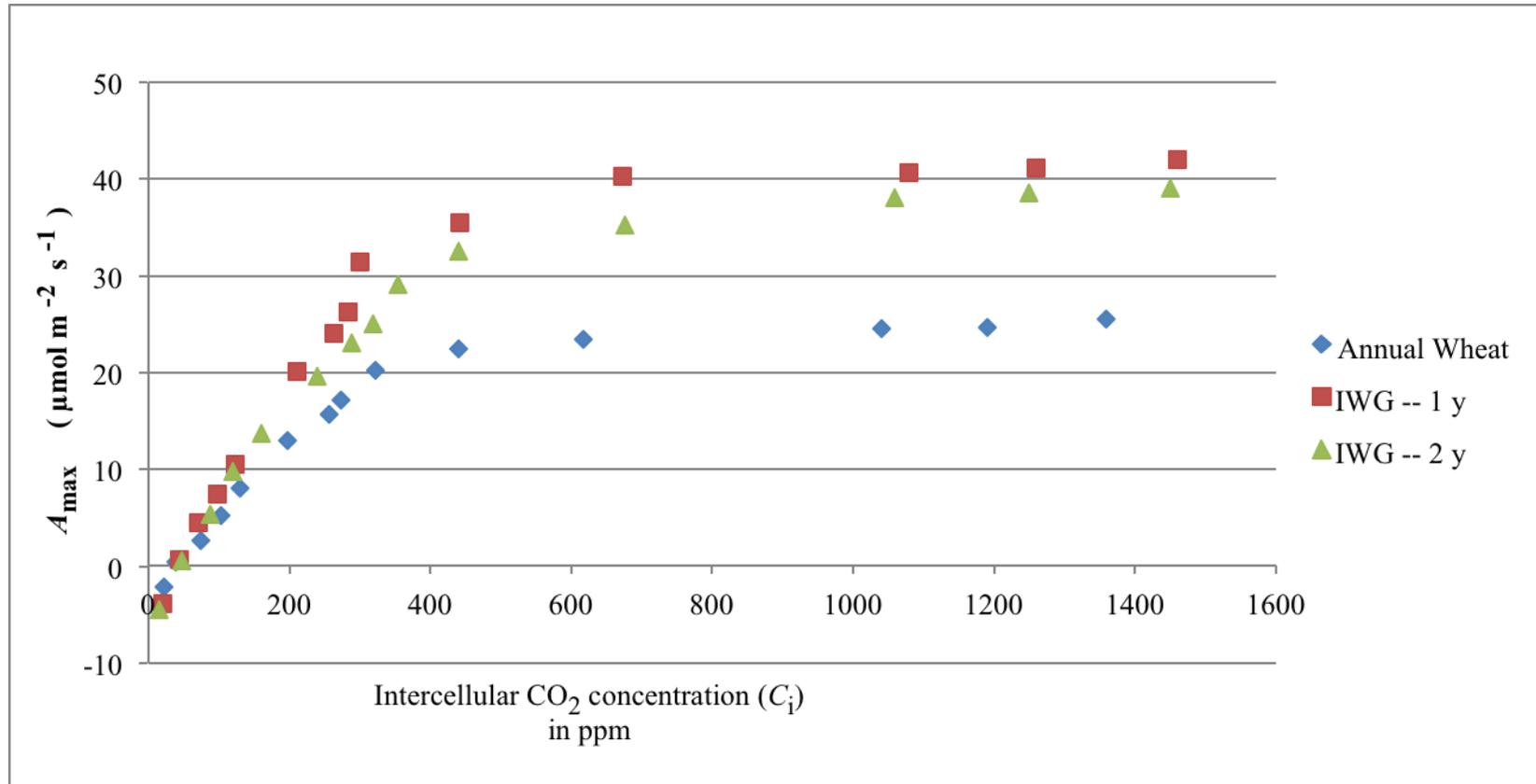


Figure 9. Photosynthetic rate (A) as a function of intercellular CO_2 (C_i) in annual rye, one year old perennial rye (*Secale cereale x montanum*: 1 y) and two year old perennial rye (2 y). Data are taken from a 2012 study of ecophysiological differences in annual and perennial cereals at Kellogg Biological Station (Hickory Corners, MI). Each of the three curves is based on measurements from a single leaf, all within one block of the experiment.

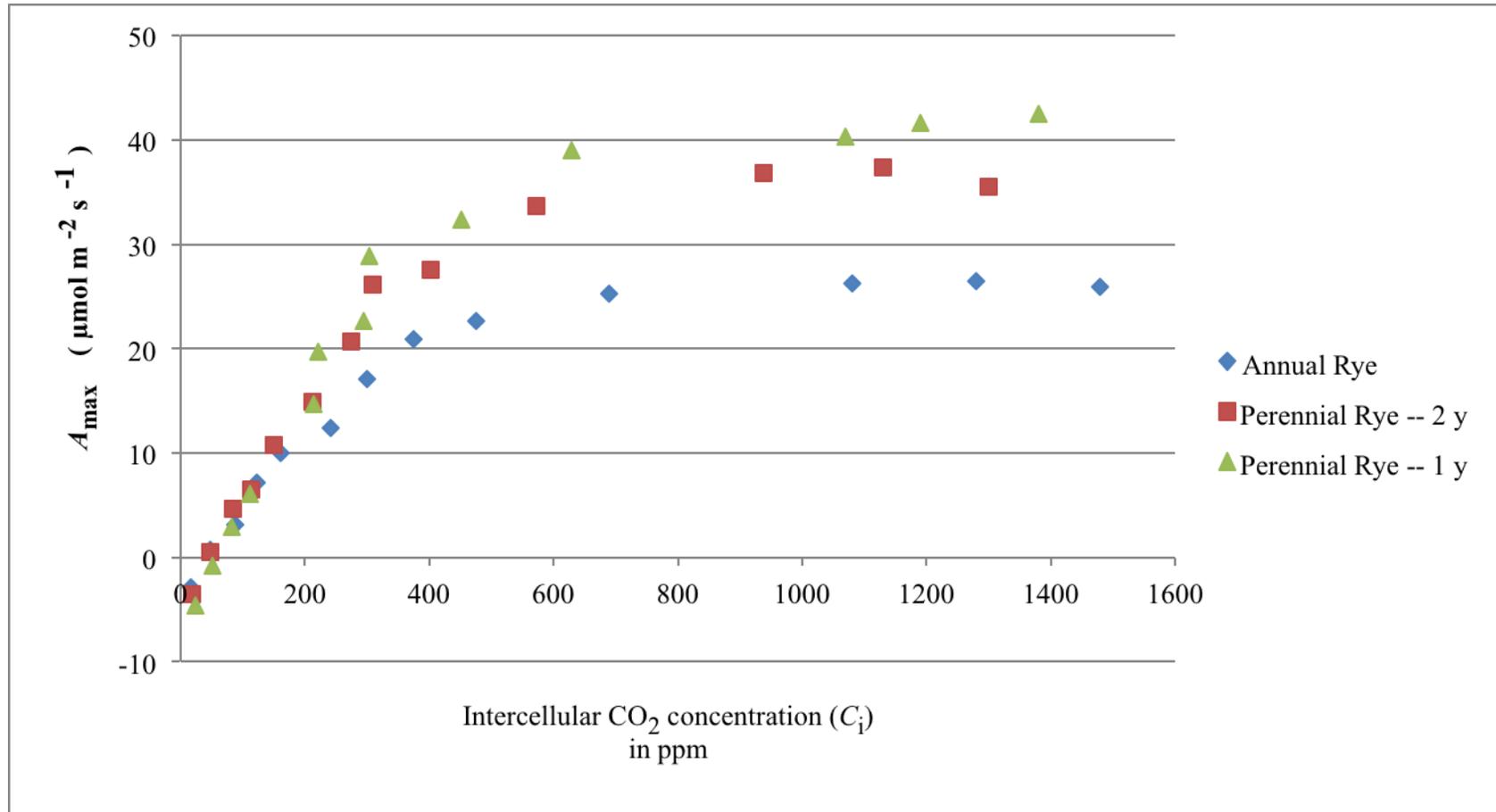


Figure 10. Photosynthetic rate (A) as a function of intercellular CO_2 (C_i) in annual wheat, one year old perennial wheat (*Thinopyrum elongatum* x *Triticum aestivum*: 1 y) and two year old perennial wheat (2 y). Data are taken from a 2012 study of ecophysiological differences in annual and perennial cereals at Kellogg Biological Station (Hickory Corners, MI). Each of the three curves is based on measurements from a single leaf, all within one block of the experiment.

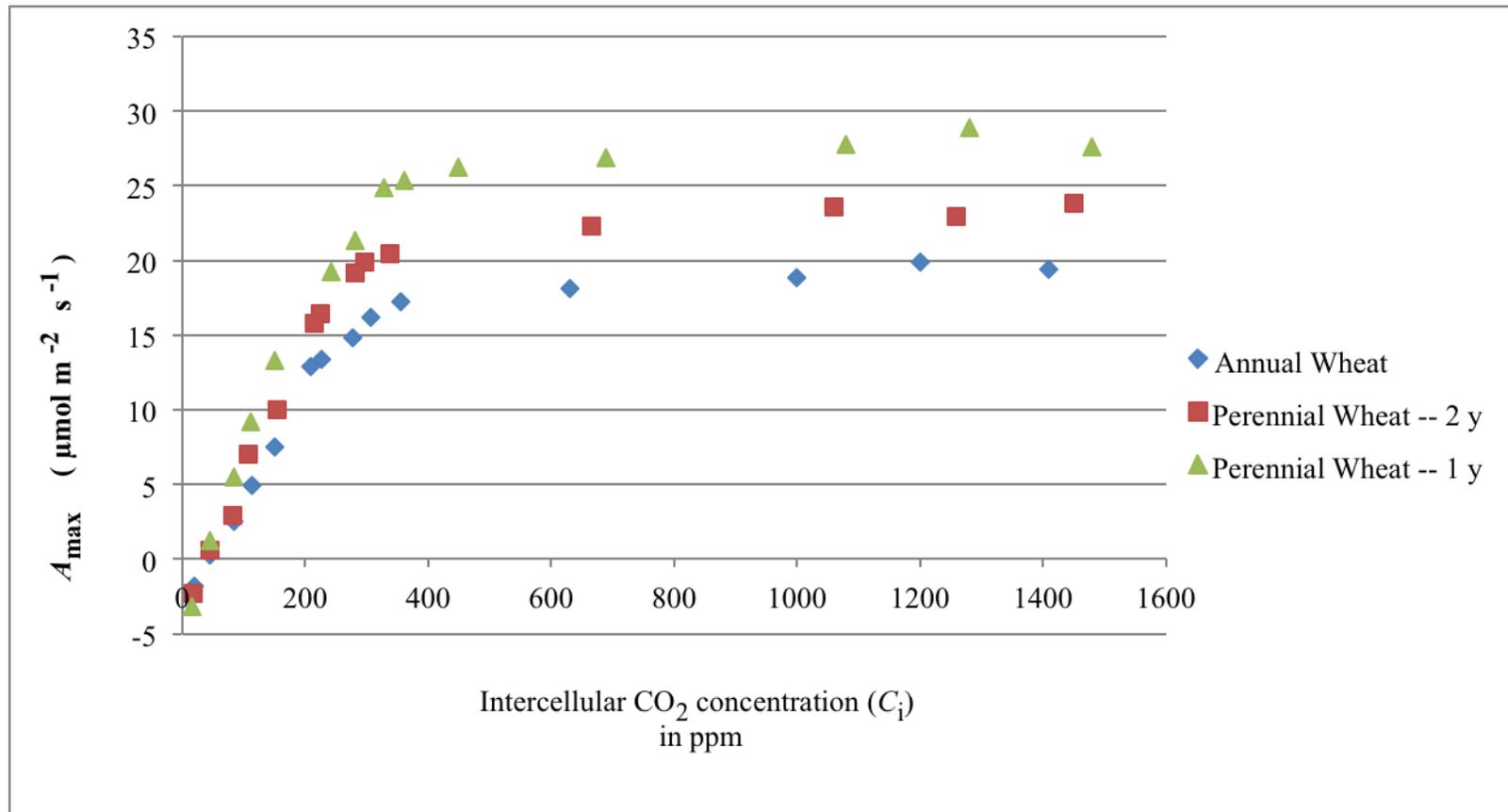


Figure 11. Photosynthetic rate (A_{\max}) in annual wheat x *Thinopyrum* amphiploids, perennial wheat x *Thinopyrum* amphiploids, annual wheat cv. ‘Caledonia’, and second-year wheatgrass (*Thinopyrum intermedium*). Data are taken from a spring 2011 study of ecophysiological differences in annual and perennial cereals at Kellogg Biological Station (Hickory Corners, MI). Light green represents *Thinopyrum* / wheat amphiploids with annual life history, dark green amphiploids with a perennial life history, brown represents annual wheat and blue represents wheatgrass.

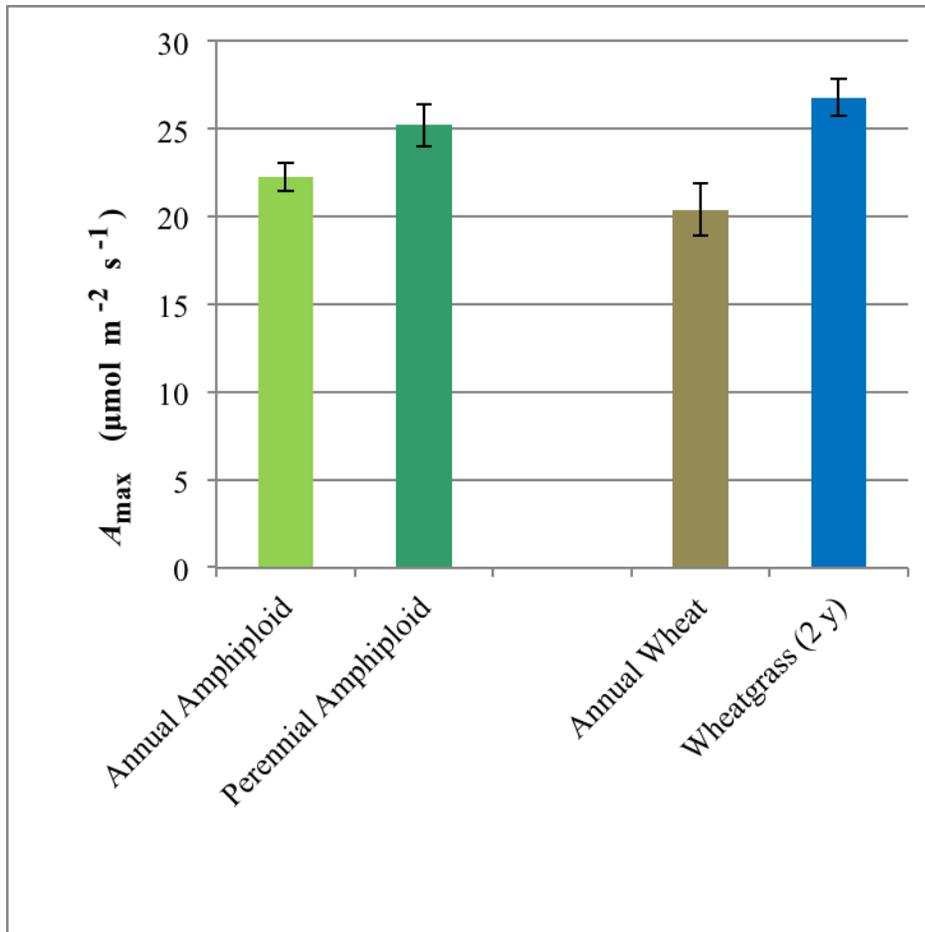


Figure 12. Photosynthetic rate (A_{\max}) in ten wheat x *Thinopyrum* amphiploids, as a function of post-sexual cycle regrowth (PSCR). Data are taken from a spring 2011 study of ecophysiological differences in annual and perennial cereals at Kellogg Biological Station (Hickory Corners, MI).

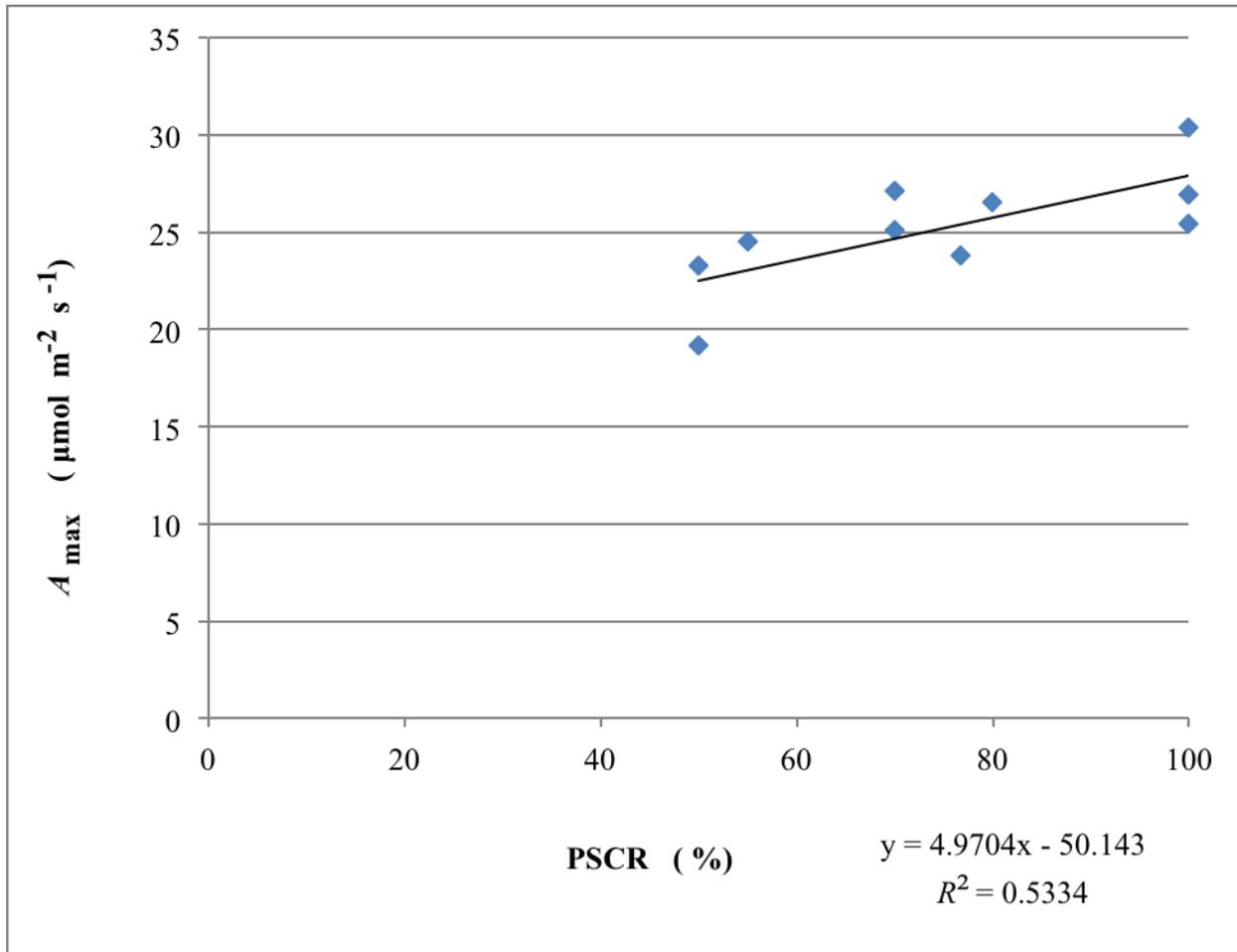


Table 7. Means (\pm SE) and *F*-values (*p*-values) for photosynthetic rates (*A*) measured at multiple time points, in spring 2012, on two annual cereal species and on first-year (1 y) and second-year (2 y) plants of three perennial cereal species. Data were taken from a 2011-2012 study. Units for A_{\max} are $\mu\text{mol m}^{-2} \text{s}^{-1}$ of CO_2 .

Species / Age	A_{\max} (Apr 20)	A_{\max} (Apr 30)	A_{\max} (May 12)
Annual Wheat	15.82 \pm 1.85	17.02 \pm 1.78	18.19 \pm 1.33
Perennial Wheat (1 y)	---	22.87 \pm 2.46** ¹	26.56 \pm 1.57*
Perennial Wheat (2 y)	---	20.46 \pm 2.05**	22.99 \pm 1.03*
Int. Wheatgrass (1 y)	20.44 \pm 2.65**	26.53 \pm 1.42**	26.35 \pm 0.55**
Int. Wheatgrass (2 y)	17.60 \pm 1.58**	21.72 \pm 2.06**	23.85 \pm 0.78*
Annual Rye	---	17.70 \pm 1.97	14.58 \pm 1.95
Perennial Rye (1 y)	---	19.51 \pm 1.73*	21.07 \pm 1.38*
Perennial Rye (2 y)	---	20.18 \pm 2.55*	24.02 \pm 1.43*
Sources of Variation			
Species	14.67 (0.003)	11.20 (0.0001)	16.84 (< 0.0001)
Age	8.64 (0.015)	3.71 (0.023)	5.55 (0.003)
Block	1.52 (0.064)	1.50 (0.066)	0.69 (0.25)

¹ Symbols ‘*’ and ‘**’ represent significant differences when compared to annual wheat (in the case of perennial wheat and wheatgrass) or annual rye (in the case of perennial rye) at $p = 0.05$ and $p = 0.01$ respectively (following the Bonferroni correction for 6 comparisons).

Table 8. Means (\pm SE) and *F*-values (*p*-values) for stomatal conductance (g_s), transpiration (*E*), ratio of leaf intercellular to ambient carbon dioxide (C_i / C_a), and degree of stomatal limitation (L_S) measured on two annual cereal species and on first-year (1 y) and second-year (2 y) plants of three perennial cereal species in spring 2012. Data were taken from a 2011-2012 study¹. Units for g_s and *E* are $\text{mmol m}^{-2} \text{s}^{-1}$ of CO_2 , values for C_i / C_a and L_S are given as %, and x_0 is expressed in ppm.

Species / Age	g_s¹	<i>E</i>	C_i / C_a	L_S	x_0
Annual Wheat	403 \pm 56	6.12 \pm 0.54	73.0 \pm 4.4	14.9 \pm 5.0	44.2 \pm 2.0
Perennial Wheat (1 y)	465 \pm 42	7.14 \pm 0.89	78.2 \pm 1.8	9.4 \pm 2.0	48.0 \pm 5.0
Perennial Wheat (2y)	377 \pm 56	4.99 \pm 0.31	75.3 \pm 2.2	19.7 \pm 4.3	42.3 \pm 2.1
Int. Wheatgrass (1 y)	848 \pm 114* ²	8.71 \pm 0.72*	77 \pm 3.0	14.3 \pm 3.2	43.6 \pm 3.8
Int. Wheatgrass (2 y)	340 \pm 56	4.12 \pm 0.43	77.3 \pm 1.9	18.7 \pm 4.4	46.6 \pm 4.6
Annual Rye	286 \pm 30	4.17 \pm 0.29	78.2 \pm 1.4	13.5 \pm 4.4	41.9 \pm 1.6
Perennial Rye (1 y)	352 \pm 80	5.64 \pm 0.50	77.0 \pm 2.2	4.9 \pm 2.0	39.0 \pm 2.5
Perennial Rye (2 y)	233 \pm 26	3.62 \pm 0.41	81.5 \pm 1.7	10.0 \pm 3.5	43.1 \pm 4.1
Sources of Variation					
Species	6.11 (0.001)	4.95 (0.003)	1.34 (0.28)	2.27 (0.082)	0.58 (0.68)
Age	13.23 (< 0.0001)	16.72 (< 0.0001)	0.98 (0.42)	2.43 (0.083)	1.08 (0.37)
Block	0.01 (0.99)	0.01 (0.99)	1.16 (0.12)	1.18 (0.12)	1.03 (0.15)

¹ All parameters measured in May 2012.

² Symbols ‘*’ and ‘**’ represent significant differences when compared to annual wheat (in the case of perennial wheat and wheatgrass) or annual rye (in the case of perennial rye) at $p = 0.05$ and $p = 0.01$ respectively (following the Bonferroni correction for 6 comparisons).

Table 9. Means (\pm SE) and *F*-values (*p*-values) for maximum carboxylation rate (V_C), electron transport rate (*J*), and triose phosphate utilization (*TPU*), on two annual cereal species and on first-year (1 y) and second-year (2 y) plants of three perennial cereal species in spring 2012. All parameters are standardized to their value at 25° C based on observed values, observed temperature at the time of measurement, and established temperature-response curves. Units for V_C , *J* and *TPU* are $\mu\text{mol m}^{-2} \text{s}^{-1}$ of CO_2 , electrons and triose phosphate respectively.

Species / Age	V_C ¹	<i>J</i>	<i>TPU</i>
Annual Wheat	102.3 \pm 6.2	191.3 \pm 17.9	15.78 \pm 1.76
Perennial Wheat (1 y)	136.0 \pm 5.1*	271.0 \pm 24.1*	21.92 \pm 2.23*
Perennial Wheat (2 y)	117.9 \pm 8.5*	222.5 \pm 20.5	18.68 \pm 2.35*
Int. Wheatgrass (1 y)	139.2 \pm 7.9**	293.3 \pm 31.8*	23.43 \pm 2.83*
Int. Wheatgrass (2 y)	120.2 \pm 4.76*	237.5 \pm 24.6*	20.95 \pm 3.51*
Annual Rye	93.2 \pm 8.7	204.5 \pm 14.1	18.62 \pm 1.28
Perennial Rye (1 y)	114.7 \pm 3.8	279.2 \pm 11.4*	25.8 \pm 1.23*
Perennial Rye (2 y)	131.5 \pm 6.0	281.8 \pm 11.8*	24.25 \pm 1.53*
Sources of Variation			
Species	10.42 (< 0.0001)	5.66 (0.001)	4.47 (0.005)
Age	4.36 (0.011)	3.38 (0.029)	1.60 (0.21)
Block	1.15 (0.12)	1.26 (0.10)	1.35 (0.089)

¹ Symbols ‘*’ and ‘**’ represent significant differences when compared to annual wheat (in the case of perennial wheat and wheatgrass) or annual rye (in the case of perennial rye) at *p* = 0.05 and *p* = 0.01 respectively (following the Bonferroni correction for 6 comparisons).

Table 10. Means (\pm SE) and *F*-values (*p*-values) for leaf mass ratio (*LMR*), percent decrease in photosynthetic rate between morning and afternoon (ΔA) and leaf mass per area (*LMA*), measured on two annual cereal species and on first-year plants of three perennial cereal species in spring 2011. Data were taken from a 2011-2012 study¹ at Kellogg Biological Station. *LMR* and ΔA are expressed as %, while *LMA* is expressed in cm² / mg.

Species / Age	<i>LMR</i>	ΔA	<i>LMA</i>
Annual Wheat	31.0 \pm 2.5	18.2 \pm 3.9	5.69 \pm 0.08
Perennial Wheat	25.1 \pm 3.3	43.5 \pm 6.6	7.24 \pm 0.24** ²
Int. Wheatgrass	39.5 \pm 7.7	36.5 \pm 5.8	7.18 \pm 0.35*
Annual Rye	34.8 \pm 1.5	0.8 \pm 7.8	6.74 \pm 0.25
Perennial Rye	33.3 \pm 1.7	11.0 \pm 5.9	7.60 \pm 0.65
Sources of Variation			
Species	1.71 (0.19)	9.16 (0.0002)	4.27 (0.01)
Block	0.04 (0.98)	0.53 (0.30)	0.30 (0.38)

¹ *LMR*, *LMA* and ΔA were measured in May 2011, thus only first year plants are present.

² Symbols ‘*’ and ‘**’ represent significant differences when compared to annual wheat (in the case of perennial wheat and wheatgrass) or annual rye (in the case of perennial rye) at *p* = 0.05 and *p* = 0.01 respectively (following the Bonferroni correction for 6 comparisons).

Table 11. Mean values for photosynthetic rate, stomatal conductance, transpiration, and ratio of leaf intercellular to ambient carbon dioxide, measured in spring 2012 on a population of 28 independent intermediate wheatgrass (*Thinopyrum intermedium*) genotypes, and on the selected genotype TLC-1 which was the subject of further experimental study. Data were taken from a 2011-2012 study¹ at Kellogg Biological Station (Hickory Corners, MI). Units for A_{max} , g_s , *E*, and C_i / C_a are $\mu\text{mol m}^{-2} \text{s}^{-1}$ of CO₂, $\text{mmol m}^{-2} \text{s}^{-1}$, $\text{mmol m}^{-2} \text{s}^{-1}$, and % respectively.

Trait	Population	TLC-1
Photosynthetic Rate (A_{max})	25.39 \pm 3.11	28.61 \pm 1.29
Stomatal Conductance (g_s)	529 \pm 32	848 \pm 114
Transpiration (<i>E</i>)	6.14 \pm 1.12	8.71 \pm 0.72
C_i / C_a	76.3 \pm 2.2	77.0 \pm 3.0

¹ Photosynthetic rates were measured in spring 2011, while other parameters were measured in spring 2012. Means are shown \pm standard deviation for the population, \pm standard error (*n* = 6) for the experimental line TLC-1.

APPENDIX B. Modeling the A / C_i Curve

C_3 plant photosynthesis, as a function of intercellular carbon dioxide (C_i) has been modeled according to several different functional forms. For example, Bethke and Drew (1992) modeled photosynthesis at levels of $C_i \leq 500$ ppm, as a Michaelis-Menten (rectangular hyperbola) function of C_i :

$$A = b (C_i - h) / (C_i - h + k) \quad \text{with } b, h, k \text{ constants.}$$

By contrast, Marenco et al. (2001) and McCormick et al. (2006) modeled the A / C_i curve as a monomolecular function:

$$A = a (1 - \exp(-bC_i) - c) \quad \text{where } \exp(X) = e^X \quad \text{and } a, b, \text{ and } c \text{ are constants.}$$

The model we used, because of its explicit mechanistic justification, its extremely widespread use in the literature (e.g. Wullschleger, 1993), and its ability to provide estimates for multiple biochemically important parameters, conceptualizes photosynthesis as a piecewise function defined by the minimum of three separate rate-limiting processes, which are in operation at different ranges of internal cellular concentration (C_i ; Farquhar et al., 1980). As C_i increases, a shift from one limiting process to another takes place (see Figure 13).

Canonically, ribulose bisphosphate (RuBP) carboxylation is expected to be limiting at low values of C_i , RuBP regeneration (which depends on the supply of reductant and therefore on light energy absorption and electron transport rate) at intermediate values of C_i , and triose phosphate utilization which depends on sink demand for carbohydrates as well as on supply of free inorganic phosphate (P_i) at high values of C_i . In any given situation, one or more of these

limitations may be absent. Under conditions of very low light, RuBP carboxylation limitation may be absent: under conditions of low temperature RuBP regeneration limitation may be absent; under conditions of high sink demand, adequate free P_i and high temperature, triose phosphate utilization (TPU) limitation is often absent (Sage and Kubien, 2007). RuBP carboxylation and RuBP regeneration are both modeled as saturating Michaelis-Menten functions, so even if the other two limiting processes were theoretically entirely absent, A would face inherent limits from any of the three processes when C_i became sufficiently high (Sharkey et al., 2007).

The rate of RuBP carboxylation can be represented as:

$$1. A = V_C [C_C - \Gamma^*] / [C_C + K_C (1 + [O_2] / K_O)] - R_d.$$

Where:

$$2. C_C = C_i - A / g_m.$$

Here V_C represents the maximum rate of RuBP carboxylation (Long and Bernacchi, 2003), C_C = concentration of carbon dioxide within the chloroplast, C_i = concentration of carbon dioxide within the leaf, K_C is the Michaelis constant for carboxylation at Rubisco, K_O is the Michaelis constant for oxygenation at Rubisco, $[O_2]$ is the concentration of oxygen at Rubisco, Γ^* is the critical carbon dioxide concentration at which photorespiration is cancelled out by carboxylation (von Caemmerer, 2000), R_d = light adapted respiration (i.e. excluding photorespiration), and g_m = mesophyll conductance. Generally, RuBP carboxylation, under well

lit and moderate temperature conditions, is expected to prevail when $C_i < 200$ ppm.

The rate of RuBP regeneration can be represented as:

$$3. A = J [C_C - \Gamma^*] / [4C_C + 8 \Gamma^*] - R_d.$$

Here J = the rate of electron transport, assuming that four electrons are required per carboxylation and oxygenation. The precise quantitative relationship between electron transport rate and photosynthesis is somewhat uncertain, and while our study used this equation, it has alternatively been suggested that a better form may be:

$$4. A = J [C_C - \Gamma^*] / [4.5C_C + 10.5 \Gamma^*] - R_d.$$

Here we chose the former equation (Eq. 3).

Electron transport is critical to RuBP regeneration since reducing power in the form of NADPH is necessary to form RuBP. As electron transport is ultimately dependent on light absorption, light availability will affect J . RuBP regeneration limitation is usually seen at C_i values > 300 ppm.

Finally, at very high values of C_i , TPU limitation is observed (Harley and Sharkey, 1991), such that:

$$5. A = 3TPU - R_d.$$

Here TPU represents the rate of use of triose phosphate molecules. In these equations, V_C is expressed in terms of $\mu\text{mol m}^{-2} \text{s}^{-1}$ of carbon dioxide molecules, J in terms of $\mu\text{mol m}^{-2} \text{s}^{-1}$ of electrons, and TPU in terms of $\text{m}^{-2} \text{s}^{-1}$ of triose phosphate molecules.

Ideally, with enough data points, it should be possible to fit curves to accurately estimate

five parameters: V_C , J , TPU , R_d and g_m . However, R_d and g_m can both be difficult to measure (in the case of R_d , partly because measurement accuracy of A at very low values of C_i becomes compromised due to chamber leaks, and partly because at extremely low levels of C_i , photorespiration interferes with the estimate of light-adapted respiration under ambient conditions). In order to increase accuracy of estimation for the other three parameters, R_d and g_m can be constrained in advance, on the basis of realistic ranges determined from previous experiments or the literature. V_C , J and TPU all show strong exponential temperature dependence, so it is necessary to standardize them to a given temperature, generally 25°C , to allow comparisons. This can be done using the observed temperature, and temperature response relationships from the literature (Harley et al., 1992; Bernacchi et al., 2001; Bernacchi et al., 2003). Alternatively, R_d could be separately measured: either by limiting O_2 to minimize photorespiration and carefully correcting for chamber leaks, or by measuring respiration at normal, ambient carbon dioxide concentration, and zero light. For improved estimation of other parameters, V_C and J can also be separately estimated, in the first case through assays of Rubisco activity and in the second by fluorescence measurements.

Two additional parameters can be estimated from the A vs. C_i curve: the carbon compensation point (x_0) and the degree of stomatal limitation (L_s). The carbon compensation point is the level of C_i at which $A = 0$, i.e. at which gross photosynthesis exactly equals respiration + photorespiration. This can be estimated through linear interpolation between the nearest two measured data points to $A = 0$ (the method we followed). It can also be calculated on

the basis of Eq. 1 and Eq. 2 once V_C has been calculated:

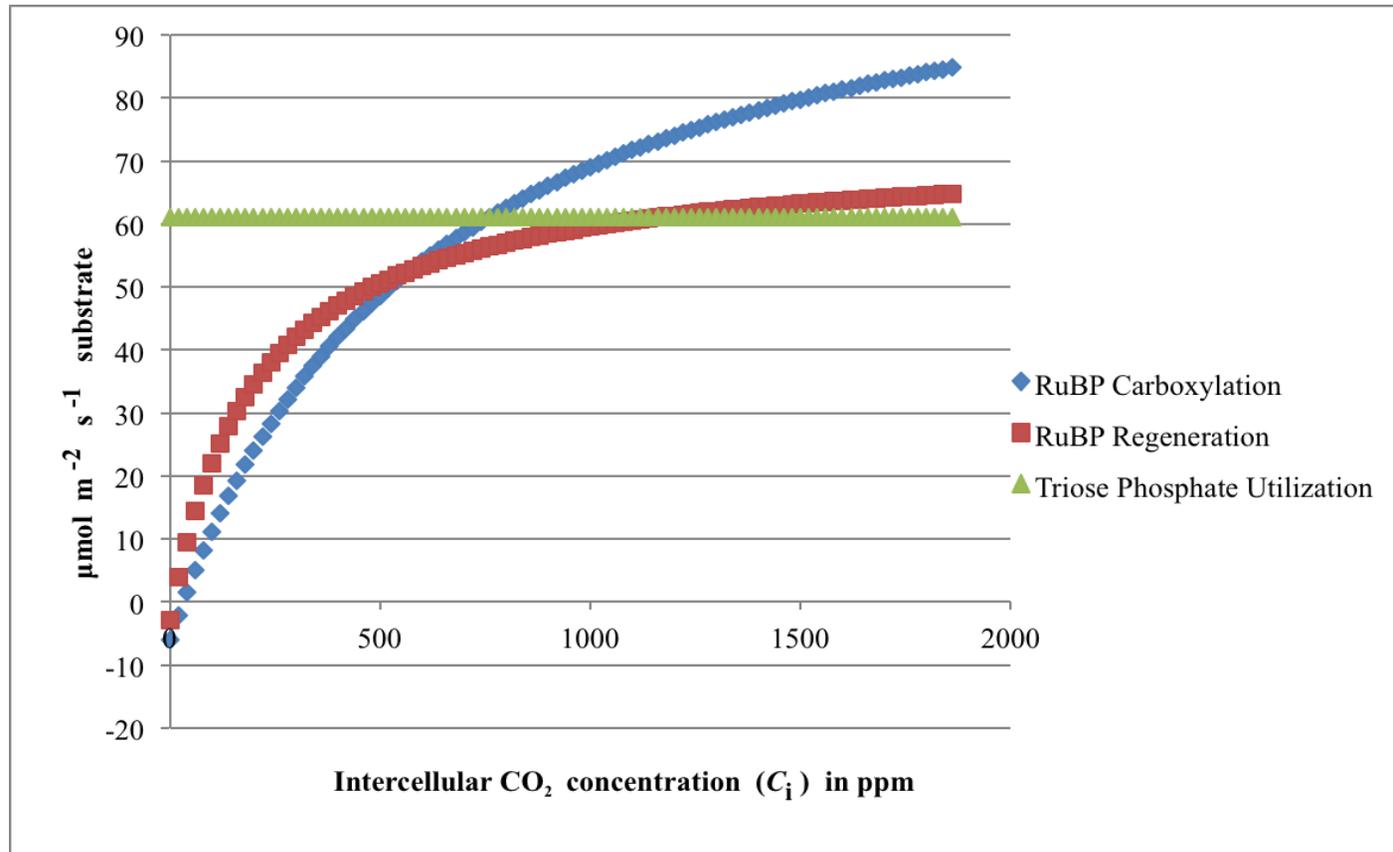
$$6. x_0 = \{ R_d [K_C (1 + [O_2] / K_O)] + V_C (\Gamma^*) \} / (V_C - R_d) .$$

The degree of stomatal limitation is an index of how much photosynthesis is under hydraulic limitation (the ability of carbon dioxide to diffuse to the chloroplast through the leaf boundary layer, stomata and mesophyll) versus biochemical limitation (the ability to fix carbon dioxide at the chloroplast). It can be calculated as follows (Farquhar and Sharkey, 1982):

$$7. L_s = 1 - A_o / A_i .$$

Here A_i is the rate of net gas exchange when $C_i = 400$, and A_o the rate of net gas exchange when $C_a = 400$. Since C_i cannot be controlled exactly, A_i can be estimated from the curve through linear interpolation between the nearest two data points to $C_i = 400$, or alternatively calculated from Eq. 3.

Figure 13. Graphical depiction of theoretical A / C_i curve. Three idealized functions model the three rate-limiting processes that determine photosynthetic rate in C_3 plants. RuBP carboxylation (blue curve) represents the rate of Rubisco-mediated reaction of free CO_2 with its substrate, ribulose-1,5-bisphosphate (RuBP). RuBP regeneration (red) represents the rate of regeneration of substrate, a function of light absorption, NADPH generation and subsequent electron transport. Finally, triose phosphate utilization (green) represents the rate of synthesis and export of triose phosphate. Photosynthesis is defined as the minimum of these three functions.



REFERENCES

REFERENCES

- Aragón CF, Méndez M, Escudero A. 2009. Survival costs of reproduction in a perennial plant: live hard, die young. *American Journal of Botany* 96: 904-911.
- Baker NR, Harbinson J, Kramer DM. 2007. Determining the limitations and regulation of photosynthetic energy transduction in leaves. *Plant, Cell and Environment* 30: 1107-1125.
- Becker R, Wagoner P, Hanners GD, Saunders RM. 1991. Compositional, nutritional and functional evaluation of intermediate wheatgrass (*Thinopyrum intermedium*). *Journal of Food Processing and Preservation* 15: 63-77.
- Bernacchi CJ, Singaas EL, Pimentel C, Portis AR, Long SP. 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant, Cell and Environment* 24: 253-259.
- Bernacchi CJ, Pimentel C, Long SP. 2003. In vivo temperature response functions of parameters required to estimate RuBP-limited photosynthesis. *Plant, Cell and Environment* 26: 1419-1430.
- Bethke PC, Drew MC. 1992. Stomatal and nonstomatal components to inhibition of photosynthesis in leaves of *Capsicum annuum* during progressive exposure to NaCl salinity. *Plant Physiology* 99: 219-226.
- Bond BJ. 2000. Age-related changes in photosynthesis of woody plants. *Trends in Plant Science* 5: 349-353.
- DeHaan LR, Van Tassel DL, Cox TS. Breeding perennial grain crops: a synthesis of ecology and plant breeding. *Renewable Agriculture and Food Systems* 20: 5-14.
- Ehrlinger JR, Cerling TE. 1995. Atmospheric CO₂ and the ratio of intercellular to ambient CO₂ levels in plants. *Tree Physiology* 15: 105-111.
- Farquhar GD, von Caemmerer S, Berry JD. 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* 149: 78-90.
- Farquhar GD, Sharkey TD. 1982. Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology* 33: 317-345.
- Flore JA, Layne DR. 1999. Photoassimilate production and distribution in cherry. *HortScience* 34: 1015-1019.
- Fredericksen TS, Steiner KC, Skelly JM, Joyce BJ, Kolb TE, Kouterick KB, Ferdinand JA. 1996. Diel and seasonal patterns of leaf gas exchange and xylem water potentials of

- different sized *Prunus serotina* Ehrh. trees. *Forest Science* 42: 359-365.
- Garnier E. 1992. Growth analysis of congeneric annual and perennial grass species. *Journal of Ecology* 80: 665-675.
- Grime JP. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111: 1169-1194.
- González-Paleo L, Ravetta D. 2011a. Indirect changes associated with a selection program for increased seed yield in wild species of *Lesquerella* (Brassicaceae): Are we developing a phenotype opposite to the expected ideotype? *Industrial Crops and Products* 34: 1372-1380.
- González-Paleo L, Ravetta D. 2011b. Relationships between reproductive output, morpho-physiological traits, and lifespan in *Lesquerella* (Brassicaceae). *Industrial Crops and Products* 34: 1372-1380.
- Grulke NE, Miller PR. 1994. Changes in gas exchange characteristics during the life span of giant sequoia: implications for response to current and future concentrations of atmospheric ozone. *Tree Physiology* 14: 659-668.
- Harley PC, Sharkey TD. 1991. An improved model of C₃ photosynthesis at high CO₂: reversed O₂ sensitivity explained by lack of glycerate reentry into the chloroplast. *Photosynthesis Research* 27: 169-178.
- Harley PC, Thomas RB, Reynolds JF, Strain BR. 1992. Modelling photosynthesis of cotton grown in elevated CO₂. *Plant, Cell and Environment* 15: 271-282.
- Helsel ZR, Thomas JW. 1987. Small grains for forage. *Journal of Dairy Science* 70: 2330-2338.
- Houle D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. *Evolution* 45: 630-648.
- Huebner FR, Bietz JA, Nelsen T, Bains GS, Finney PL. 1999. Soft wheat quality as related to protein composition. *Cereal Chemistry* 76: 650-655.
- Jaikumar NS, Snapp SS, Murphy KM and Jones SS. 2012. An agronomic assessment of some accessions of perennial wheat and rye. *Agronomy Journal*. In press.
- Kemanian AR, Stöckle CO, Huggins DR, Viega LM. 2007. A simple method to estimate harvest index in grain crops. *Field Crops Research* 103: 208-216.
- Long SP, Bernacchi CJ. 2003. Gas exchange measurements: what can they tell us about the underlying limits to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany* 54: 2393-2401.

- Manter DK, Kerrigan J. 2004. A / C_i curve analysis across a range of woody plant species: influence of regression analysis parameters and mesophyll conductance. *Journal of Experimental Botany* 55: 2581-2588.
- Marenco RA, Goncalves JFD, Vieira G. 2001. Leaf gas exchange and carbohydrates in tropical trees differing in successional status in two light environments in central Amazonia. *Tree Physiology* 21: 1311-1318.
- McCormick AJ, Cramer MD, Watt DA. 2006. Sink strength regulates photosynthesis in sugarcane. *New Phytologist* 171: 759-770.
- McGinley MA, Charnov EL. 1988. Multiple resources and the optimal balance between size and number of offspring. *Evolutionary Ecology* 2: 77-84.
- Monson RK, Szarek SR. 1981. Life cycle characteristics of *Machaeranthera gracilis* (Compositae) in desert habitats. *Oecologia* 49: 50-55.
- Murphy KM, Hoagland LA, Reeves PG, Baik B-K, Jones SS. 2009. Nutritional and quality characteristics expressed in 31 perennial wheat breeding lines. *Renewable Agriculture and Food Systems* 24: 285-292.
- Murphy KM, Lyon SR, Balow KA, Jones SS. 2010. Post-sexual cycle regrowth and grain yield in *Thinopyrum elongatum* x *Triticum aestivum* amphiploid. *Plant Breeding* 129: 480-483.
- Obeso JR. 2002. The costs of reproduction in plants. *New Phytologist* 155: 321-348.
- Oñate M, Munné-Bosch S. 2009. Influence of plant maturity, shoot reproduction and sex in the dioecious plant *Urtica dioica*. *Annals of Botany* 104: 945-956.
- Pieters AJ, Paul MJ, Lawlor DW. 2001. Low sink demand limits photosynthesis under P_i deficiency. *Journal of Experimental Botany* 52: 1083-1091.
- Ploschuk EL, Slafer GA, Ravetta DA. 2005. Reproductive allocation of biomass and nitrogen in annual and perennial *Lesquerella* crops. *Annals of Botany* 96: 127-135.
- Reekie EG, Bazzaz FA. 1987a. Reproductive effort in plants. 1. Carbon allocation to reproduction *American Naturalist* 129: 876-896.
- Reekie EG, Bazzaz FA. 1987b. Reproductive effort in plants. 2. Does carbon reflect the allocation of other resources? *American Naturalist* 129: 897-906.
- Rijkers T, Pons TL, Bongers F. 2000. The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance. *Functional Ecology* 14: 77-86.

- Sage RF, Kubien DS. 2007. The temperature response of C₃ and C₄ photosynthesis. *Plant, Cell and Environment* 30: 1086-1106.
- Sage RF, Sharkey TD. 1987. The effect of temperature on the occurrence of O₂ and CO₂ insensitive photosynthesis in field grown plants. *Plant Physiology* 72: 409-413.
- Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL. 2007. Fitting photosynthetic carbon dioxide response curves for C₃ leaves. *Plant, Cell and Environment* 30: 1035-1040.
- Taylor SH, Hulme SP, Rees M, Ripley BS, Woodward FI, Osborne CP. 2010. Ecophysiological traits in C₃ and C₄ grasses: a phylogenetically controlled screening experiment. *New Phytologist* 185: 780-791.
- Sobrado MA. 2011. Leaf gas exchange and fluorescence of two teosinte species: *Zea mays* ssp. *parviglumis* and *Z. diploperennis*. *Journal of Tropical Agriculture* 41: 91-94.
- Steppe K, Niinemets U, Teskey RO. 2011. Tree size- and age-related changes in leaf physiology and their influence on carbon gain. *Tree Physiology* 4: 235-253.
- Van Noordwijk AJ, DeJong G. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. *American Naturalist* 128: 137-142.
- Van Auken OW, Bush JK. 2011. Photosynthetic rates of two species of Malvaceae, *Malvaviscus arborea* var. *drummondii* (wax mallow) and *Abutilon theophrasti* (velvetleaf). *The Southwestern Naturalist* 56: 325-332.
- Van Tassel DL, DeHaan LR, Cox TS. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications* 3: 434-452.
- Von Caemmerer S. 2000. *Biochemical Models of Leaf Photosynthesis* pp. 1-165. CSIRO Publishing, Collingwood, Victoria, Australia.
- Wheelwright NT, Logan BA. 2004. Previous year reproduction reduces photosynthetic capacity and slows lifetime growth in females of a neotropical tree. *Proceedings of the National Academy of Sciences* 101: 8051-8055.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHT, Diemer M, etc. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821-827.
- Wullschlegel SD. 1993. Biochemical limitations to carbon assimilation in C₃ plants: a retrospective analysis of the A / C_i curves from 109 species. *Journal of Experimental Botany* 44: 907-920.

Xia G, Cheng G, Lakso A, Goffinet M. 2009. Effects of nitrogen supply on source-sink balance and fruit size of 'Gala' apple trees. *Journal of the American Society for Horticultural Science* 134: 126-133.

Zangerl AR, Bazzaz FA. 1983. Plasticity and genotypic variation in photosynthetic behavior of an early and a late successional species of *Polygonum*. *Oecologia* 57: 270-273.

Zhao M, Botwright Acuña TL, Lafitte HR, Dimayuga G, Sacks E. 2008. Perennial hybrids of *Oryza sativa* x *O. rufipogon*: Carbon exchange and assimilate partitioning. *Field Crops Research* 106: 214-223.

Zhao M, Ding Z, Lafitte R, Sacks E, Dimayuga G, Holt D. 2010. Photosynthetic characteristics in *Oryza* species. *Photosynthetica* 48: 234-240.

Zotz G, Richter A. 2006. Changes in carbohydrate and nutrient contents throughout a reproductive cycle indicate that phosphorus is a limiting nutrient in the epiphytic bromeliad, *Werauhia sanguinolenta*. *Annals of Botany* 97: 745-754.

CHAPTER 4.

SOURCE VS. SINK REGULATION IN PERENNIAL AND ANNUAL CEREALS

Introduction

The extent to which resource flow in plants is regulated by source strength (capacity to generate carbohydrates) as opposed to sink strength (capacity to accumulate carbohydrates) is an ongoing focus of research. Sink regulation occurs when changes in carbohydrate demand by flowers, fruits, or other ‘sinks’ lead to compensatory upregulation or downregulation of photosynthesis (Reekie and Bazzaz, 1987). Sink-regulated photosynthesis exists in many cultivated woody perennials including apple (Zhou and Quebedeaux, 2003), peach (Gucci et al., 1991), cherry (Layne and Flore, 1995; Flore & Layne, 1999), citrus (Iglesias et al., 2002), mango (Urban and Lechaudel, 2005) and coffee (DaMatta et al., 2008), as well in grasses including wheat (Borras et al., 2004) and sugarcane (McCormick et al., 2006). Other species, such as peach (Grossman et al., 2006) and maize (Hiyane et al., 2010) can be source limited or colimited (where both sink and source strength play a role in regulating resource flow). Source and sink limitation are only relevant terms within a given environment and level of allocation: a plant that is sink-limited at a particular level of seed production, or in a particular environment, could become source-limited at higher levels of seed production or under certain environmental stresses (Flore and Layne, 1999).

There has been little consideration, however, of how source vs. sink limitation may vary as a function of annual vs. perennial life history. There is some evidence that perennials can be more sink-limited than annuals, which could be due to longer periods for resource accumulation, and to past selection for more conservative reproductive strategies (Gaines et al., 1974; Masnatta and Ravetta, 2011). Alternatively, Arp (1991) hypothesized that perennials, due to larger vegetative sinks, will be more source-limited than closely related annuals. Recently developed

perennial wheat and rye are genetically closely related to their annual analogues, and offer an ideal system to consider interactions between life history and source/sink dynamics (Jaikumar et al., 2012). These species combine high reproductive allocation, perennial life history and herbaceous growth form, a combination of traits that is rare either in nature or in cultivated agroecosystems (Van Tassel et al., 2010). The findings of Roumet and Roy (1996) suggest that both life history and selection for high seed yield could profoundly affect the degree of source vs. sink limitation (as measured by sensitivity to elevated CO₂). For example, annual rye might be less source-limited than annual wheat, since it has not experienced equally intense selection for high harvest index.

Our goal in this study was to determine whether carbon metabolism in perennial cereals is sink-limited under low-stress, high-fertility conditions, subjected to modest alterations in source/sink ratio ($\pm 50\%$ alterations in spikelet to leaf ratio) from their current levels, and whether sink limitation in these two perennials is greater than in annual relatives. While past studies have considered effects of source-sink manipulation in annual wheat, our study makes a novel contribution by examining source vs. sink limitation in high yielding herbaceous perennials, as well as in the economically important annual rye, and by considering how life history affects source vs. sink limitation. Parameters indicating source vs. sink limitation include photosynthetic rate (A_{\max}), seed size, nonstructural carbohydrate content (NSC), and subsequent ability for regrowth. Changes in NSC often precede sink-regulated changes in photosynthetic rate, and serve as a mechanism of feedback inhibition (Franck et al., 2006). However, these effects are not always observed (DaMatta et al., 2008; Yan et al., 2011) and other mechanisms may be involved. By measuring NSC we can assess the involvement of this mechanism in sink regulation. We estimated source/sink balance using the ratio of the leaf area to

the number of spikelets (Usenik et al., 2010).

In field experiments, we experimentally manipulated the source/sink ratio in two perennial cereals (perennial wheat and perennial rye) and their annual analogues, at three time points, to determine the extent of source vs. sink limitation. Responses measured included photosynthetic rate, NSC, seed size, and fall regrowth. We hypothesized: 1) perennial and annual cereals will exhibit enhanced A_{\max} under low source/sink ratios, and reduced A_{\max} under high ratios, with perennials responding more strongly than annuals; 2) levels of NSC in all species will decrease under low source/sink ratios and increase under high ratios, and will generally be higher in perennials than in annuals; and 3) perennial cereals, following the hypothesis of Masnatta and Ravetta (2011) should fully compensate for changes in source/sink ratio and thus show no changes in single seed mass or fall regrowth. Each hypothesis is equivalent to assuming that perennial cereals are sink-limited. Lack of change in seed size, in response to changed source/sink ratio, would indicate that plants are able to fully compensate for the decrease in carbohydrate availability (or have no need for excess carbohydrates beyond those achieved by current levels of photosynthesis). The ability to compensate for part, but not all, of the change in source/sink ratio would indicate colimitation (Alvaro et al., 2008), while inability to compensate (e.g. no change in photosynthetic rate, change in seed mass proportionate to change in source/sink ratio) would indicate source limitation.

Materials and Methods

Site and Plant Materials

We conducted this study at the W. K. Kellogg Biological Station in southwest Michigan, USA, 50 km east of Lake Michigan (42°24' N, 85°24' W, elevation 288 m) on soils developed from glacial outwash deposited 12000 years ago. Soils are fine to coarse loamy, mixed mesic Typic Hapludalfs. The area receives *ca.* 897 mm of precipitation annually (based on a 24-year average up to 2010), with approximately half occurring as snow. Mean annual temperature is 9.7° C. Four species were involved: annual wheat, perennial wheat, annual rye, perennial rye. The annual wheat cultivar (*Triticum aestivum* cv. 'Frankenmuth', PVP 8000165: Huebner et al, 1999) is used as a benchmark for yield in breeding studies at Michigan State University. The perennial wheat line, 'P19' (*Triticum aestivum* cv. "Chinese Spring" \times *Thinopyrum elongatum* // *T. aestivum* cv. "Madsen"), was developed by Washington State University researchers in 2005 (Murphy et al., 2010) and has been tested for agronomic performance in Michigan (Jaikumar et al., 2012). Also included was an annual rye cultivar adapted to Michigan (*Secale cereale* cv. 'Wheeler': Hesel and Thomas, 1987), and a perennial hybrid rye (*Secale cereale* \times *montanum* cv. 'Rival': Peters Seed Company, Oregon); these genotypes have shown good yield potential in southwest Michigan (Jaikumar et al., 2012).

Experimental Design

A field study was repeated in time, with Experiment 1 carried out in 2009-2010 and Experiment 2 in 2010-2011. Experimental design, species, and statistical model were the same in both experiments, though each included measurements not present in the other. Both experiments

followed a RCBD split-split plot design, with species the main plot factor, growth stage the subplot factor, and treatment (source/sink ratio) the sub-subplot factors. In Experiment 1, each species was planted in October 2009 in 6.9 m^2 plots ($n = 5$ blocks), at $1.5 \text{ million seeds ha}^{-1}$, 3.8 cm deep, following fertilization with $102 \text{ kg ha}^{-1} \text{ N}$. In Experiment 2, each species was planted in November 2010, in 2.25 m^2 plots ($n = 6$ blocks) at $1.75 \text{ million seeds ha}^{-1}$, 3.75 cm deep, following fertilization with $90 \text{ kg ha}^{-1} \text{ N}$. In 2010, dates of anthesis were June 1 (annual rye), June 7 (annual wheat), June 11 (perennial rye) and June 17 (perennial wheat). In 2011, dates of anthesis were May 31 (annual rye), June 7 (annual wheat), June 8 (perennial rye), and June 19 (perennial wheat). Weeding was done by hand, four times during the season in Experiment 1 and four times during the season in Experiment 2.

To quantify TPU and second year seed production in response to source/sink manipulation, a separate pilot experiment was carried out in 2008-2009. This experiment was carried out before our main source/sink experiment, and did not include annual species; it is of supplementary interest, however, as it includes two parameters not measured in subsequent experiments: effects of source/sink manipulation on triose phosphate utilization (TPU) and on seed production the following year. This experiment involved two perennial wheat genotypes only, and no annual wheat or rye. One perennial wheat genotype (P19) was mentioned in the description of Experiment 1 above. The other (P3) was a genotype with generally more ‘grass-like’ spikes. Analysis of grain yields and other agronomic parameters showed no statistical differences between the genotypes in terms of yield or regrowth. This experiment was laid out as an RCBD split plot with genotype as the whole plot factor and treatment as the sub plot factor. There were 5 blocks, each with one plot of P19 and one plot of P3. Each species was planted on

Oct 25, 2008 in 6.9 m^2 plots at the rate of $1.5 \text{ million seeds ha}^{-1}$ about 2.4 cm deep, using an Almaco small plot planter. The field was fertilized in Oct 2008 with about $102 \text{ kg ha}^{-1} \text{ N}$ in the form of composted dairy manure. Weeding was done by hand, four times during the season in the pilot experiment.

Source / Sink Manipulations

Manipulations of source/sink ratio involved “Low” and “High” treatments, each compared against the control. “Low” and “High” will for the rest of this chapter, refer to source-reduced and sink-reduced treatments respectively. In the Low treatment, leaf area was reduced by painting the distal 33% of all leaves within a 0.28 m^2 microplot with a nontoxic, solvent-free white paint (BioShield: Santa Fe, NM). This rendered painted portions photosynthetically inactive (Karlsson, 1994; Niva et al., 2003). In the High treatment, reproductive sinks were reduced by manually reducing the number of spikelets on each plant, within an 0.28 m^2 microplot, by 50%. Plants in the control treatment were left intact, and monitored within a corresponding 0.28 m^2 microplot. Following DaMatta et al. (2008) and Masnatta and Ravetta (2011), leaf area/spikelet ratio (i.e., source/sink ratio) was 67% of the control in the Low treatment and 200% of control in the High treatment. Source / sink manipulations are shown in Figures 23-25 (Appendix 2 of this chapter).

Treatments were imposed at three time points: boot stage (12-14 days before anthesis), pre-anthesis (one week before anthesis) and post-anthesis (one day after anthesis), or alternatively at approximately stages 45, 55 and 65 on the Zadoks scale (Zadoks et al., 1974). In Experiment 1, A_{max} was measured on the perennial species during all three stages and on annual

rye during the final two stages: due to adverse weather we were able to measure annual wheat only during the middle stage. In Experiment 2, A_{\max} was measured during all three stages for all species. These three sets of manipulations will be referred to as “Boot”, “Pre-Anthesis” and “Post-Anthesis” stages respectively. During the final two stages, all three treatments (High, Low, and Control) were imposed; at boot stage, since plants had not flowered, only Low and Control treatments were imposed.

Survival

In 2011 (i.e., Experiment 2) we monitored “survival to harvest” as a measure of plant health. In early May, 2011, we counted the number of living plants within each microplot prior to the first set of manipulations. We then counted the number of living plants again in July, 2011, to determine the percentage of plants in each microplot that survived until harvest

Photosynthetic Rates

Photosynthetic rates (A_{\max}) were measured on a sunny day 5-8 days after treatments were imposed for each growth stage. Plants were chosen through a stratified random method, choosing healthy sections of the plot (with deep green leaves) and selecting three leaves randomly within this section. Photosynthetic rate (A_{\max}) was measured between 2:30 and 4:30 p.m., as afternoon is often the time at which feedback inhibition of photosynthesis is most evident (Flore and Layne, 1999). Measurements were taken with a Licor 6400 gas-exchange system (Licor Biosciences: Lincoln, NE), using approximately ambient CO_2 (392 ppm), $\text{PAR} = 1200 \mu\text{mol m}^{-2} \text{s}^{-1}$, at ambient air temperature (ranging from 10°C for boot stage treatments to

30^o C for some post-anthesis treatments) and at 45-55% humidity.

Nonstructural Carbohydrates

To provide insights into photosynthetic feedback processes, NSC of leaves and stems were monitored in Experiment 2. Enzymatic assays for individual carbohydrates were used to assess NSC (Andersen & Sorensen, 1999). We opted to use an enzymatic approach, converting each main type of carbohydrate separately to glucose and assaying the glucose with a spectrophotometer, in preference to a gas chromatography approach due to the greater ease and lower cost of the procedure.

In June 2011, (i.e., Experiment 2) stem and leaf tissue samples were collected for NSC measurements. Four plants from each treatment, for each species, were clipped 10 days following the pre-anthesis treatment. Flowering heads were removed, leaves (totaling at least 3 g per sample) and stems (totaling at least 5 g per sample) were separated, and all painted portions of leaves discarded. Samples were flash frozen at -70^o C to inactivate invertase and glycosidases (which could destroy or polymerize carbohydrates), and stored in -20^o C freezer. In September 2011 samples were removed, lyophilized for two days, and ground through a Christy-Turner mill with a 0.5 mm screen. To quantify total source strength, leaf mass ratio (leaf dry mass / whole plant dry mass), was measured on the basis of these samples.

Samples of up to 200 mg of powdered leaf or stem tissue were used for analysis, bulking across all four plants (in some cases, there was less than 200 mg available). Soluble carbohydrates were extracted four times in hot water at 85^o C (3 ml of hot water each time) with five minutes of centrifugation (3000 rpm) following each extraction. The pellets remaining after

the four sequential extractions were digested with 50 units mL⁻¹ alpha-amylglucosidase (from *Aspergillus niger*) and 40 units mL⁻¹ fructanase (Megazyme Co: Dublin, Ireland) in 6 mL total solution at 60^o C for 30 min. Following digestion, each carbohydrate was assayed using the following six enzyme combinations: 1) hexokinase, glucose 6-P dehydrogenase, NADP, Mg²⁺ on an aliquot from the supernatant to assay glucose; 2) hexokinase, glucose 6-P dehydrogenase, NADP, Mg²⁺ and phosphoglucose isomerase on an aliquot from the supernatant to assay fructose; 3) hexokinase, glucose 6-P dehydrogenase, NADP, Mg²⁺ and invertase on an aliquot from the supernatant to assay sucrose; 4) hexokinase, glucose 6-P dehydrogenase, NADP, Mg²⁺, phosphoglucose isomerase and fructanase on an aliquot from the supernatant to assay soluble fructans; 5) hexokinase, glucose 6-P dehydrogenase, NADP, Mg²⁺, and phosphoglucose isomerase on an aliquot from the pellet to assay insoluble fructans; and 6) hexokinase, glucose 6-P dehydrogenase, NADP, and Mg²⁺ on an aliquot from the pellet to assay starch. Each aliquot was about 1.5 mL total volume, and included generally 10-40 microliters of leaf extract solution. Absorbance of NADPH at 340 nm for each fraction was measured using a Shimadzu UV-2600 spectrophotometer (Shimadzu Corporation: Kyoto, Japan). Summed together, these form the NSC fraction.

This method is relatively new, and constitutes a novel adaptation of existing enzymatic approaches to assay plant NSC. Previous enzymatic assay procedures, particularly in studies of source/sink balance, have generally focused on estimating starch and soluble sugars (e.g. Flore and Layne, 1999). Cool-season grasses (Poaceae) pose a problem for such procedures, as they

may maintain large amounts of their total carbohydrate reserves in the form of small to large size fructose polymers built on a sucrose substrate (fructans). Fructans may range in size from as few as 8 to as many as 100,000 fructose units (Vijn and Smeekens, 1999) and fructan production is a trait cool season grasses share along with certain other important plant families including Asteraceae (the sunflower family), Amaryllidaceae (onion family), Agavaceae (agave family), Asparagaceae (asparagus family), Iridaceae (iris family) and Boraginaceae (borage family). It is believed that fructans play an important role in cold tolerance, cryoprotection of membranes, and osmotic adjustment, and they may also play a role in helping to store carbohydrates within vacuoles, in a way that reduces feedback inhibition of photosynthesis (Kim S-H, personal communication).

Standard methods for assaying NSC are poorly adapted for assays of fructan concentration, as fructans show a range of water solubilities (high for small chains, low for large chains) and thus partition partly within the supernatant and partly within the pellet, depending on the conditions of extraction. They are also acid-labile and can break down under the acidic conditions used in some protocols for assaying starch. Thus in order to accurately estimate fructan concentrations it is necessary to include a step which specifically breaks fructans down to fructose, to avoid strongly acidic starch digestion conditions, and to assay fructans both within aliquots from the supernatant as well as the pellet. Our modification of previous enzymatic NSC assay procedures consists of the incorporation of a fructan digestion step. Our method was validated using samples of sunchoke (*Helianthus tuberosus*) tubers and Kentucky bluegrass (*Poa pratensis*) leaves, comparing our estimate of each type of carbohydrate to published literature.

Seed Mass and PSCR

In Experiment 1, plants were harvested on July 21 (annuals) and on August 15 (perennials). Seed heads were removed and cleaned using a customized tabletop thresher. Subsamples of 20 seeds per treatment were randomly selected and weighed to determine single seed mass. Harvest was conducted on July 21 (annuals) and August 10 (perennials) for experiment 2, while post-sexual cycle regrowth (PSCR, percent of plants regrowing) was visually estimated for each microplot on Oct 15.

Fall Flowering and Fall Photosynthesis

One unusual feature of perennial wheat and rye, which may be indicative of the fact that these species derive from an annual x perennial cross, is that they may experience a second round of unproductive, abortive flowering in the fall, if they have been able to grow large enough during the post-sexual cycle regrowth (PSCR) period. This may occur if weather conditions are favorable. Fall flowering is generally unproductive, as it is interrupted by the onset of cold weather which kills the flowers. However, as only a minority of the perennial cereal plants in our experiment initiated fall flowering in 2011, this allowed us to conduct a natural experiment to determine how closely photosynthetic rate was coupled with flowering. By comparing photosynthetic rates in flowering and non-flowering plants at the same moment in time, we could determine whether flowering was associated with higher photosynthetic rates. We carried out photosynthetic measurements on October 15 – 17, 2011, on both perennial wheat and rye, between 9:00 – 11:30 a.m., following the same protocol above.

Pilot Experiment

In the pilot experiment, the Low, High and Control treatments were imposed only at one of the three growth stages (pre-anthesis). Photosynthetic rates (A_{\max}) were measured on P19 and P3, using the methods specified above, 1 week after treatment impositions. In addition, triose phosphate utilization (TPU) was measured 10 days following treatment imposition. TPU is a measure of the total capacity of plants to process and export triose phosphate molecules: it represents the maximum rate at which photosynthesis can proceed, as plants which generate carbohydrates faster than they can be exported will experience depletion of free phosphate and feedback inhibition of photosynthesis. TPU was modeled as $A_{\text{sat}} - R_d$, where A_{sat} is defined by observed net photosynthesis at saturating concentrations of CO_2 , and R_d represents day respiration (Sharkey et al., 2007). Here day respiration was assumed to be $-2.00 \mu\text{mol m}^{-2} \text{s}^{-1}$, and 1600 ppm CO_2 was assumed to be saturating.

Following imposition of treatments, the microplots where treatments had been imposed were labeled with colored flags and tracked into the following year. In July 2011, seed heads from each of the labeled microplots were harvested, threshed through a customized tabletop seed cleaner, and weighed. The purpose was to determine whether source-sink ratio manipulation had delayed effects on the reproductive fitness of plants in the following year.

Statistical Analysis

The model for photosynthetic rates and seed size was an RCBD split-split plot ANOVA:
$$A_{\max} = \mu + \textit{Species} + \textit{Block} + \textit{Species} \times \textit{Block} + \textit{Species} \times \textit{Block} \times \textit{Stage} + \textit{Stage} + \textit{Treatment} + \textit{Species} \times \textit{Stage} + \textit{Species} \times \textit{Treatment} + \textit{Species} \times \textit{Stage} \times \textit{Treatment}.$$

Stage (i.e., growth stage at the time of treatment imposition), species and treatment were fixed factors and block was considered a random factor. Contrasts of High and Low treatments against the control, for a given species and/or species x stage combination, were made using Dunnett's post-hoc test. Experiments 1 and 2 were examined separately, using PROC MIXED in SAS 9.2 (SAS Institute, 2008). Post sexual cycle regrowth (PSCR) was measured on all six source/sink manipulation treatments at the same time, so we did not include 'stage' as a variable. Rather, we treated each stage x manipulation combination as a separate treatment for this analysis, (e.g., "Pre-Anthesis Low" and "Post-Anthesis Low") were considered different treatments. Our model for regrowth (logit-transformed to meet assumptions of ANOVA) was thus:

$$\log [(0.1 + PSCR) / (1.1 - PSCR)] =$$

$$\mu + \textit{Species} + \textit{Block} + \textit{Species} \times \textit{Block} + \textit{Treatment} + \textit{Species} \times \textit{Treatment}.$$

NSC was analyzed separately for stem tissues and leaf tissue samples. For each, we used the same model as for PSCR, but with only three treatments (i.e., Low, High and Control).

The overall model in the pilot experiment was as follows:

$$A = \mu + \textit{Genotype} + \textit{Block} + \textit{Genotype} \times \textit{Block} + \textit{Genotype} \times \textit{Treatment} .$$

Results

Survival

No differences in survival to harvest were seen between different treatments, indicating

that treatments did not increase immediate mortality (data not shown).

Spring Photosynthetic Rates

In Experiment 1, photosynthetic rates (A_{\max}) were affected by source-sink ratio: there was also a species x treatment interaction, meaning that species responded differently to source/sink manipulation (Table 12). As there was not a three-way interaction, we averaged A_{\max} for each species x treatment combination across growth stages (Figure 14). In perennial rye, the Low treatment had 17.5% higher A_{\max} than the control, while the High treatment was 14% lower. In perennial wheat, the Low treatment had 11% higher A_{\max} than the control, while the High treatment did not differ from controls. Neither annual species showed treatment effects. Photosynthetic rates (A_{\max}) ranged between 21-26 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for perennial wheat, 18-20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for annual wheat, 15-23 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for perennial rye, and 10-23 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for annual rye. Responses were similar between the boot and pre-anthesis stages (means shown in Table 12) and the post-anthesis stage (Table 13).

Photosynthetic responses in Experiment 2 were similar (Table 14). Due to the three-way interaction we considered treatment responses separately for each growth stage. Photosynthetic rate (A_{\max}) for control plants, averaged across the three growth stages (Figure 15) were between 13-19 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for perennial wheat, 11-17 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for annual wheat, 16-19 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for perennial rye, and 15-17 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for annual rye. Leaf mass ratio (leaf dry mass / total dry mass) did not vary between perennial and annual wheat (23 – 33%), or perennial and

annual rye (32 – 36%).

Source/sink manipulation, at boot stage, influenced A_{\max} for every species except annual wheat (Table 14). The Low treatment showed 10-12% higher A_{\max} than the control in annual and perennial rye, and 18% higher A_{\max} than the control in perennial wheat. At the pre-anthesis stage, A_{\max} responded to source/sink manipulation only in perennials (Table 14). The Low treatment showed 27% and 17% higher A_{\max} than the control, in perennial rye and perennial wheat, respectively. The High treatment showed no response in perennial rye and 12% lower A_{\max} in perennial wheat. Finally, source/sink manipulations at the post-anthesis stage affected photosynthetic rate in all species (Table 15). The Low treatment showed 13-14% higher A_{\max} than the control in the perennials, and 8-11% higher in the annuals. The High treatment showed 14% lower A_{\max} than the control in perennial rye, 24% lower in perennial wheat, 12% lower in annual rye, and did not differ from control in annual wheat.

Nonstructural Carbohydrates

In all four species, leaf NSC showed similar responses to source/sink manipulations, with NSC concentrations increasing in ‘High’ source/sink treatments (14 – 84%), and decreasing in ‘Low’ treatments (12 – 33%). Although treatment responses in leaf tissue were more pronounced in the two *Triticum* species than in the *Secale* species, there were no consistent differences in treatment response between annual and perennial species, and the perennials generally showed similar responses to annuals (Table 17: Figure 16). In the Low source/sink treatment, leaf NSC was reduced by 33% in annual wheat, and 12-14% in all other species (Figure 16). The High

treatment increased leaf NSC by 23% in annual wheat, 84% in perennial wheat, 22% in annual rye and 14% in perennial rye. Treatment had an effect on concentrations of sucrose ($P = 0.036$) and soluble fructans ($P < 0.0001$) but not on other carbohydrates: thus changes in NSC appear to be mostly driven by changes in soluble fructans (32-42% of the total: Table 16) and in sucrose (14-19% of the total: Table 17). This could reflect the fact that these two carbohydrates are quantitatively the largest pools, accounting for over half the total: alternatively, it could be related to the fact that sucrose and fructan metabolism are closely related, with sucrose serving as the initial substrate for fructan formation (Vijn and Smeekens, 1999).

Stem NSC showed the same trend for annual and perennial wheat, increasing in the High treatment (43% -45%) and decreasing in the Low treatment (11% in annual wheat, 38% in perennial wheat): however, stem NSC was not affected by treatment in annual or perennial rye (Table 19: Figure 17). As with the leaf tissues, buildup of NSC under high source/sink ratio and depletion of NSC under low source/sink ratio could be explained entirely by changes in soluble fructan and sucrose concentration (the two largest carbohydrate pools). Stem NSC was 34 – 43% soluble fructans (Table 18), and 16 – 23% sucrose (Table 19). Starch, fructose, glucose and insoluble fructan concentrations were not affected by source-sink treatments.

Overall, total NSC concentrations in unmanipulated (control) plants did not differ between stem and leaf tissues, ranging from 13 – 19% of dry matter in leaves (Table 17; Figure 16) and 15 – 25% in stems (Table 19; Figure 17). Leaf NSC was 28% higher in perennial compared to annual wheat ($t = 3.09$, $p = 0.027$), but did not differ between perennial compared to annual rye (Table 13). Likewise, stem NSC in control plants was 31% higher in perennial compared to annual wheat ($t = 3.26$, $p = 0.022$), but was no different in perennial and annual rye (Table 14). Thus the wheat species, but not the rye species, support our hypothesis that

carbohydrate reserves would be greater in the perennial species. This is consistent with the results of Gonzalez-Paleo and Ravetta (2011) who found higher leaf NSC in perennial as compared to annual *Lesquerella*. It also suggests an overall trend for a more conservative resource strategy in perennial wheat: perennial wheat, relative to annual wheat, appears to be maintaining reproductive allocation at a fairly low level, with additional photosynthetic capacity and additional stored carbohydrates that are not being utilized for reproduction.

Differences in leaf NSC between species appear to be driven by changes in glucose content (15 – 26% of total NSC in leaves; Table 16) rather than by other carbohydrate pools. The same is true of stems, where glucose constitutes 11 – 25% of total NSC (Table 18). The glucose contents were unusually high, while starch and sucrose contents were low; these results may be indicative of some breakdown of starch and sucrose, through residual amylase and invertase activity, during the transferring and processing of our samples.

Seed Mass and Regrowth

In Experiment 1, seed mass was affected by stage x treatment interaction ($F = 7.82$, $P = 0.001$), and by species x treatment interaction ($F = 3.35$, $P = 0.005$; Figure 18). Since there was no three-way interaction we averaged across growth stages for each species x treatment combination. Seed mass of control plants was on average 36 mg in annual wheat, 27 mg in perennial wheat, 36 mg in annual rye and 28 mg in perennial rye. Source/sink ratio affected seed size for annual wheat, and annual rye, perennial rye, but not perennial wheat. In annual rye, pooled across the three growth stages, the Low treatment reduced seed size by 12%, while the High treatment increased it by 12%. In perennial rye, the Low treatment reduced seed size by 14%, while the High treatment did not increase it. In annual wheat, the Low treatment reduced

seed size by 17%, while the High treatment increased seed size by 7% (Figure 18). Much of the treatment effect was driven by effects at boot stage, when annual wheat, annual rye and perennial rye showed large responses. For the annual species examined here, increased source/sink ratio led to a 30% decline in seed size. Regrowth percentage was not affected by treatment in either perennial species although, perennial wheat did tend to have a lower percentage of regrowth (63 – 83%) than perennial rye (90 – 96%: Table 14).

Fall Flowering and Photosynthesis

Fall flowering in perennial rye was very low (across the six blocks, only 2.5% of perennial rye plants flowered on average). Because of the low flowering rates, we could not compare photosynthesis in nonflowering and flowering individuals. In perennial wheat, between 6 – 60% of plants produced flowering heads in the fall, although no mature seed heads were produced due to the onset of frost in late fall. Flowering individuals in the perennial wheat plots maintained 35% higher photosynthetic rates than non-flowering individuals ($t = 8.26$, $p = 0.0016$ following the Bonferroni correction for $k = 2$ comparisons: Figure 19). This does not demonstrate causality, since it is unclear whether higher rates of resource acquisition drive flowering or vice versa. However, it does provide further evidence of close coupling between resource supply and demand in these plants.

Pilot Experiment

In the 2009 pilot experiment, photosynthetic rate was measured only on the two perennial wheat genotypes. In one of the plots the Low treatment was heavily damaged by rodents and no measurements could be taken. Photosynthetic rate (A_{\max}) and TPU were both strongly affected by treatment. There was no main effect of genotype on either parameter, nor an effect of

treatment x genotype, and treatment effects were significant for both species (Table 20). To simplify the model for A_{\max} and TPU we pooled both genotypes (since there was little difference between the genotypes). Treatment affected A_{\max} when pooled across genotypes ($F = 6.33, p = 0.04$): the Low treatment had 20% higher photosynthetic rates than the control ($t = 5.51, p < 0.01, n = 9$), while the High treatment did not differ from the control (Table 21). Treatment also affected TPU when pooled across genotypes ($F = 53.78, p < 0.0010$). The Low treatment had 18% higher TPU than the control ($t = 6.25, p < 0.01, n = 9$) while the High treatment was 11% lower than the control ($t = 3.79, p < 0.01$).

In the pilot experiment, neither total second-year seed yield from treated plants (i.e. seed yield in 2010, in the year following treatments), nor single seed mass, was affected by treatment, genotype or the interaction of genotype and treatment (Table 20). Thus the manipulations of source/sink ratio had no effect on kernel mass or on seed yield in the following year (Table 21).

Discussion

Annual cereals showed colimitation rather than sink limitation during flowering and grain filling, and compensated for only part of the source/sink change (e.g., seed size declined by only 16% when source/sink ratio declined by 33%, indicating the lack of complete source limitation). This is suggested by the lack of consistent compensatory photosynthesis, as well as by changes in seed size. When manipulations were implemented during boot stage, both species showed clear source limitation, with seed mass reflecting source/sink ratio. These results match previous studies, in which annual wheat varies between being sink-limited, and colimited, during grain

filling (Borras et al., 2004; Reynolds et al., 2005). Wheat has also been found to be sink limited during vegetative stage (Harrison et al., 2010) but source limited shortly before anthesis, as illustrated by stimulatory effects of elevated CO₂ on yield (Amthor, 2001). The mechanism of switching between source and sink limitation is that carbohydrate concentrations at anthesis affect the number of florets fertilized, and plants fertilize only as many florets as can be filled largely on the basis of stored reserves (Cruz-Aguado et al., 1999). The effect sizes we found in annual wheat are consistent with existing literature on changes in photosynthetic rate (Zhenlin et al., 1998; Reynolds et al., 2005), seed size (Borras et al., 2004; Alvaro et al., 2008), and NSC content (Kuhbauch and Thome, 1989; Ma et al., 2009).

To the best of our knowledge, no studies have explicitly considered source vs. sink limitation of photosynthesis in annual rye. However, a previous study found that defoliation during reproductive period caused 7 – 17% decreases in seed mass, with greater effects when manipulations took place earlier in the season (Rho et al., 1990); in the wheat-rye hybrid triticale, overall seed mass decreases were greater than in rye. These data give some indication that rye shows colimitation before and after anthesis, that the degree of source limitation is less than in wheat (since triticale, with its introduction of wheat genetics, shows more source limitation than rye) and that source limitation becomes less important later during reproductive development. Our results, which found greater compensatory changes in A_{\max} and stability of seed mass in annual rye compared to wheat, are consistent with these conclusions.

In contrast to the source/sink colimitation seen in annual wheat and annual rye, perennial wheat showed complete sink limitation during all three growth stages. This was indicated by larger changes in A_{\max} relative to annuals, stability of seed size and large buildup of NSC.

Increased photosynthesis and NSC depletion allowed perennial wheat to tolerate low source/sink ratio with no change in seed mass. Perennial rye showed sink limitation during flowering and grain filling, but colimitation during boot stage where A_{\max} increased. Seed size also declined in response to source/sink reduction in perennial rye, although less so than in the annuals. Perennial rye did not show changes in stem NSC in response to changed source/sink ratio. This could be because perennial rye shows both greater upregulation of photosynthetic rate (including the highest increase in A_{\max} in our study) and small declines in seed size in response to decreased source/sink ratio, which could allow it to avoid depleting stem NSC reserves (Figure 17). Similarly, increased source/sink ratio results in interrelated compensatory responses, including (in some cases) reductions in photosynthesis, buildup of TNC in leaves, and no change in seed mass. In neither species was fall regrowth affected by moderate source/sink perturbations, suggesting that perennial cereals could tolerate selection for moderately increased sink strength without losing the ability to perennialize. Thus our hypotheses were generally supported, with perennial cereals showing more sink limitation than annuals.

Perennial wheat and rye, with their high A_{\max} , small seeds and lower grain yield compared to annuals, demonstrated a stronger form of the conservative reproductive strategy observed previously in annual wheat (Reynolds et al., 2009). This stands in contrast to the study by Masnatta and Ravetta (2011) which found equal responsiveness to source-sink manipulations in annual and perennial *Lesquerella*. A conservative strategy appears to act as a partial (in perennial rye) or full (in perennial wheat) buffer against reductions in effective leaf area. In general, wild perennial grasses that pursue a ‘conservative’ strategy face fewer risks than annual cereals, as low reproductive output in one year can be compensated for in the future. Perennials

also face risks from a ‘liberal’ strategy, since they are not subjected to human interventions that can ameliorate effects of environmental stresses after anthesis. Increased sink capacity, coupled with sink limitation, could be a means for some perennial cereals to achieve higher A_{\max} and resource acquisition rates than annuals. Previous research has indicated that some perennial rice species maintain higher A_{\max} than annual rice (Zhao et al., 2008). If the findings from this study indicating sink limitation hold for other perennial cereals, this supports the hypothesis that increased allocation to vegetative structures could promote increased photosynthesis relative to annuals (Zhao et al., 2008).

Not only do perennial cereals appear to maintain relatively stable seed size during the harvest immediately following source/sink ratio manipulation, but stability of seed size appears to be maintained during the following year as well. Our pilot experiment indicated that increasing or decreasing the source sink ratio in perennial cereal plants did not affect the seed yield of those plants in the following year. Thus there do not appear to be effects of carbohydrate depletion persisting post harvest, and during the year following source sink manipulation plants appear to be able to compensate fully for changes in source sink ratio and carbohydrate reserves, both in the immediate and the longer term. Changes in source sink ratio do not appear to affect immediate survival, fall regrowth, or seed production the following year, and have only modest (in perennial rye) or no effects (in perennial wheat) on seed size within the same year.

Physiological responses to sink reduction and source reduction in perennial fruit trees and grasses are often larger than the effect sizes observed in our study (DaMatta et al., 2008; Park and Kim, 2011). For example, heavy shading of sugarcane (source reduction) caused a 27% decrease in soluble sugar content in unshaded leaves, with concomitant 50-65% increases in A_{\max} (McCormick et al., 2008a). Conversely, cold-girdling of sugarcane (sink reduction)

increased soluble sugar concentrations by 80% and A_{\max} by 40% (McCormick et al., 2008b). Crested wheatgrass (*Agropyron cristatum*) showed an even larger response: photosynthesis increased by 190% and 160% in response to 70% and 85% biomass removal, respectively (Peng et al., 2007). In a study on apple trees (*Malus domestica*) source reduction led to a 33% increase in photosynthesis, whereas root sink removal through cold-girdling of apple trees resulted in a 50% decrease in A_{\max} (Zhou and Quebedeaux, 2003; Fan et al., 2010). Similarly, in cherry, increased source/sink ratios lead to 30-50% increases in A_{\max} (Layne and Flore, 1995). Thus the physiological responses to source-sink ratio in our study were modest compared to many perennials.

The specific mechanisms by which source-sink ratio manipulation affects photosynthetic rate in perennial cereals is unclear. Buildup of assimilates and subsequent feedback inhibition of photosynthesis, possibly through depletion of free phosphates and declines in ATP/ADP ratio, is a mechanism that has been widely proposed (Sharkey, 1985), and indeed many plants do show buildup of soluble carbohydrates in advance of the decline in photosynthetic rates (Iglesias et al., 2002; Flore and Layne, 1999). It is also known that changes in sink/source ratio can affect phosphate pools in leaf tissue, which could be a mechanism involved in feedback inhibition. However, buildup in carbohydrate concentrations prior to declines in photosynthesis are not always seen, and other mechanisms are almost certainly involved in many cases. In addition, as around 75% of plants, under normal environmental conditions, are not at the C_i level where they are limited by TPU (Wullschleger, 1993) it is likely that in most cases effects of sink limitation, while they may most directly and immediately reduce TPU, also have indirect effects on other determinants of photosynthetic rate. Buildup of carbohydrates may lead to stomatal closure (Yan et al., 2011), or decreases in Rubisco activity and electron transport rate (Lewis, James D.,

personal communication). For example, Ainsworth et al. (2004) found declines in Rubisco activity under elevated CO₂ in soybean, while Krapp and Stitt (1995) found a 45% decrease in Rubisco activity in cold girdled spinach leaves, which was not attributable to declines in total amount of Rubisco but most likely to changes in the activation state. A study in soybean (Kasai, 2008) found that high source/sink ratios and buildup of soluble carbohydrates were directly associated with declines in the activation state of Rubisco. Schaefer et al. (1992) found similar results in *Chenopodium rubrum*, where buildup of carbohydrates through glucose feeding led to decreases in Rubisco activity. Foyer et al. (1990) also hypothesized that electron transport could be affected by sink-regulated changes in free phosphate, through regulation of the redox state of intermediate electron carriers.

In the case of these perennial cereals, little work was done on elucidating the mechanisms of sink regulation of photosynthesis. We did find in our pilot experiment, that at least in perennial wheat, changes in source/sink ratio affect TPU as well as photosynthetic rate, and that the changes in TPU are of similar magnitude to changes in A_{\max} . However, we also observed that TPU was much higher than A_{\max} at ambient CO₂. This suggests that downregulation of TPU and sink activity decrease photosynthetic rate indirectly, and that declines in TPU have secondary effects on other processes regulating photosynthesis (either stomatal closure, electron transport rate or Rubisco activity). Our examination of the impacts of source-sink ratio separately on TPU and on A illustrate that sink regulation of photosynthetic activity appears to be a complex process that involves coordinated changes in several parameters, and that regulates A so that it is maintained well below maximal TPU capacity.

Conclusion

Perennial wheat and rye show more sink regulation of metabolism than annual equivalents, and demonstrate compensatory responses that help buffer seed size against environmental fluctuation. This indicates that perennial cereals currently operate further below their maximum photosynthetic capacity than annual wheat and rye, and follow a conservative reproductive strategy. Our results suggest that higher sink capacity could help explain why some perennial species maintain higher photosynthetic rates than closely related annuals, and illustrate how life history and physiology affect each other in these emerging representatives of a novel plant growth form.

Further work is necessary to determine the precise mechanisms by which source/sink ratio regulates photosynthetic rate and metabolism in perennial cereals, as well in other species where the mechanisms are not fully clear. One approach towards elucidating mechanisms of sink regulation, that could build upon the work done in this study, would be to generate A vs. C_i (photosynthetic rate vs. intercellular carbon dioxide concentration) curves for perennial and annual cereal plants subjected to higher and lower source/sink ratios. These curves allow estimation of stomatal limitation, electron transport rates, TPU and Rubisco-catalyzed carboxylation of ribulose biphosphate carboxylation (Sharkey et al., 2007; Farquhar and Sharkey, 1982). By observing which specific parameters differed between High, Low and Control treatments, as well as observing overall intercellular/ambient carbon dioxide ratios and stomatal conductance, it would be possible to more closely elucidate the mechanisms of sink regulation.

A further continuation of this study could be to look at how source/sink ratio affects not simply the rate of photosynthesis, but its duration. It is known that in some plants, changes in

source/sink ratio affect the timing of leaf senescence: for example, in wheat, higher source/sink ratios associated with elevated CO₂ cause earlier leaf senescence (Zhu et al., 2009) while buildup of carbohydrates is associated with later leaf senescence in the spring geophyte *Erythronium americanum* (Gandin et al., 2011). If plants with low source/sink ratios keep their leaves green and photosynthetically functional for longer, this could serve as an additional strategy by which they could compensate for the increased carbohydrate demand relative to leaf area. In our study it was observed that the changes in photosynthetic rate, while significant, were relatively modest compared to some woody perennials or wild grasses, and were quantitatively too small to fully compensate for the changes in carbohydrate demand relative to supply. This was reflected, among other things, in the fact that high sink strength did lead to carbohydrate depletion, and high source/sink ratio led to carbohydrate buildup. Changes in the timing of leaf senescence, such that plants with low source/sink ratios maintain green leaves for longer, could serve as an additional compensatory mechanism, and further studies are needed to determine the extent to which senescence and length of growing season respond to source/sink ratio.

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APPENDICES

APPENDIX A. Tables and Figures for Chapter 4

Table 12. Experiment 1 (2010) means (SE), *F*-values and *P*-values for photosynthetic rates (A_{\max}) following boot stage and pre-anthesis manipulations. Photosynthetic rates were measured 5-8 days following treatment imposition, for annual wheat, perennial wheat, annual rye and perennial rye. All plants were subjected to three levels of source/sink ratio treatments (“Low” = 67% of control, “Control”, and “High” = 200% of control) at two or three growth stages. Only the means for the first two growth stages are shown in this table, but *F*-values are reported for the experiment as a whole. Symbols ‘*’ and ‘**’ represent significant differences from the control, following Dunnett’s post-hoc testing, at $P = 0.05$ and $P = 0.01$ respectively. Units for A_{\max} are $\mu\text{mol m}^{-2} \text{s}^{-1}$ of CO_2 .

Species	Boot Low	Boot Control	Pre Low	Pre Control	Pre High
Annual wheat	-----	-----	19.9 (1.1)	18.0 (1.0)	18.3 (0.7)
Perennial wheat	25.3 (0.9)**	23.0 (0.4)	26.1 (0.8)**	22.8 (0.8)	22.9 (1.0)*
Annual rye	-----	-----	22.5 (1.0)	19.7 (1.4)	20.0 (1.0)
Perennial rye	23.3 (1.2)**	20.6 (0.9)	22.6 (1.6)**	19.3 (1.6)	17.1 (1.6)

ANOVA results	<i>F</i> -ratio	<i>P</i> -value
Species	38.22	< 0.001
Treatment	14.93	< 0.001
Stage	50.61	< 0.001
Sp x Trt	2.41	0.036
Sp x Stage	23.64	< 0.0007
Trt x Stage	0.89	0.50
Sp x Trt x Stage	0.61	0.69

Table 13. Experiment 1 (2010) means (SE) for photosynthetic rates, measured 5-8 days following post-anthesis source/sink manipulations, for annual wheat, perennial wheat, annual rye and perennial rye. All plants were subjected to three levels of source/sink ratio treatments (“Low” = 67% of control, “Control”, and “High” = 200% of control) at 1 day post-anthesis. This experiment was conducted at Kellogg Biological Station, Hickory Corners, MI. Symbols ‘*’ and ‘**’ represent significant differences from the control, following Dunnett’s post-hoc testing, at $P = 0.05$ and $P = 0.01$ respectively. Units for A_{\max} are $\mu\text{mol m}^{-2} \text{s}^{-1}$ of CO_2 .

Species	Post Low	Post Control	Post High
Annual wheat	-----	-----	-----
Perennial wheat	23.9 (0.7)**	21.7 (0.6)	21.0 (0.8)**
Annual rye	10.3 (1.4)**	10.0 (1.1)	10.2 (1.2)
Perennial rye	20.8 (0.9)**	16.9 (0.4)	15.5 (1.0)**

Table 14. Experiment 2 (2011) means (SE), *F*-values and *P*-values for photosynthetic rates (A_{\max}) measured 5-8 days following boot stage and pre-anthesis source/sink manipulation, in annual wheat, perennial wheat, annual rye and perennial rye and post-sexual cycle regrowth (*PSCR*) in perennial wheat and rye. All plants were subjected to three levels of source/sink ratio treatments (“Low” = 67% of control, “Control”, and “High” = 200% of control) at two or three growth stages (“Boot” = boot stage, “Pre” = 1 week pre-anthesis, and “Post” = 1 day post-anthesis). Only means for the first two stages, and analysis of variance results for photosynthetic rates, are shown in this table. The experiment was conducted at Kellogg Biological Station, Hickory Corners, MI. Symbols “*” and “**” represent significant differences from the control, following Dunnett’s post-hoc testing, at $P = 0.05$ and $P = 0.01$ respectively. Units for A_{\max} are $\mu\text{mol m}^{-2} \text{s}^{-1}$ of CO_2 , while *PSCR* is expressed as a %.

	Boot Low	Boot Control	Pre Low	Pre Control	Pre High
Photosynthetic Rate					
Annual wheat	15.1 (1.3)	14.0 (1.2)	11.7 (1.0)	12.3 (1.5)	10.7 (1.3)
Perennial wheat	18.0 (1.5)**	15.4 (1.8)	21.7 (2.0)**	18.7 (1.8)	16.5 (1.8)*
Annual rye	21.4 (1.4)**	19.6 (1.4)	13.3 (1.0)	13.8 (0.9)	13.4 (1.0)
Perennial rye	22.6 (1.0)**	20.3 (1.2)	20.0 (2.1)**	15.8 (1.5)	16.6 (1.9)
<i>PSCR</i>					
Perennial wheat	66 (14)	65 (14)	69 (6)	65 (14)	85 (8)
Perennial rye	97 (2)	93 (2)	93 (3)	93 (2)	89 (2)
ANOVA results (A_{\max})				<i>F</i>-ratio	<i>P</i>-value
Species				5.92	0.007
Treatment				69.20	< 0.001
Stage				2.81	0.072
Sp x Trt				4.36	< 0.001
Sp x Stage				4.63	0.001
Trt x Stage				2.89	0.039
Sp x Trt x Stage				2.07	0.04

Table 15. Experiment 2 (2011) means (SE), *F*-values and *P*-values for photosynthetic rates (A_{\max}) measured 5-8 days following post-anthesis source-sink manipulation in annual wheat, perennial wheat, annual rye and perennial rye, and post-sexual cycle regrowth (*PSCR*) in perennial wheat and rye. All plants were subjected to three levels of source/sink ratio treatments (“Low” = 67% of control, “Control”, and “High” = 200% of control) at two or three growth stages (“Boot” = boot stage, “Pre” = 1 week pre-anthesis, and “Post” = 1 day post-anthesis). Only means for the post-anthesis growth stage, and analysis of variance results for *PSCR*, are shown in this table. The experiment was conducted at Kellogg Biological Station, Hickory Corners, MI. Symbols ‘*’ and ‘**’ represent significant differences from the control, following Dunnett’s post-hoc testing, at $P = 0.05$ and $P = 0.01$ respectively. Units for A_{\max} are $\mu\text{mol m}^{-2} \text{s}^{-1}$ of CO_2 , while *PSCR* is expressed as a %.

	Post Low	Post Control	Post High
Photosynthetic Rate			
Annual wheat	17.0 (0.8)**	15.8 (0.7)	14.5 (1.0)
Perennial wheat	14.9 (2.2)**	13.2 (2.2)	10.4 (2.3)**
Annual rye	20.6 (1.1)**	18.6 (0.8)	16.4 (1.9)
Perennial rye	18.8 (0.8)**	16.5 (0.6)	14.2 (0.5)**
<i>PSCR</i> %			
Perennial wheat	68 (13)	65 (14)	86 (6)
Perennial rye	93 (3)	93 (2)	94 (3)
<i>PSCR</i>			
ANOVA results		<i>F</i>-ratio	<i>P</i>-value
Species		0.15	0.44
Treatment		4.85	0.001
Sp x Trt		7.07	0.001

Table 16. Experiment 2 (2011) means for unmanipulated plants (percent of dry wt \pm SE), *F*-values and *P*-values for various carbohydrate pools (glucose, starch, fructose, soluble fructans) measured around anthesis, on leaf tissue from annual wheat, perennial wheat, annual rye and perennial rye. All plants were subjected to three levels of source/sink ratio treatments (“Low” = 67% of control, “Control”, and “High” = 200% of control) at approximately 1 week pre-anthesis. The experiment was conducted at Kellogg Biological Station, Hickory Corners, MI. Only means for unmanipulated (“Control”) plants are shown. The symbol ‘*’ indicates significant differences compared to the annual species (annual wheat or rye) at $\alpha = 0.05$.

Species	Glucose %	Starch %	Fructose %	Sol. Fructans %
Annual Wheat	2.54 \pm 0.42	0.40 \pm 0.08	2.80 \pm 0.62	5.11 \pm 1.15
Perennial Wheat	4.62 \pm 1.29*	0.41 \pm 0.15	2.24 \pm 0.51	7.27 \pm 1.86
Annual Rye	2.34 \pm 0.36	0.23 \pm 0.06	3.68 \pm 0.83	4.70 \pm 1.51
Perennial Rye	3.34 \pm 0.49*	0.28 \pm 0.09	4.82 \pm 1.05	7.01 \pm 1.99

Source of Variation	Glucose	Starch	Fructose	Sol. Fructans
Species	5.77 (0.008)	2.34 (0.11)	4.40 (0.021)	1.75 (0.20)
Treatment	0.31 (0.73)	1.07 (0.35)	1.04 (0.36)	17.60 (< 0.0001)
Species x Treatment	0.44 (0.84)	1.01 (0.43)	0.45 (0.84)	0.59 (0.74)
Block	0.43 (0.33)	0.24 (0.40)	0.01 (0.99)	0.01 (0.99)
Block x Treatment	0.85 (0.20)	0.01 (0.99)	0.01 (0.99)	1.24 (0.11)

Table 17. Experiment 2 (2011) means for unmanipulated plants (percent of dry wt \pm SE), *F*-values and *P*-values for various carbohydrate pools (insoluble fructans, sucrose, and total NSC) measured around anthesis, on leaf tissue from annual wheat, perennial wheat, annual rye and perennial rye. All plants were subjected to three levels of source/sink ratio treatments (“Low” = 67% of control, “Control”, and “High” = 200% of control) at approximately 1 week pre-anthesis. The experiment was conducted at Kellogg Biological Station, Hickory Corners, MI. Only means for unmanipulated (“Control”) plants are shown. The symbol ‘*’ indicates significant differences compared to the annual species (annual wheat or rye) at $\alpha = 0.05$.

Species	Insol. Fructans %	Sucrose %	Total %
Annual Wheat	0.41 \pm 0.17	1.51 \pm 0.42	12.76 \pm 1.44
Perennial Wheat	0.11 \pm 0.06	2.13 \pm 0.54	16.78 \pm 0.81*
Annual Rye	0.30 \pm 0.08	3.40 \pm 0.54	14.64 \pm 1.71
Perennial Rye	0.43 \pm 0.15	3.92 \pm 1.07	19.82 \pm 1.36*

Source of Variation	Insol. Fructans	Sucrose	Total
Species	1.02 (0.41)	1.71 (0.21)	7.29 (0.003)
Treatment	2.32 (0.11)	3.47 (0.04)	41.19 (< 0.0001)
Species x Treatment	1.36 (0.25)	3.73 (0.005)	4.11 (0.003)
Block	0.21 (0.42)	0.01 (0.99)	0.72 (0.24)
Block x Treatment	0.01 (0.99)	0.01 (0.99)	1.72 (0.04)

Table 18. Experiment 2 (2011) means for unmanipulated plants (percent of dry wt \pm SE), *F*-values and *P*-values for various carbohydrate pools (glucose, starch, fructose, soluble fructans) measured around anthesis, on stem tissue from annual wheat, perennial wheat, annual rye and perennial rye. All plants were subjected to three levels of source/sink ratio treatments (“Low” = 67% of control, “Control”, and “High” = 200% of control) at approximately 1 week pre-anthesis. The experiment was conducted at Kellogg Biological Station, Hickory Corners, MI. Only means for unmanipulated (“Control”) plants are shown.

Species	Glucose	Starch	Fructose	Sol. Fructans
	%	%	%	%
Annual Wheat	2.45 \pm 0.13	0.29 \pm 0.11	4.73 \pm 0.62	10.83 \pm 1.31
Perennial Wheat	5.89 \pm 0.40**	0.60 \pm 0.14	3.90 \pm 1.01	10.39 \pm 0.71
Annual Rye	2.90 \pm 0.22	0.27 \pm 0.05	4.42 \pm 1.27	6.14 \pm 1.73
Perennial Rye	2.21 \pm 0.55	0.20 \pm 0.06	4.24 \pm 1.00	2.72 \pm 1.15

Source of Variation	Glucose	Starch	Fructose	Sol. Fructans
Species	13.04 (0.0002)	6.75 (0.004)	2.11 (0.14)	6.55 (0.005)
Treatment	0.09 (0.91)	1.30 (0.28)	0.78 (0.48)	19.38 (< 0.0001)
Species x Treatment	0.36 (0.90)	1.52 (0.18)	1.54 (0.17)	1.25 (0.28)
Block	0.01 (0.99)	0.56 (0.28)	0.01 (0.99)	0.01 (0.99)
Block x Treatment	0.23 (0.41)	0.37 (0.36)	0.01 (0.99)	0.01 (0.99)

Table 19. Experiment 2 (2011) means for unmanipulated plants (percent of dry wt \pm SE), *F*-values and *P*-values for various carbohydrate pools (insoluble fructans, sucrose, and total NSC) measured around anthesis, on stem tissue from annual wheat, perennial wheat, annual rye and perennial rye. All plants were subjected to three levels of source/sink ratio treatments (“Low” = 67% of control, “Control”, and “High” = 200% of control) at approximately 1 week pre-anthesis. The experiment was conducted at Kellogg Biological Station, Hickory Corners, MI. Only means for unmanipulated (“Control”) plants are shown.

Species	Insol. Fructans	Sucrose	Total
	%	%	%
Annual Wheat	0.49 \pm 0.10	2.05 \pm 0.33	20.85 \pm 1.46
Perennial Wheat	0.88 \pm 0.24	5.06 \pm 2.02	26.66 \pm 2.17*
Annual Rye	0.33 \pm 0.04	2.85 \pm 0.63	16.91 \pm 1.36
Perennial Rye	0.67 \pm 0.20	4.25 \pm 1.00	15.94 \pm 2.47

Source of Variation	Insol. Fructans	Sucrose	Total
Species	0.40 (0.76)	3.62 (0.04)	11.65 (0.0003)
Treatment	0.46 (0.63)	10.48 (< 0.0001)	24.27 (< 0.0001)
Species x Treatment	0.78 (0.59)	5.89 (< 0.0001)	2.60 (0.02)
Block	0.01 (0.99)	0.29 (0.39)	0.51 (0.30)
Block x Treatment	0.01 (0.99)	0.63 (0.26)	0.01 (0.99)

Table 20. Pilot Experiment (2009) analysis of variance results for photosynthetic rates (A_{\max}) and triose phosphate utilization rates (TPU) measured 7-10 days following treatment imposition, as well as seed yield and single seed mass measured the following year, for three perennial cereals. Perennial accessions include ‘Rival’ perennial rye, ‘WSU-P19’ perennial wheat and ‘WSU- P3’ perennial wheat. All plants were subjected to three levels of source/sink ratio treatments (“Low” = 67% of control, “Control”, and “High” = 200% of control) at approximately 1 week pre-anthesis. The experiment was conducted at Kellogg Biological Station, Hickory Corners, MI.

Sources of Variation	A_{\max}		TPU		<i>Seed Yield</i>		<i>Single Seed Mass</i>	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Accession	18.02	< 0.0001	0.76	0.45	0.25	0.78	1.18	0.36
Treatment	0.51	0.52	63.86	< 0.0001	1.41	0.26	1.50	0.25
Accession x Treatment	1.90	0.19	2.48	0.13	0.68	0.61	1.46	0.25
Block	0.65	0.25	0.06	0.47	0.01	0.99	0.01	0.99
Accession x Block	0.44	0.33	0.80	0.21	1.07	0.14	0.79	0.21

Table 21. Pilot Experiment (2009) means (\pm SE) for photosynthetic rates (A_{\max}) and triose phosphate utilization rates (TPU) measured 7-10 days following treatment imposition, as well as seed yield and single seed mass measured the following year, for three perennial cereals. Perennial accessions include ‘Rival’ perennial rye, ‘WSU-P19’ perennial wheat and ‘WSU- P3’ perennial wheat. All plants were subjected to three levels of source/sink ratio treatments (“Low” = 67% of control, “Control”, and “High” = 200% of control) at approximately 1 week pre-anthesis. The experiment was conducted at Kellogg Biological Station, Hickory Corners, MI. Symbols ‘*’ and ‘**’ represent significant differences from the control, following Dunnett’s post-hoc testing, at $P = 0.05$ and $P = 0.01$ respectively. Units for A_{\max} and TPU are $\mu\text{mol m}^{-2} \text{s}^{-1}$ of CO_2 and triose phosphate respectively, while seed yield is expressed in grams m^{-1} and seed mass in mg.

Accession	Low	Control	High
---A_{\max}---			
Perennial Wheat P3	21.9 \pm 1.1**	17.4 \pm 0.9	17.2 \pm 0.5
Perennial Wheat P19	21.3 \pm 0.5**	18.3 \pm 0.4	19.1 \pm 1.4
---TPU---			
Perennial Wheat P3	14.0 \pm 1.5**	11.3 \pm 1.4	10.5 \pm 0.33**
Perennial Wheat P19	13.5 \pm 0.5**	12.3 \pm 0.2	10.7 \pm 0.4**
---Seed Yield---			
Perennial Wheat P3	21.8 \pm 2.0	21.8 \pm 0.9	21.8 \pm 0.9
Perennial Wheat P19	19.8 \pm 1.4	19.4 \pm 1.5	20.0 \pm 1.9
Perennial Rye	28.5 \pm 5.0	20.0 \pm 2.2	21.1 \pm 2.4
---Single Seed Mass---			
Perennial Wheat P13	23.7 \pm 0.4	16.7 \pm 0.2	17.6 \pm 0.2
Perennial Wheat P19	16.5 \pm 0.1	16.2 \pm 0.1	16.7 \pm 0.2
Perennial Rye	18.2 \pm 0.2	18.5 \pm 0.1	18.2 \pm 0.1

Figure 14. Light-saturated photosynthetic rate (A_{max}) as a function of source/sink ratio in annual wheat (AW), perennial wheat (PW), annual rye (AR), and perennial rye (PR), measured in Experiment 1 (2010). Photosynthetic rates (A_{max}) was measured 5-8 days after treatment imposition on two different dates, and averaged across dates. Three treatments are compared: Low (source/sink ratio = 67% of control), Control (100% of control) and High (200% of control). The symbols “*” and “**” represent differences from the control with $P < 0.05$ or $P < 0.01$ respectively. Data are from Experiment 1 (2010).

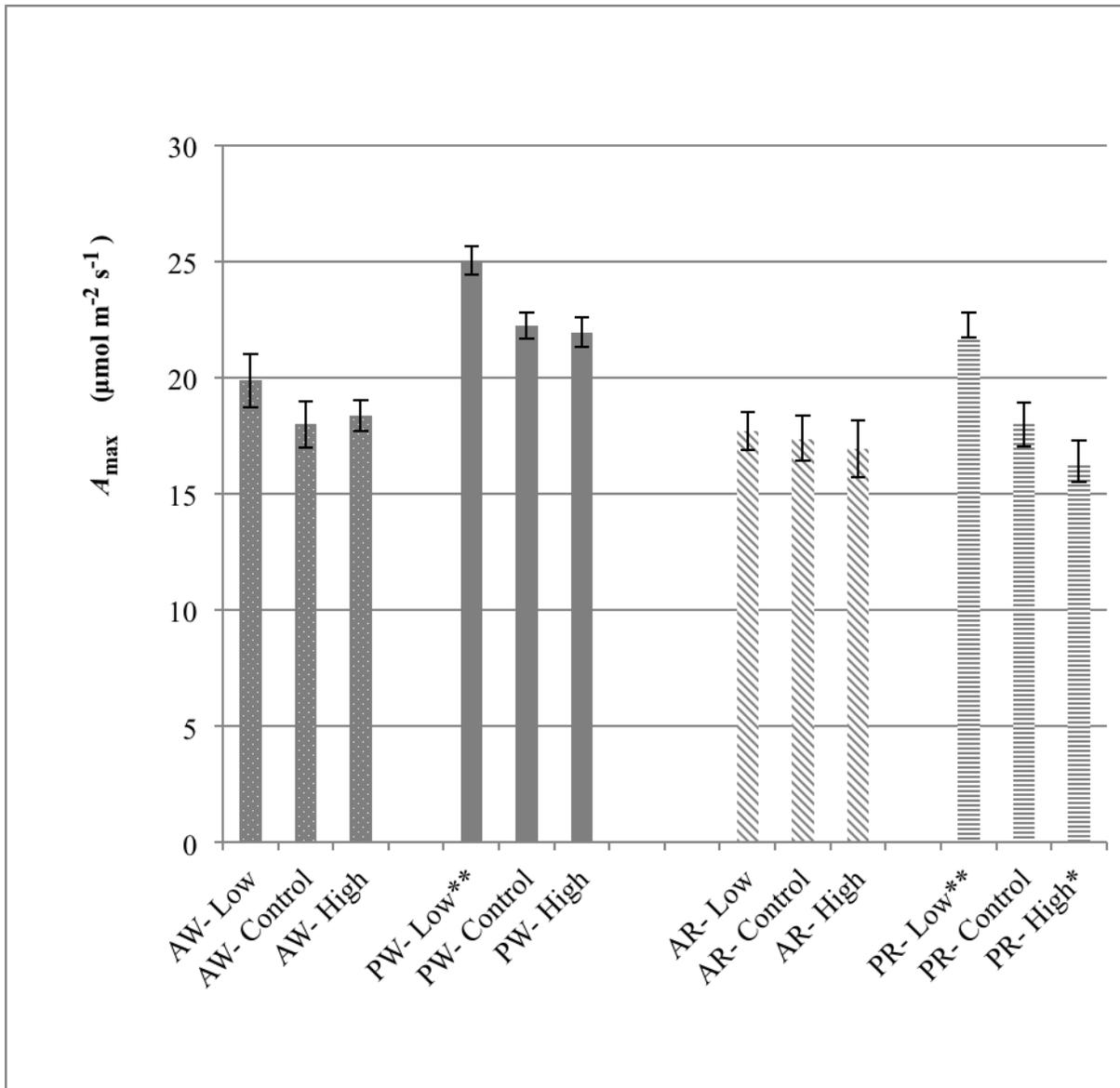


Figure 15. Light-saturated photosynthetic rate (A_{\max}) as a function of source/sink ratio in annual wheat (AW), perennial wheat (PW), annual rye (AR), and perennial rye (PR), measured in Experiment 2 (2011). Photosynthetic rates (A_{\max}) was measured 5-8 days after treatment imposition on two different dates, and averaged across dates. Three treatments are compared: Low (source/sink ratio = 67% of control), Control (100% of control) and High (200% of control). The symbols “*” and “**” represent differences from the control with $P < 0.05$ or $P < 0.01$ respectively.

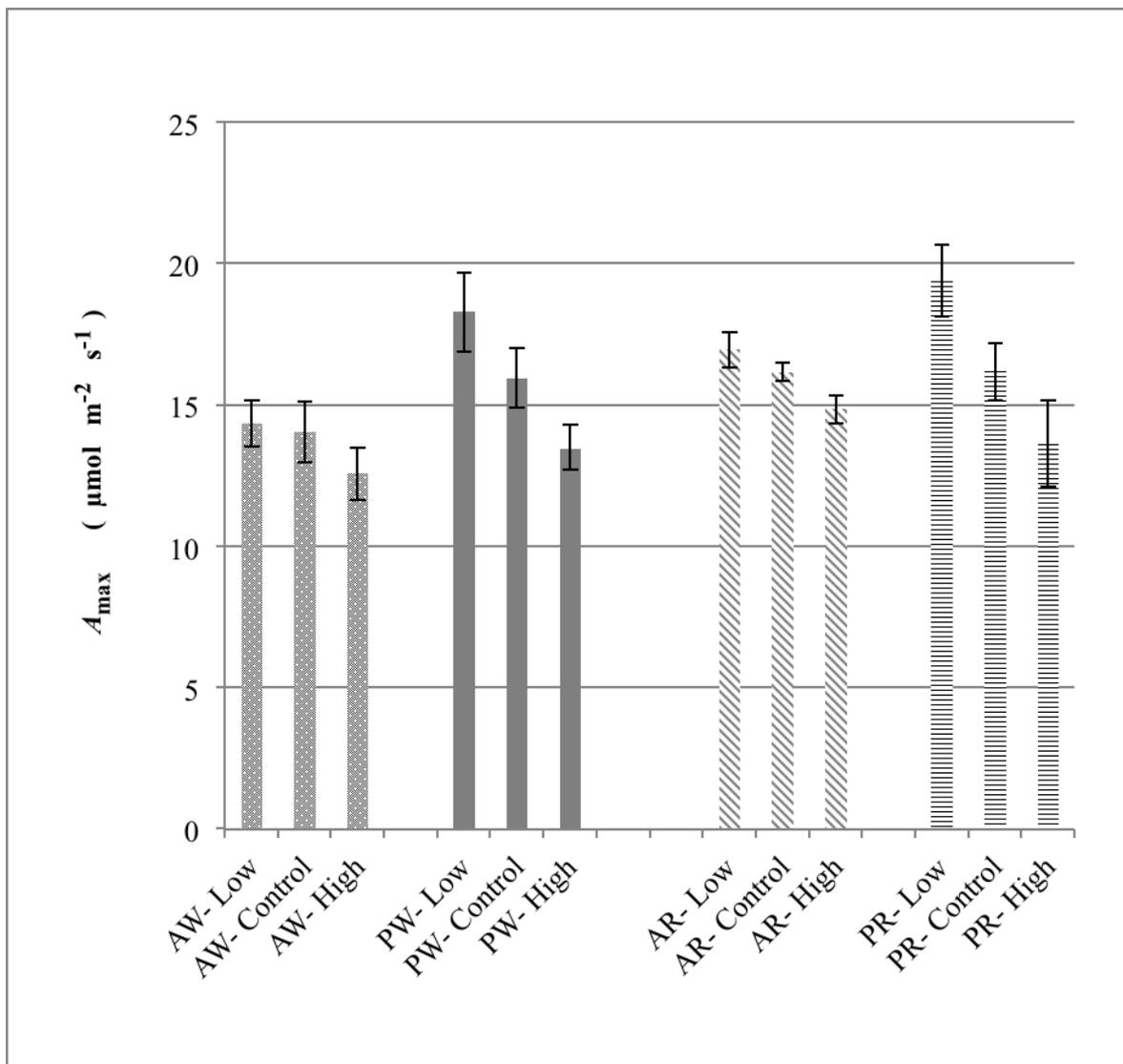


Figure 16. Leaf nonstructural carbohydrate content (NSC) as a function of source/sink ratio in annual wheat (AW), perennial wheat (PW), annual rye (AR) and perennial rye (PR), in a 2011 field experiment. Low (source/sink ratio = 67% of control), Control (100% of control) and High (200% of control). * and ** represent differences from the control with $P < 0.05$ or $P < 0.01$ respectively.

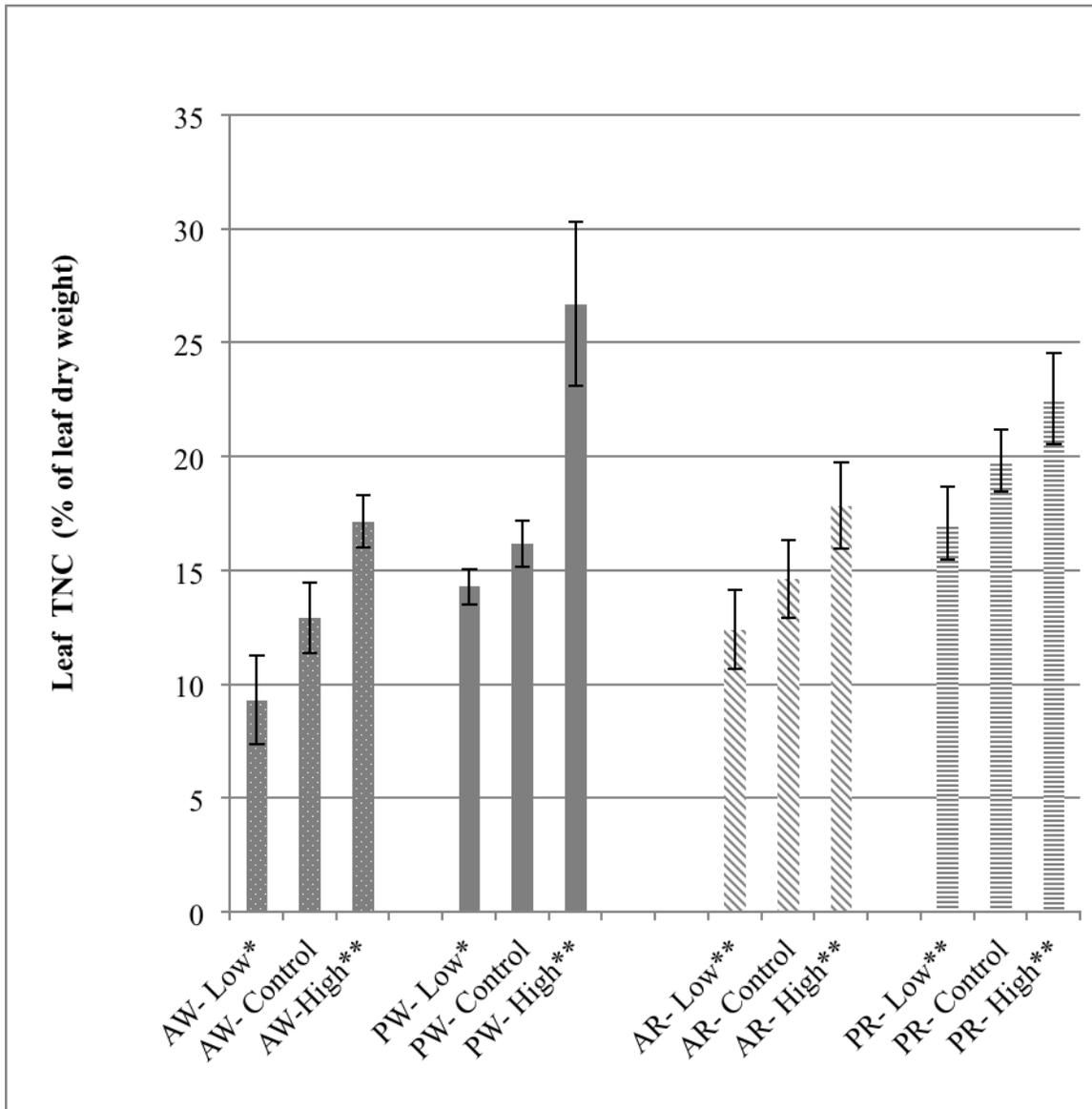


Figure 17. Stem nonstructural carbohydrate content (NSC) as a function of source/sink ratio in annual wheat (AW), perennial wheat (PW), annual rye (AR) and perennial rye (PR), in a 2011 field experiment. Low (source/sink ratio = 67% of control), Control (100% of control) and High (200% of control). * and ** represent differences from the control with $P < 0.05$ or $P < 0.01$ respectively.

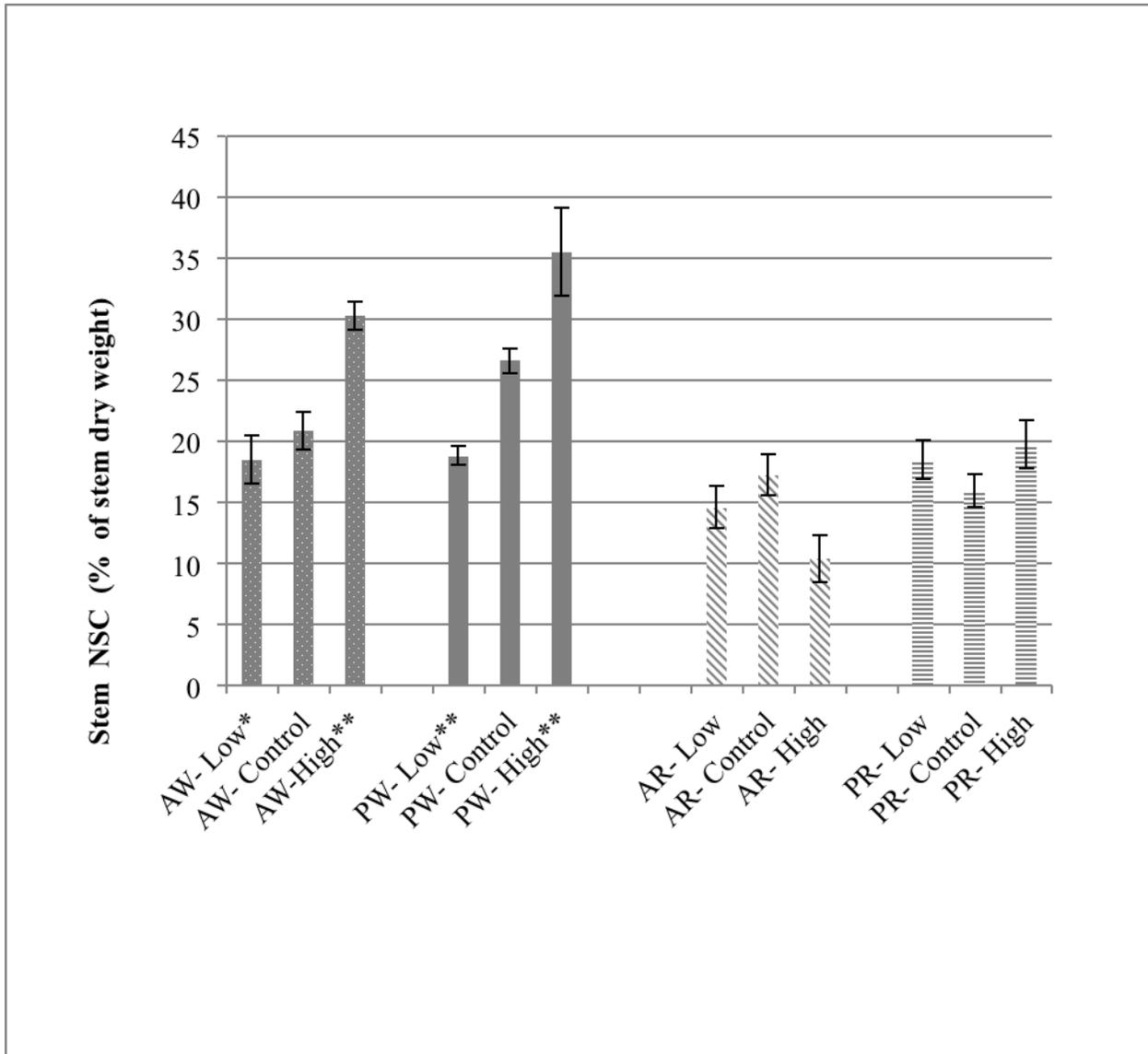


Figure 18. Kernel mass as a function of source/sink ratio in annual wheat (AW), perennial wheat (PW), annual rye (AR) and perennial rye (PR), in a 2010 field experiment. Kernel mass is compared between three treatments: Low (source/sink ratio = 67% of control), Control (100% of control) and High (200% of control), each averaged over treatments imposed at 2-3 time points. * and ** represent differences from the control with $P < 0.05$ or $P < 0.01$ respectively.

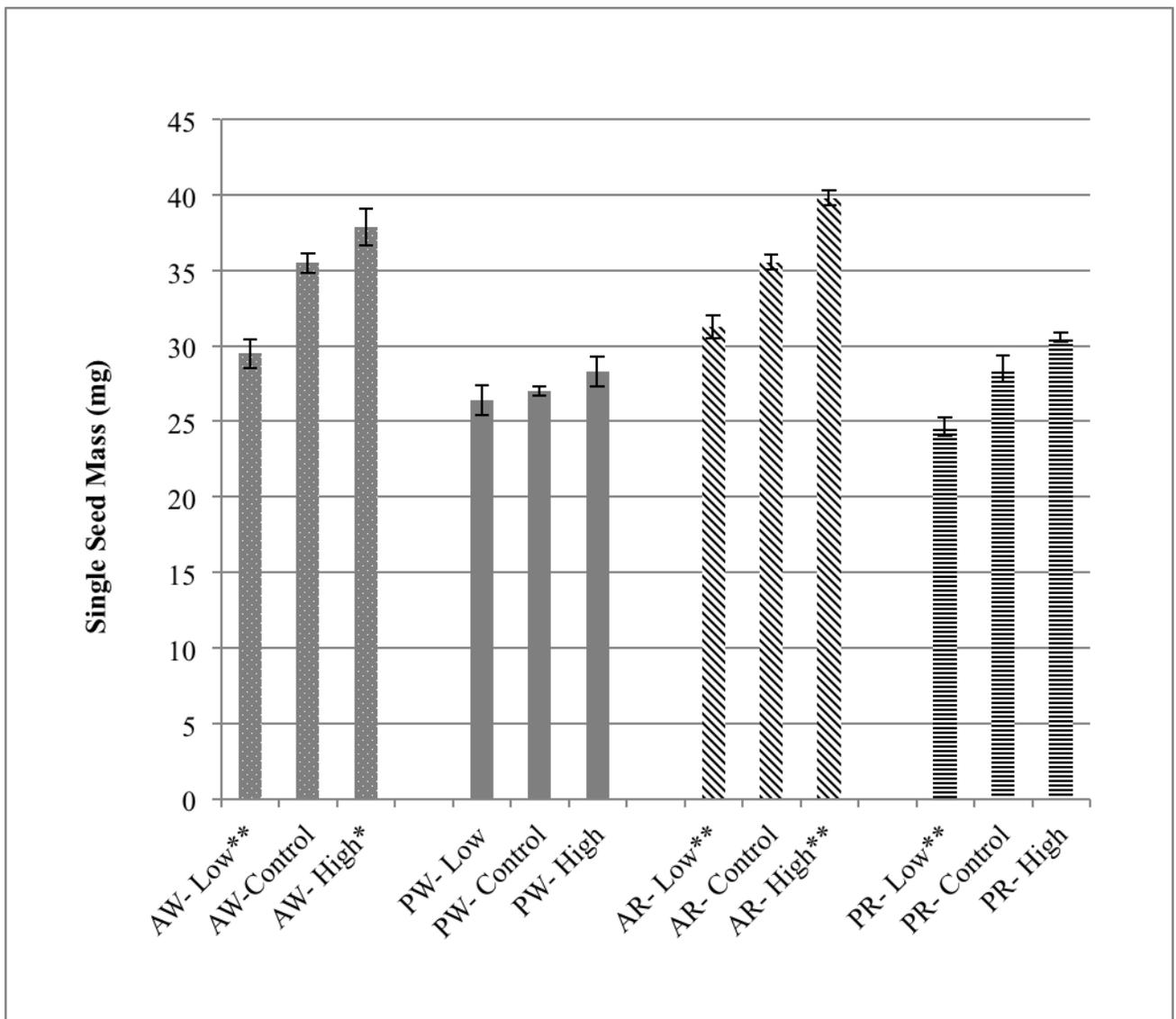
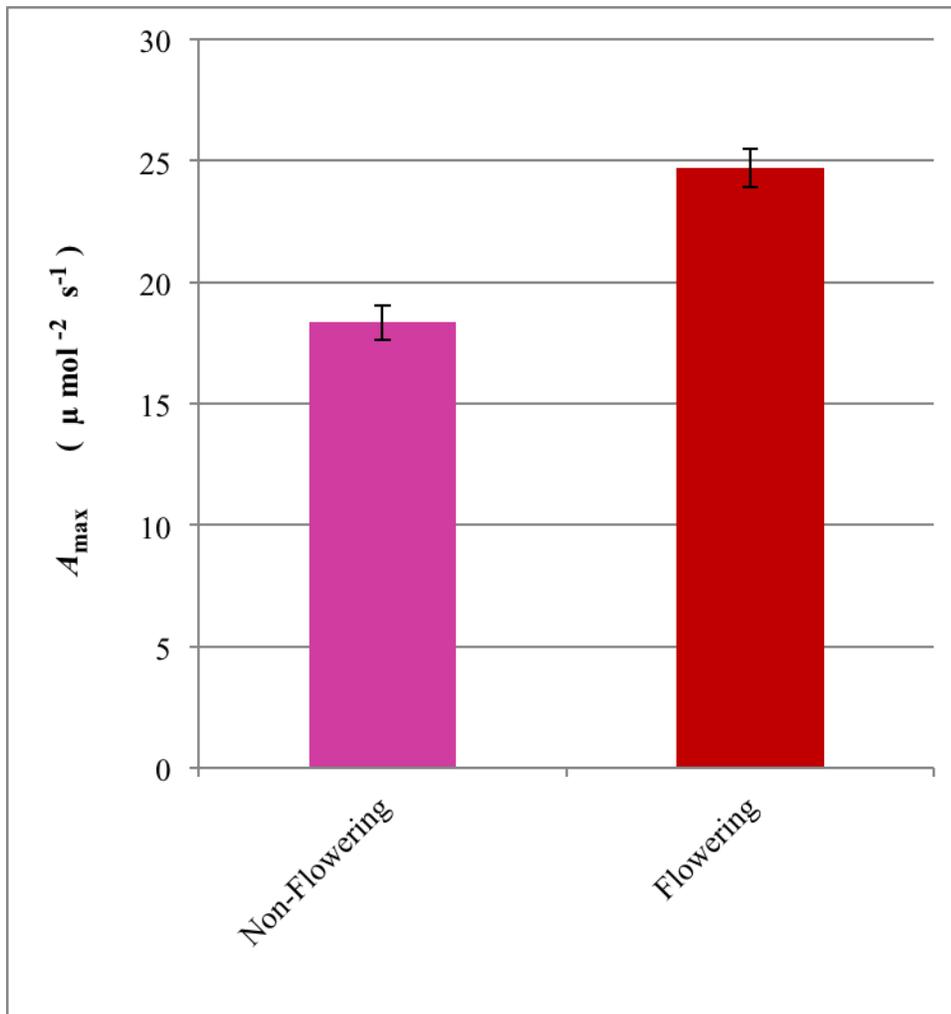


Figure 19. Light-saturated photosynthetic rate (A_{\max}) in flowering and non-flowering microplots of perennial wheat, during the fall post-sexual cycle regrowth period, in a 2011 field experiment. Pink and red represent flowering and non-flowering plants respectively.



APPENDIX B. Pictures of Perennial Cereal Experiments

Figure 20. Perennial wheat plots at late elongation stage, in a spring 2011 study of ecophysiological differences in annual and perennial cereals (Chapter 3, Experiment 2).



Figure 21. Perennial wheat plots at early elongation stage, in a spring 2011 study of ecophysiological differences in annual and perennial cereals (Chapter 3, Experiment 2).



Figure 22. Intermediate wheatgrass plants at early elongation stage, in a spring 2011 study of ecophysiological traits in intermediate wheatgrass (Chapter 3, Experiment 3).



Figure 23. “Low” treatment (source / sink ratio = 67% of control) in perennial wheat plants, imposed at boot stage, in a 2011 study of physiological responses to source/sink manipulation in annual and perennial cereals (Chapter 4, Experiment 2).



Figure 24. “High” treatment (source / sink ratio = 200% of control) in perennial rye plants, imposed at boot stage, in a 2011 study of physiological responses to source/sink manipulation in annual and perennial cereals (Chapter 4, Experiment 2).



Figure 25. Photosynthetic measurements in perennial wheat plants, in a 2011 study of physiological responses to source/sink manipulation in annual and perennial cereals (Chapter 4, Experiment 2).



REFERENCES

REFERENCES

- Ainsworth EA, Rogers A, Nelson R, Long SP. 2004. Testing the “source sink” hypothesis of downregulation of photosynthesis in elevated CO₂ in the field with single gene substitutions in *Glycine max*. *Agricultural and Forest Meteorology* 122: 85-94.
- Alvaro F, Royo C, del Moral LFG, Villegas D. 2008. Grain filling and dry matter translocation responses to source-sink modifications in a historical series of durum wheat. *Crop Science* 48: 1523-1531.
- Amthor JS. 2001. Effects of atmospheric CO₂ concentration on wheat yield: review of results from experiments using various approaches to control CO₂ concentration. *Field Crops Research* 73: 1-34.
- Andersen R, Sorensen A. 1999. An enzymatic method for the determination of fructans in foods and food products - Comparison of the results by high performance anion exchange chromatography with pulsed amperometric detection. *European Food Research and Technology* 210: 148-152.
- Arp WJ. 1991. Effects of source-sink relations on photosynthetic acclimation to elevated CO₂. *Plant, Cell & Environment* 14: 869-875.
- Borras L, Slafer GA, Otegui ME. 2004. Seed dry weight response to source-sink manipulations in wheat, maize and soybean: a quantitative reappraisal. *Field Crops Research* 86: 131-146.
- Cruz-Aguado JA, Reyes F, Rodes R, Peres I, Dorado M. 1999. Effect of source-to-sink ratio on partitioning of dry matter and ¹⁴C-photoassimilates in wheat during grain filling. *Annals of Botany* 83: 655-665.
- DaMatta FM, Cunha RL, Antunes WC, Martins SCV, Araujo WL, Fernie AR, Moraes GABK. 2008. In field-grown coffee trees source-sink manipulation alters photosynthetic rates, independently of carbon metabolism, via alterations in stomatal function. *New Phytologist* 178: 348-357.
- Fan PG, Li LS, Duan W, Li WD, Li SH. 2010. Photosynthesis of young apple trees in response to low sink demand under different air temperatures. *Tree Physiology* 30: 313-325.
- Flore JA, Layne DR. 1999. Photoassimilate production and distribution in cherry. *HortScience* 34: 1015-1019.
- Foyer C, Furbank R, Harbinson J, Horton P. 1990. The mechanisms contributing to photosynthetic control of electron transport by carbon assimilation in leaves. *Photosynthetic*

Research 25: 83-100.

Franck N, Vaast P, Genard M, Dauzat J. 2006. Soluble sugars mediate sink feedback down-regulation of leaf photosynthesis in field-grown *Coffea arabica*. *Tree Physiology* 26: 517-525.

Gaines MS, Vogt KJ, Hamrick JL, Caldwell J. 1974. Reproductive strategies and growth-patterns in sunflowers (*Helianthus*). *American Naturalist* 108: 889-894.

Gandin A, Gutjahr S, Dizengremel P, Lapointe L. 2011. Source sink imbalance increases with growth temperature in the spring geophyte *Erythronium americanum*. *Journal of Experimental Botany* 62: 3467-3479.

Grossman JM, Sheaffer C, Wyse D, Bucciarelli B, Vance C, Graham PH. 2006. An assessment of nodulation and nitrogen fixation in inoculated *Inga oerstediana*, a nitrogen-fixing tree shading organically grown coffee in Chiapas, Mexico. *Soil Biology and Biochemistry* 38: 769-784.

Gucci R, Xiloyannis C, Flore JA. 1991. Gas exchange parameters, water relations and carbohydrate partitioning in leaves of field-grown *Prunus domestica* following fruit removal. *Physiologia Plantarum* 83(3): 497-505.

Harrison M, Kelman WM, Moore AD, Evans JR. 2010. Grazing winter wheat relieves plant water stress and transiently raises photosynthesis. *Functional Plant Biology* 37: 726-736.

Helsel ZR, Thomas JW. 1987. Small grains for forage. *Journal of Dairy Science* 70: 2330-2338.

Hiyane R, Hiyane S, Tang AC, Boyer JS. 2010. Sucrose feeding reverses shade-induced kernel losses in maize. *Annals of Botany* 106: 395-403.

Huebner FR, Bietz JA, Nelsen T, Bains GS, Finney PL. 1999. Soft wheat quality as related to protein composition. *Cereal Chemistry* 76: 650-655.

Iglesias DJ, Lliso I, Tadeo FR, Talon M. 2002. Regulation of photosynthesis through source: sink imbalance in citrus is mediated by carbohydrate content in leaves. *Physiologia Plantarum* 116: 563-572.

Jaikumar N, Snapp SS, Murphy K, Jones S. 2012. A field assessment of the agronomic potential of two novel perennial cereal crops. *Agronomy Journal* In press.

Kasai M. 2008. Regulation of leaf photosynthetic rate correlating with leaf carbohydrate status and activation state of Rubisco under a variety of photosynthetic source/sink balances. *Physiologia Plantarum* 134: 216-228.

Karlsson PS. 1994. The significance of internal nutrient cycling in branches for growth and reproduction of *Rhododendron iaponicum*. *Oikos* 70: 191-200.

- Krapp A, Stitt M. 1995. An evaluation of direct and indirect mechanisms for the "sink regulation" of photosynthesis in spinach: Changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady state transcript levels after cold girdling source leaves. *Planta* 195: 315-323.
- Kuhbauch W, Thome U. 1989. Nonstructural carbohydrates of wheat stems as influenced by sink-source manipulations. *Journal of Plant Physiology* 134: 243-250.
- Layne DR, Flore JA. 1995. End-product inhibition of photosynthesis in *Prunus-cerasus* L. in response to whole-plant source-sink manipulation. *Journal of the American Society for Horticultural Science* 120: 583-599.
- Ma Y, Li J, Li X, Tang X, Liang Y, Huang S, Wang B, Liu H, Yang X. 2009. Phosphorus accumulation and depletion in soils in wheat–maize cropping systems: Modeling and validation. *Field Crops Research* 110: 207-212.
- Masnatta WJ, Ravetta DA. 2011. Seed-yield and yield components response to source sink ratio in annual and perennial species of *Lesquerella* (*Brassicaceae*). *Industrial Crops and Products* 34: 1393-1398.
- McCormick AJ, Cramer MD, Watt DA. 2006. Sink strength regulates photosynthesis in sugarcane. *New Phytologist* 171: 759-770.
- McCormick AJ, Cramer MD, Watt DA. 2008. Changes in photosynthetic rates and gene expression of leaves during a source-sink perturbation in sugarcane. *Annals of Botany* 101: 89-102.
- McCormick AJ, Cramer MD, Watt DA. 2008. Regulation of photosynthesis by sugars in sugarcane leaves. *Journal of Plant Physiology* 165: 1817-1829.
- Murphy KM, Lyon SR, Balow KA, Jones SS. 2010. Post-sexual cycle regrowth and grain yield in *Thinopyrum elongatum* x *Triticum aestivum* amphiploids. *Plant Breeding* 129: 480-483.
- Niva M, Svensson BM, Karlsson PS. 2003. Nutrient resorption from senescing leaves of the clonal plant *Linnaea borealis* in relation to reproductive state and resource availability. *Functional Ecology* 17: 438-444.
- Park SJ, Kim YK. 2011. Defruiting effect of young Fuyu persimmon (*Diospyros kaki*) on assimilate partitioning in-season and early growth the next season. *Scientia Horticulturae* 130: 197-202.
- Peng Y, Jiang GM, Liu XH, Niu SL, Liu MZ, Biswas DK. 2007. Photosynthesis, transpiration and water use efficiency of four plant species with grazing intensities in Hunshandak Sandland, China. *Journal of Arid Environments* 70: 304-315.
- Reekie EG, Bazzaz FA. 1987. Reproductive effort in plants. 3. Effect of reproduction on

vegetative activity. *American Naturalist* 129: 907-919.

Reynolds M, Foulkes MJ, Slafer GA, Berry P, Parry MAJ, Snape JW, Angus WJ. 2009. Raising yield potential in wheat. *Journal of Experimental Botany* 60: 1899-1918.

Reynolds MP, Pellegrineschi A, Skovmand B. 2005. Sink-limitation to yield and biomass: a summary of some investigations in spring wheat. *Annals of Applied Biology* 146: 39-49.

Rho CW, Cha YH, Kim TS, Kwon KC, Park YK. 1990. Effect of defoliation on seed development of rye and triticale cultivar. *Korean Journal of Crop Science* 35: 211-217.

Roumet C, Roy J. 1996. Prediction of the growth response to elevated CO₂: A search for physiological criteria in closely related grass species. *New Phytologist* 134: 615-621.

SAS Institute. 2008. *The SAS System for Windows*. 9.2. Cary, NC: SAS Institute, Inc.

Schaefer C, Simper H, Hofmann B. 1992. Glucose feeding leads to coordinated changes in chlorophyll content, ribulose 1,5-bisphosphate carboxylase-oxygenase activity, and photosynthetic activity in photoautotrophic suspension cultured cells of *Chenopodium rubrum*. *Plant, Cell and Environment* 15: 343-350.

Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL. 2007. Fitting photosynthetic carbon dioxide response curves for C₃ leaves. *Plant, Cell and Environment* 30: 1035-1040.

Urban L., Léchaudel M. 2005. Effect of leaf-to-fruit ratio on leaf nitrogen content and net photosynthesis in girdled branches of *Mangifera indica* L. *Trees- Structure and Function* 19(5): 564-571.

Usenik V, Orazem P, Stampar F. 2010. Low leaf to fruit ratio delays fruit maturity of 'Lapins' sweet cherry on Gisela 5. *Scientia Horticulturae* 126: 33-36.

Van Tassel DL, DeHaan LR, Cox TS. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications* 3: 434-452.

Vijn I, Smeekens S. 1999. Fructan: More than a reserve carbohydrate? *Plant Physiology* 120: 351-359.

Wullschlegel SD. 1993. "Biochemical limitations to carbon assimilation in C₃ plants: a retrospective analysis of the A / C_i curves from 109 species." *Journal of Experimental Botany* 44: 907-920.

Yan ST, Li XD, Li WD, Fan PG, Duan W, Li SH. 2011. Photosynthesis and chlorophyll fluorescence response to low sink demand of tubers and roots in *Dahlia pinnata* source leaves. *Biologia Plantarum* 55: 83-89.

Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth stages of cereals. *Weed Research* 14: 415-421.

Zhao M, Acuna TLB, Lafitte HR, Dimayuga G, Sacks E. 2008. Perennial hybrids of *Oryza sativa* x *Oryza rufipogon* - Part II. Carbon exchange and assimilate partitioning. *Field Crops Research* 106: 214-223.

Zhenlin W, Yanping Y, Mingrong H, Hongming C. 1998. Source-sink manipulation effects on postanthesis photosynthesis and grain setting on spike in winter wheat. *Photosynthetica* 35: 453-459.

Zhou R, Quebedeaux B. 2003. Changes in photosynthesis and carbohydrate metabolism in mature apple leaves in response to whole plant source-sink manipulation. *Journal of the American Society for Horticultural Science* 128: 113-119.

Zhu C, Zhu J, Zeng Q, Liu G, Xie Z, Tang H, Cao J, Zhao X. 2009. Elevated CO₂ accelerates flag leaf senescence in wheat due to ear photosynthesis which causes greater ear nitrogen sink capacity and ear carbon sink limitation. *Functional Plant Biology* 36: 291-299.

CHAPTER 5.
RESISTANCE TO FUSARIUM HEAD BLIGHT IN THREE PERENNIAL CEREAL
CROPS

Introduction

Novel perennial cereals are in the process of being developed as alternatives to annual grain crops (Cox et al. 2006). Promising candidates for the cold temperate zone include perennial wheat (*Triticum aestivum* x *Thinopyrum* spp.), perennial rye (*Secale cereale* x *montanum*), and intermediate wheatgrass (*Thinopyrum intermedium*). Perennial wheat and perennial rye have been developed by crossing annual wheat and rye with wild relatives (Acharya et al. 2004; Murphy et al. 2010). Intermediate wheatgrass is currently being domesticated through selection for high yield (Cox et al. 2006).

Because of their perennial life history, these emerging species face the challenge of pathogen accumulation, since these plants live for multiple years and could potentially represent a reservoir for disease. Some existing strategies to manage disease in annuals (e.g. crop rotation) cannot be used with perennials. One serious disease threat to perennial cereals is *Fusarium* head blight (scab, FHB) caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*: Xue et al. 2009). Winter survival of *Fusarium* on crop residues is the primary inoculum source (Zhang L. et al. 2011), which underscores the importance of selecting for FHB resistance in perennial cereal crops. FHB resistance can be categorized into Type I (resistance to initial infection), Type II (resistance to spread within an infected spike), Type III (resistance to kernel infection), Type IV (yield tolerance) and Type V (ability to degrade mycotoxins: Mesterházy 1995; Schroeder and Christensen 1963).

Previous greenhouse studies of FHB resistance in *Thinopyrum* and in *Thinopyrum* / wheat crosses indicate that the genus can contribute Type II resistance, though not all *Thinopyrum* / wheat crosses display resistance and there is high variability within the F1 generation (Cai et al.

2005). For example, approximately 25% of wheat-alien species derivatives (mostly *Thinopyrum* / wheat) in one study grouped with the ‘resistant’ checks ‘Sumai 3’, ‘Wangshuibai’ and ‘Alsen’ (Oliver et al. 2005) while in a later study 75% of derivatives showed equivalent resistance to ‘Sumai 3’ (Oliver et al. 2006). A further study of *Th. intermedium* / wheat derivatives found that one out of the seven studied, had better type II resistance than ‘Sumai 3’ (Han et al. 2003). *Th. intermedium* addition lines into a background of ‘Vilmorin’ wheat reduced average FHB disease ratings to 3.6 (on a 0-9 scale) compared to 4.6 for ‘Vilmorin’ and 2.8 for *Thinopyrum* (Gilbert et al. 1996). Similarly, a reduction of 75% to 90% in FHB as compared with the annual wheat parent followed the additions of *Th. elongatum* chromosomes within a background of susceptible ‘Chinese Spring’ (Wang et al. 2010).

Most studies of FHB resistance have not considered whether the crosses studied displayed perenniality or high seed yield: this makes them helpful for improving FHB resistance in annual wheat, but less so for understanding the potential for FHB resistance in a new perennial cereal species. Until now, no FHB resistance studies have been carried out on the set of *Thinopyrum* and *Thinopyrum* / wheat lines which have undergone intense selection over the last ten years for perennial regrowth as well as high seed production. Furthermore, to the best of our knowledge, none have been conducted on perennial rye. Because FHB resistance in *Thinopyrum* / wheat amphiploids has been shown to vary greatly among genotypes, it is important for breeding purposes to determine whether these specific lines show resistance, or whether selection for high yield in a low rainfall environment may have caused the loss of FHB resistance, through narrowing the genetic base and subsequent genetic drift. It is also possible, if *Thinopyrum*-derived FHB resistance is genetically linked to *Thinopyrum* QTLs that depress yield, that selecting for higher yield could result in lowered FHB resistance; it is also possible

that there may be direct metabolic tradeoffs between resistance and yield.

Our main goal in this study, which distinguishes it from previous studies of FHB in *Thinopyrum* / wheat amphiploids, was to determine whether a set of perennial cereal lines (*Thinopyrum* / wheat amphiploids, perennial rye, and *Thinopyrum intermedium*), that had undergone several cycles of selection for high yield and regrowth, would show the high levels of resistance comparable to those previously shown in some *Thinopyrum* / wheat amphiploids and in forage accessions of *Th. intermedium*. Secondly, our goal was to identify lines that are highly resistant to FHB, for breeding purposes. Our guiding hypotheses were that some of the perennial cereal lines would show type II resistance equal to the “Resistant” checks.

Materials and Methods

Experimental Design

We conducted two experiments to determine FHB resistance and susceptibility in a range of perennial cereal accessions. Experiment 1 examined responses to FHB inoculation in the greenhouse. Experiment 2 considered natural occurrence of FHB on plants growing in the field, during a year characterized by a cool and wet spring. Experiment 1 included more accessions than Experiment 2, and compared against both positive and negative controls (susceptible and resistant wheat lines): Experiment 2 included a subset of lines from Experiment 1, observed the prevalence of FHB on non-inoculated plants, and compared against a single, moderately susceptible annual wheat cultivar adapted to Michigan climate.

Experiment 1: Plant Materials

We used 17 genotypes in our study (Table 22). Four annual wheat checks were included, two resistant to FHB and two susceptible. Resistant checks included winter wheat ‘Truman’ (PI 634824; McKendry et al. 2005) and spring wheat ‘Sumai 3’ (PI 481542). Sumai 3 is currently considered a highly resistant wheat cultivar and the most widely used source of resistance traits for breeding purposes (Zhou et al. 2010). Susceptible checks included winter wheat ‘Ambassador’ (PI 656845; Lewis et al. 2010) and spring wheat ‘Wheaton’ (PI 469271; Busch et al. 1984). ‘Ambassador’ was a white wheat cultivar bred at MSU, which yielded 7% higher than the mean across 29 lines in a 2007 variety trial (Lewis J. M. unpublished data). ‘Truman’ was a soft red variety, relatively late maturing, bred at the University of Missouri and released in 2003; it yielded 6% lower than the mean across 29 lines in the aforementioned 2007 Michigan State variety trial.

Eleven *Thinopyrum* / wheat amphiploids, one perennial rye and one intermediate wheatgrass line were chosen to represent a wide range of ancestry.

- Four of these were chosen, in fall 2010, out of a preliminary screening of 15 *Thinopyrum* / wheat amphiploids developed at Washington State University (S.S. Jones and K.M. Murphy, personal communication, 2009). We selected four lines out of the 15 on the following basis: P10-2009 demonstrated the highest yield and P15-2009 the most vigorous PSCR, while P3-2009 and P5-2009 were moderate yielding lines. The parentage of these lines was *Thinopyrum elongatum* / *Triticum aestivum* cv. ‘Chinese Spring’ // *T. aestivum* cv. ‘Madsen’ /3/ *T. aestivum* / *Thinopyrum elongatum* cv. ‘Spitzer’ (Oliver et al. 2008).

- We included four *Thinopyrum* / wheat amphiploids from earlier materials obtained from WSU in 2005, numbered P3-2005, P11-2005, P15-2005, and P19-2005. These four were part of a set of 31 F5 lines tested at Pullman, WA in 2005 (Murphy et al. 2010) that had demonstrated the ability to perennialize in Michigan (Jaikumar et al., unpublished data). The parentage of these lines was *Thinopyrum elongatum* / *T. aestivum* cv. ‘Chinese Spring’ // *T. aestivum* cv. ‘Madsen’.
- Three perennial wheat accessions (LI-19, LI-47 and LI-48) were picked from a set of accessions developed at the Land Institute, (Salina, KS) in 2009. LI-19 was a hybrid of *Triticum durum* ‘Afuwan’ (PI 634318) x *Thinopyrum junceiforme* (PI 414667), while LI-47 and LI-48 were both derived from crosses between wheat (PI 386154) and pubescent wheatgrass (*Thinopyrum intermedium barbulatum*: DeHaan, L.R. personal communication). All three were selected from a larger set of lines, on the basis of demonstrated PSCR in a pilot trial. Thus the *Thinopyrum* / wheat amphiploids contained ancestry from three different *Thinopyrum* species.
- We included one perennial rye cultivar, *Secale cereale x montanum*, cv. ‘Rival’ (Peters Seed Company, Oregon, USA).
- Finally, we included one intermediate wheatgrass line (*Thinopyrum intermedium*). The wheatgrass, ‘TLI-C1’ was from a breeding population at the Land Institute following a cycle of selection for larger grain size (DeHaan LR, personal communication).

Experiment 1: Planting Protocol

All accessions were planted on 7 planting dates at the MSU East Lansing campus, every 4 days from November 1 through December 7, 2010. Every accession was planted on each planting date: the purpose was to allow a reasonable number of inoculation dates for each accession, so that each inoculation date could be treated as a replicate. Fifteen seeds of each accession were planted into an approximately 500 mL container filled with Perlite potting mix, and fertilized with around 10 g Osmocote slow-release pellets (14-14-14 NPK). After 10 days the pots were transported into a cold chamber for vernalization, and were maintained for 8 weeks at 6° C with 10.5 hours/day lighting.

Following vernalization, plants were removed and kept at room temperature (18° C) for 3 days, before being transplanted. Ten plants from each accession were selected and transplanted into 700 mL conical pots. These pots were fertilized with approximately 10 g of Osmocote pellets and then placed in the Michigan State University glasshouses (East Lansing, MI). Plants were subject to natural light through the sun roof and supplemented with artificial lighting for a total 16 hour day: they were watered daily and fertilized weekly with 300 ppm Peters Professional Water Soluble Fertilizer (NPK = 20:20:20) added to the water. Insects were controlled as needed following Michigan State University recommended protocol: tetraconazole was sprayed in early February, sulfur in early April and mid-April, and pyrometrazine in early May.

Overall plants grew between 40 – 90 cm tall and had multiple flowering stems. As an indication of overall plant vigor, chlorophyll readings (measured using the Minolta SPAD-502 chlorophyll meter) were comparable to plants growing in the field (readings of 50 – 55 on the

SPAD).

Experiment 1: Inoculation and Observation of Response

Inoculum was generated by culturing *Fusarium graminearum* isolate PH1 (NRRL31084) in Carboxymethyl Cellulose liquid medium (Cappelli and Peterson 1965). Ten microliters of stock culture was added to autoclaved media (100ml in 250 ml flasks). The culture was shaken at 200-250 rpm and 25° C for four days. Mycelia were removed by filtration with a sterile cheesecloth. Spores were counted using a haemocytometer and the culture was diluted to a final concentration of 5×10^5 spores/ml.

We used the single floret inoculation method to inoculate the plants (Schroeder and Christensen 1963; Mackintosh et al. 2007). Here a 'spike' refers to the entire inflorescence on a single wheat stalk; a 'spikelet' refers to one of the multiple smaller bundles of florets which make up a spike, protected by glumes; and a floret refers to an individual flower within a spikelet. Twelve inoculation dates were used, between mid-March and mid-May. On each date, plants at mid-anthesis were selected and one floret per plant (generally midway along the spike) was infected with 10 µL of inoculum containing *Fusarium graminearum* spores (5×10^5 spores / mL) using a micropipette. The inoculated spikes were then covered with plastic sandwich bags and tied shut to maintain a humid environment for 48 hours, after which the bags were removed and the plants monitored for disease expression.

After 14 days, plants were assessed for disease symptoms. The two parameters measured were spikelet infection (proportion of diseased spikelets/ total spikelets) and rachis infection (proportion of diseased rachis segments/ total rachis segments). Here a 'rachis segment' refers to

the length along the rachis between one spikelet and the adjacent spikelet. A spikelet, or a segment of rachis, was considered diseased if it showed brownish discoloration (this varied from light tan color to dark brown, compared with a greenish color for healthy tissue), or visible fungal growth. Any significant sign of brown discoloration was interpreted as infection. Infection was scored as a proportion between 0 and 1, e.g. number of diseased spikelets / number of total spikelets. Because only plants at mid-anthesis were selected, not every genotype is represented at each inoculation date. In general, between 0-5 plants per genotype were inoculated at each planting date (generally 2-3) and each plant was represented at between 6-11 inoculation dates (generally 7-9).

Experiment 1: Post-sexual Cycle Regrowth

In addition to quantifying FHB resistance among the accessions in our study, we also carried out a field trial to measure post-sexual cycle regrowth (PSCR). The purpose of this trial was to determine whether the highly FHB-resistant accessions in our study also showed good regrowth. Part of the goal of this experiment was to identify useful, FHB-resistant accessions for use in future breeding efforts in perennial wheat. However, in order to determine that these lines would be well suited for breeding efforts, it is necessary to establish that they show adequate levels of regrowth. We planted eight lines (those for which sufficient seed existed: Table 17) in a field trial to quantify PSCR. The area for planting was chisel plowed on Sept 29, 2010 and fertilized on Oct 11 with 100 kg ha^{-1} nitrogen in the form of poultry manure. A soil finisher was used to prepare plots on Oct 12, and on Oct 13 each line was planted at $1.75 \text{ million seeds ha}^{-1}$, in 2.30 m^2 plots, replicated 4 times, with a small plot planter (Allen Machine Company: Nevada,

IA). Weeding was carried out three times. Plots were harvested on Aug 1, 2011. On September 29, 2011, PSCR was visually evaluated by estimating the percentage of plants that showed green regrowth in the central two rows of a plot.

Experiment 2: FHB Symptoms on Naturally Infected Plants in the Field

In addition to our greenhouse experiment investigating Type II resistance, we also sought to observe the susceptibility of perennial cereals to FHB infection under non-treated, ‘natural’ field conditions. This was a smaller, observational study, supplementary to Experiment 1. Here we did not explicitly distinguish between Type I and Type II resistance, but rather observed the incidence, severity and disease index of FHB on non-inoculated, naturally infected plants.

In this experiment, we used the following subset of seven genotypes: the four lines received from Washington State University in 2009 (P3-2009, P5-2009, P10-2009, P15-2009), one line received in 2005 (P19-2005), the perennial rye (PR-Rival) and the intermediate wheatgrass accession (TLI-C1). As described above, they were chisel plowed in September 2010, fertilized with 100 kg ha^{-1} N, soil finished and then planted at $1.7 \text{ million seeds ha}^{-1}$ in a IBD design. The four lines P3-2009, P5-2009, P10-2009 and P15-2009 were replicated four times, while the other three test lines were replicated six times. The annual wheat cultivar against which perennial rye, intermediate wheatgrass and the wheat / *Thinopyrum* accessions were compared was the cultivar ‘Frankenmuth’, used as a benchmark in breeding studies at Michigan State University (Huebner et al., 1999; Lewis JM, personal communication). This was the same annual wheat cultivar used in Chapters 2-4 of this dissertation, and is considered to be moderately susceptible to FHB; it was replicated six times. Plots were weeded three times during the 2011 season. Anthesis date for each plot was observed through regular observations during

late spring and early summer, and generally varied between June 6 (for Frankenmuth) to July 4 (for intermediate wheatgrass).

FHB symptoms were assessed 21 days after anthesis on each plot. We randomly chose two ‘typical’ microplots within each plot, generally located towards the center, and assessed FHB symptoms on 10 plants in each microplot. Characteristic whitish to tan discoloration, or dark brown streaking similar to those seen on FHB inoculated plants in the greenhouse, were considered symptomatic of FHB. We recorded three parameters:

- Number of plants showing symptoms / number of total plants (*incidence*)
- Number of infected spikelets / number of total spikelets on each plant (*severity*)
- Incidence x average severity per plant (*disease index*).

Statistical Analysis

In Experiment 1, disease symptoms were analyzed as an incomplete block design with PROC MIXED in SAS, using a two-way ANOVA with genotype as a fixed factor and date as a random factor. Disease symptoms and PSCR were logit-transformed:

$$x' = \log [(0.1 + x) / (1.1 - x)].$$

Pairwise comparisons were made individually against the four annual wheat checks, using the Dunnett procedure. To increase power, the three other checks were omitted from the analysis during each comparison to a check. Statistical significance was assessed at $\alpha = 0.05$.

We considered a genotype “Resistant” if its levels of rachis and spikelet infection were both statistically similar to ‘Truman’ and ‘Sumai 3’ and different from ‘Ambassador’ and ‘Wheaton’. “Susceptible” genotypes had levels of rachis and spikelet infection similar to both ‘Ambassador’ and ‘Wheaton’ and different from ‘Truman’ and ‘Sumai 3’. Genotypes which were

statistically equivalent to a resistant check in one test and to a susceptible check in another, or were consistently different than both, were considered “Intermediate”. To determine whether PSCR was correlated with spikelet or rachis infection, we treated each genotype as one data point, and carried out a regression of first logit-transformed average spikelet infection and then logit-transformed average rachis infection (y') against logit-transformed PSCR (x'). Values reported in Table 1 and Fig. 1-2 are untransformed means.

In Experiment 2, disease index, severity and incidence were transformed using a logit transformation, and analyzed as an incomplete block design ANOVA, with genotype as a fixed factor and block as a random factor. Comparisons of each genotype against ‘Frankenmuth’ were made using Dunnett’s test, with $\alpha = 0.05$.

Results

Experiment 1: Overall ANOVA

Genotype strongly affected the percentage of infected spikelets in the greenhouse ($F = 15.35, p < 0.0001$) but inoculation date did not ($F = 1.44, p = 0.075$). Similarly the percentage of infected rachis was significantly affected by genotype ($F = 10.12, p < 0.0001$), but not by inoculation date ($F = 1.55, p = 0.060$).

Experiment 1: Resistant and Susceptible Lines

P3-2005, P11-2005, P15-2005 and P19-2005 all demonstrated a similar level of resistance to ‘resistant’ checks (Sumai 3 and Truman) in terms of spikelet infection (Table 23, Figure 26) and rachis infection (Table 24, Figure 27), as did *Thinopyrum intermedium* TLI-C1,

and LI-19. Two other lines, P10-2009 and P3-2009, grouped with the ‘susceptible’ checks. The other lines (Rival Rye, LI-47, LI-48, P15-2009 and P5-2009) generally showed intermediate levels of spikelet infection (Fig. 26) and rachis infection (Fig. 27), with P15-2009 being the lowest and P5-2009 the highest. The annual ‘resistant’ checks showed very low levels of rachis and spikelet infection (7-14%), while the ‘susceptible’ checks were in the 80-90% range. The one perennial rye genotype was not significantly different from the perennial wheat lines. The *Th. intermedium* line bred at the Land Institute for high grain production (TLI-C1) had extremely low FHB infection, comparable with the resistant wheat lines, and displayed excellent type II resistance (around 12% of spikelets infected). P15-2009 grouped with the resistant checks in terms of spikelet infection and with the intermediate group in terms of rachis infection, but otherwise the two parameters always agreed.

Experiment 1: Regrowth

PSCR ratings ranged from zero regrowth in three WSU-2009 lines to 100% in TLI-C1 (**Table 22**). As an exploratory procedure, we ran linear regressions of spikelet and rachis infection against PSCR to determine if there was a correlation. We found that spikelet infection was negatively correlated with PSCR, i.e. less disease symptoms was associated with greater PSCR. A 10% increase in the odds of a plant regrowing corresponded to a 4.5% decrease in the odds of a particular spikelet on that plant being infected ($y' = -0.454x' - 0.098; r^2 = 0.788, df = 6, p = 0.004$). Similarly, percentage rachis infection was negatively correlated with PSCR, with a 10% increase in the odds of a plant regrowing being associated with a roughly 5% decrease in the odds of a particular rachis segment being infected. For the 8 lines in our study, the observed relationship was $y' = -0.479x' - 0.095 (r^2 = 0.723, df = 6, p < 0.01)$. Lines P15-

2005, P19-2005 and TLI-C1 combined very high PSCR with high levels of resistance.

Experiment 2: Natural Occurrence of FHB in the Field

Incidence varied between genotypes ($p < 0.0001$) as did severity ($p = 0.049$) and disease index ($p = 0.0007$). Intermediate wheatgrass (TLI-C1) showed 86% lower incidence ($t = 3.87, p < 0.01$), 74% lower severity ($t = 4.74, p < 0.01$) and 79% lower disease index ($t = 4.02, p < 0.01$) than ‘Frankenmuth’, and was the only genotype to outperform ‘Frankenmuth’ on all three parameters. Perennial wheat accession P19-2005 had 86% lower incidence than ‘Frankenmuth’, but equal severity and disease index. The line P5-2009 had 360% greater incidence ($t = 7.35, p < 0.01$) and 380% greater disease index than ‘Frankenmuth’ ($t = 3.16, p < 0.05$), while perennial rye, P3-2009 and P10-2009 generally had similar disease symptoms. Thus, in partial corroboration of our results from the greenhouse, perennial rye was found to be moderately susceptible to FHB, while intermediate wheatgrass was highly resistant and perennial wheat accessions P19-2005 and P15-2009 were moderately resistant (Table 25).

Discussion

Our study found that six of the accessions studied (*Th. intermedium* ‘TLI-C1’, wheat / *Th. junceiforme*, and four wheat / *Thinopyrum elongatum* F5 lines, from 2005) showed resistance equivalent to the resistant checks. Other lines were highly or moderately susceptible. Our findings were consistent with studies that showed that addition or substitution of *Thinopyrum* chromosomes into annual wheat could confer FHB resistance (Oliver et al. 2005; Gilbert et al.

1996). We found high levels of FHB resistance in 6/11 of the hybrids, despite having undergone intense selection for high seed yield and perenniality in the field, within a low-FHB environment. Selection for yield, up to 2005, did not cause loss of FHB resistance.

Perennial rye showed intermediate resistance rather than grouping with the resistant checks. While no previous work has considered impacts of FHB on perennial rye, or of other diseases on this particular cultivar, studies do suggest the perennial rye cultivars ‘ACE-1’ and ‘Permontra’ have shown little susceptibility to disease in the field, with the notable exception of ergot (*Claviceps purpurea*: Acharya et al. 2004; Cox et al. 2005). In contrast to other common regional pathogens, FHB (along with ergot) appears from our study to be an important threat that perennial rye breeders must overcome.

Thinopyrum-derived resistance to FHB is strongly influenced by QTLs on chromosome 7e12 of *Th. elongatum* and *Th. intermedium*, as well as by chromosome 2 of *Th. intermedium*. The QTL on 7e12 is located between markers XBE445653 and Xcfa2240, shows partial dominance, and explains 30% of variation in the F2 generation (Shen et al. 2004; Shen and Ohm 2007; Zhang X. et al. 2011). There is evidence that QTLs on the homoeologous annual wheat chromosomes 7A, 7B or 7D condition susceptibility to FHB, based on substitution experiments (Fu et al. 2011). Chromosome 2D of *Th. intermedium* has also been found to contribute to FHB resistance in wheat / *Thinopyrum* amphiploids (Fedak and Han, 2005).

Neither of these two QTLs appears to be directly linked to the ability to perennialize, which is controlled by genes on the short arm of chromosome 4E from *Thinopyrum* (Lammer et al. 2004). It is unlikely, therefore, that FHB resistance has strong genetic links to the ability for post-sexual cycle regrowth. Resistance to FHB spread in wheat is influenced to some extent by QTLs on chromosomes 4A, B, and D, homoeologous to the 4E chromosome that confers

perenniality (Buerstmayr et al. 2009; Liu et al. 2009). Thus is it possible there might be some weak links between perenniality and regrowth, as our study indicated, that might be linked to the presence of chromosome 4E from *Thinopyrum*.

FHB resistance has, in the past, been linked to perennial growth habit in a range of wild Triticeae. Perennial *Hordeum* spp. ranked 0 or 1 (completely or highly resistant) on a 0-4 scale, while annual wild *Hordeum* spp. were all highly susceptible, and most cultivated *Hordeum* accessions tended to rank 2-3 on the scale. Perennial genera (e.g. *Agropyron*) predominated among the most resistant accessions, while the least resistant tended to be annual genera. Rather than propose a direct mechanistic link, however, the authors suggested that perenniality had been selected for under the same cool moist conditions that favored FHB, while annual Triticeae, associated with drier climates, had not been subject to selection for FHB resistance (Wan et al. 1997). Similarly, it is likely that in our study, the strongly perennial lines showed greater FHB resistance simply because they had more overall genetic material from *Thinopyrum*: as yet there is no data or theoretical reason to believe that genes controlling PSCR are closely linked to the most important QTLs involved in FHB resistance. It is unlikely that the traits are closely linked, and therefore selection for high PSCR in future may not necessarily result in high FHB resistance. Explicit screening for FHB resistance will be necessary to ensure good resistance in perennial wheat lines in future. We did find that intermediate wheatgrass accession TLI-C1, as well as the perennial wheat lines P15-2005 and P19-2005 all showed a combination of high FHB resistance (comparable to the 'Resistant' annual wheat checks) and high PSCR (above 80%). Thus these three accessions would seem to provide good parents for breeding efforts directed towards developing FHB-resistant, strongly perennial cereal lines. The perennial 'Rival' rye, however, shows moderately high susceptibility to FHB, thus using 'Rival' for future breeding

efforts may make it more difficult to achieve good levels of FHB resistance.

Our comparison of susceptibility to natural infection in the field has numerous limitations. Most importantly, a number of environmental and morphological factors could contribute to the higher resistance seen in intermediate wheatgrass and some perennial wheat lines. To begin with, there were pronounced phenological differences between flowering dates for annual wheat vs. the perennial species. Annual wheat flowered around June 6, approximately 7-14 days before first year perennial wheat, and 24 days before intermediate wheatgrass. June 2011 was characterized by generally warm and dry weather, and thus it is likely that spikes were generally drier around the time of perennial wheat and intermediate wheatgrass flowering compared to annual wheat. Although plant height was not measured, casual observations also suggested that perennial wheat and wheatgrass were taller than annual wheat: as plant height is associated with resistance to FHB, this could have contributed to lower infection in the perennial species. Finally, the perennial species generally had tougher and thicker glumes (much tougher in the case of intermediate wheatgrass, such that threshing was very difficult, and somewhat tougher in the case of perennial wheat). Tougher, more recalcitrant glumes that wrapped more tightly around the floret could also present a physical barrier to FHB infection. In addition, there might be physiological mechanisms that suppress the spread of FHB infection. Thus it is impossible at this point to disentangle phenological, morphological and physiological factors that could all help explain differential susceptibility to FHB between annual wheat, perennial wheat and intermediate wheatgrass.

Selection for high yield and PSCR in *Thinopyrum* / wheat amphiploids over several generations does not appear, up to 2005, to have selected against FHB resistance. However, the set of lines from WSU in 2009 were substantially less resistant, suggesting that resistance may

have been lost over the four cycles of breeding after 2005. It is unlikely that the perennial parents had low resistance in the first place, as *Thinopyrum* spp. have been reliably used as a source of resistance traits in the past, and observational evidence from wheatgrass breeders in Kansas (DeHaan LR, personal communication) suggest that FHB disease pressure is low. The decline in resistance hints that further selection for yield in FHB-free environments could result in the loss of FHB resistance, and that further breeding efforts should screen for FHB resistance through inoculation assays. The positive correlation between FHB resistance and PSCR suggests that selection for increased perenniality in future could favour increased FHB resistance. The very high levels of resistance found in some perennial cereal lines offer hopeful indications that perennial wheat and wheatgrass could be grown successfully as perennial food grains even in environments prone to infection by FHB.

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APPENDIX

Tables and Figures for Chapter 5

Table 22. Genotypes involved in a 2010-2011 greenhouse study of Type II resistance to Fusarium Head Blight (East Lansing, MI). Annual wheat checks and perennial cereal test lines are included. ‘Amphiploid test lines’ include all lines with perennial grass ancestry.

<i>Annual Wheat Checks</i>				
Line Name	Life History	Resistance	Reference	
Sumai	Annual spring	Resistant	Bai and Suenaga, 2000	
Wheaton	Annual spring	Susceptible	Busch et al., 1984	
Truman	Annual winter	Resistant	McKendry <i>et al.</i> , 2005	
Ambassador	Annual winter	Susceptible	Lewis <i>et al.</i> , 2010	
<i>Amphiploid Test Lines</i>				
Line Name	Life Form	Perennial Ancestry	PSCR	Reference
TLI-C1	Perennial wheatgrass ^a	100%	1.00	DeHaan <i>et al.</i> , 2010
Rival Rye	Rye amphiploid	50%	0.86	***
LI-48	Wheat amphiploid	50%	0.2*	DeHaan <i>et al.</i> , 2010
LI-47	Wheat amphiploid	50%	0.1*	DeHaan <i>et al.</i> , 2010
LI-19	Wheat amphiploid	50%	0.4*	DeHaan <i>et al.</i> , 2010
P3-2009	Wheat amphiploid	37.5%	0.00	Murphy, K.M. (unpublished)
P5-2009	Wheat amphiploid	37.5%	0.20	Murphy, K. M. (unpublished)
P10-2009	Wheat amphiploid	37.5%	0.00	Murphy, K. M. (unpublished)
P15-2009	Wheat amphiploid	37.5%	0.76	Murphy K. M. (unpublished)
P3-2005	Wheat amphiploid	25%	0.68**	Murphy <i>et al.</i> , 2010
P11-2005	Wheat amphiploid	25%	0.64**	Murphy <i>et al.</i> , 2010
P15-2005	Wheat amphiploid	25%	0.90	Murphy <i>et al.</i> , 2010
P19-2005	Wheat amphiploid	25%	0.80	Murphy <i>et al.</i> , 2010

*Unreplicated observation from 1 m row.

**Data obtained from separate pilot study in 2007-2008.

*** Obtained from Peters Seed Company, Oregon, USA.

Table 23. Results from comparing percent of *Fusarium*-infected spikelets in various perennial grass and grass-cereal amphiploid lines to annual susceptible and resistant controls. Data were taken from a 2010-2011 greenhouse study of susceptibility to *Fusarium* head blight (FHB) in perennial cereals, conducted in East Lansing, MI.

* (H) indicates significantly higher than the indicated control

* (L) indicates significantly lower than the indicated control

n.s. indicates no significant difference from the indicated control (at $\alpha = 0.05$).

“Resistant”, “Susceptible” and “Intermediate” classification are as defined in “Methods” section.

Genotype	vs. Ambassador	vs. Wheaton	vs. Truman	vs. Sumai 3	Overall Rating
TLI-1	* (L)	* (L)	n.s.	n.s.	Resistant
P3-2005	* (L)	* (L)	n.s.	n.s.	Resistant
P11-2005	* (L)	* (L)	n.s.	n.s.	Resistant
P15-2005	* (L)	* (L)	n.s.	n.s.	Resistant
P19-2005	* (L)	* (L)	n.s.	n.s.	Resistant
P3-2009	n.s.	n.s.	* (H)	* (H)	Susceptible
P5-2009	* (L)	n.s.	* (H)	* (H)	Intermediate
P10-2009	n.s.	n.s.	* (H)	* (H)	Susceptible
P15-2009	* (L)	* (L)	n.s.	n.s.	Resistant
LI-19	* (L)	* (L)	n.s.	n.s.	Resistant
LI-47	* (L)	* (L)	* (H)	* (H)	Intermediate
LI-48	* (L)	* (L)	* (H)	* (H)	Intermediate
Rival Rye	* (L)	n.s.	* (H)	* (H)	Intermediate

Table 24. Results from comparing percent of *Fusarium*-infected rachis segments in various perennial grass and grass-cereal amphiploid lines to annual susceptible and resistant controls. Data were taken from a 2010-2011 greenhouse study of susceptibility to *Fusarium* head blight (FHB) in perennial cereals, conducted in East Lansing, MI.

* (H) indicates significantly higher than the control

* (L) indicates significantly lower than the control

n.s. indicates no significant difference from the control (at $\alpha = 0.05$).

“Resistant”, “Susceptible” and “Intermediate” classification are as defined in “Methods” section.

Genotype	vs. Ambassador	vs. Wheaton	vs. Truman	vs. Sumai 3	Overall Rating
TLI-1	* (L)	* (L)	n.s.	n.s.	Resistant
P3-2005	* (L)	* (L)	n.s.	n.s.	Resistant
P11-2005	* (L)	* (L)	n.s.	n.s.	Resistant
P15-2005	* (L)	* (L)	n.s.	n.s.	Resistant
P19-2005	* (L)	* (L)	n.s.	n.s.	Resistant
P3-2009	n.s.	n.s.	* (H)	* (H)	Susceptible
P5-2009	* (L)	n.s.	* (H)	* (H)	Intermediate
P10-2009	n.s.	n.s.	* (H)	* (H)	Susceptible
P15-2009	* (L)	n.s.	n.s.	* (H)	Intermediate
LI-19	* (L)	* (L)	n.s.	n.s.	Resistant
LI-47	* (L)	n.s.	* (H)	* (H)	Intermediate
LI-48	* (L)	n.s.	* (H)	* (H)	Intermediate
Rival Rye	* (L)	n.s.	n.s.	n.s.	Intermediate

Table 25. Means (\pm SE) and *F*-values (*p*-values in parentheses) from comparing incidence, severity and total disease index of *Fusarium graminearum* infection on seed heads of perennial rye (Rival Rye), intermediate wheatgrass (TLI-C1) and five wheat / *Thinopyrum* amphiploids, growing in the field under natural and non-inoculated conditions. Data were taken from a 2010-2011 greenhouse study of susceptibility to *Fusarium* head blight (FHB) of perennial cereals in the field, conducted in Hickory Corners, MI. All lines were compared against the annual wheat check, *Triticum aestivum* cv. Frankenmuth’.

Accession	Incidence	Severity	Disease Index
<i>Frankenmuth</i>	5.8 \pm 0.8	53.1 \pm 6.4	3.4 \pm 0.4
TLI-C1	0.8 \pm 0.8	14.0 \pm 14.0	0.7 \pm 0.7
P19-2005	0.8 \pm 0.8	16.7 \pm 16.7	0.8 \pm 0.8
P3-2009	30.0 \pm 11.7	48.2 \pm 8.0	16.0 \pm 7.7
P5-2009	21.2 \pm 2.4	61.1 \pm 14.2	13.1 \pm 3.5
P10-2009	18.8 \pm 4.3	33.9 \pm 6.7	6.2 \pm 1.9
P15-2009	7.5 \pm 7.5	10.0 \pm 10.0	3.0 \pm 3.0
Rival Rye	3.0 \pm 2.0	46.5 \pm 20.9	7.0 \pm 7.0
Sources of Variation			
Species	9.76 (0.0001)	2.38 (0.049)	5.28 (0.0007)
Block	1.04 (0.15)	0.79 (0.22)	0.74 (0.23)

Figure 26. Percent of *Fusarium*-infected spikelets in 13 perennial grass and grass-cereal amphiploid lines, compared against susceptible and resistant annual wheat controls. Data were taken from a 2010-2011 greenhouse study of susceptibility to *Fusarium* head blight (FHB) in perennial cereals, conducted in East Lansing, MI. Pink = susceptible annual wheat lines, red = resistant annual wheat, blue = *Thinopyrum intermedium*, dark green = wheat x *Th. junceiforme*, green = wheat x *Th. intermedium*, light green = wheat x *Th. elongatum*, brown = perennial rye.

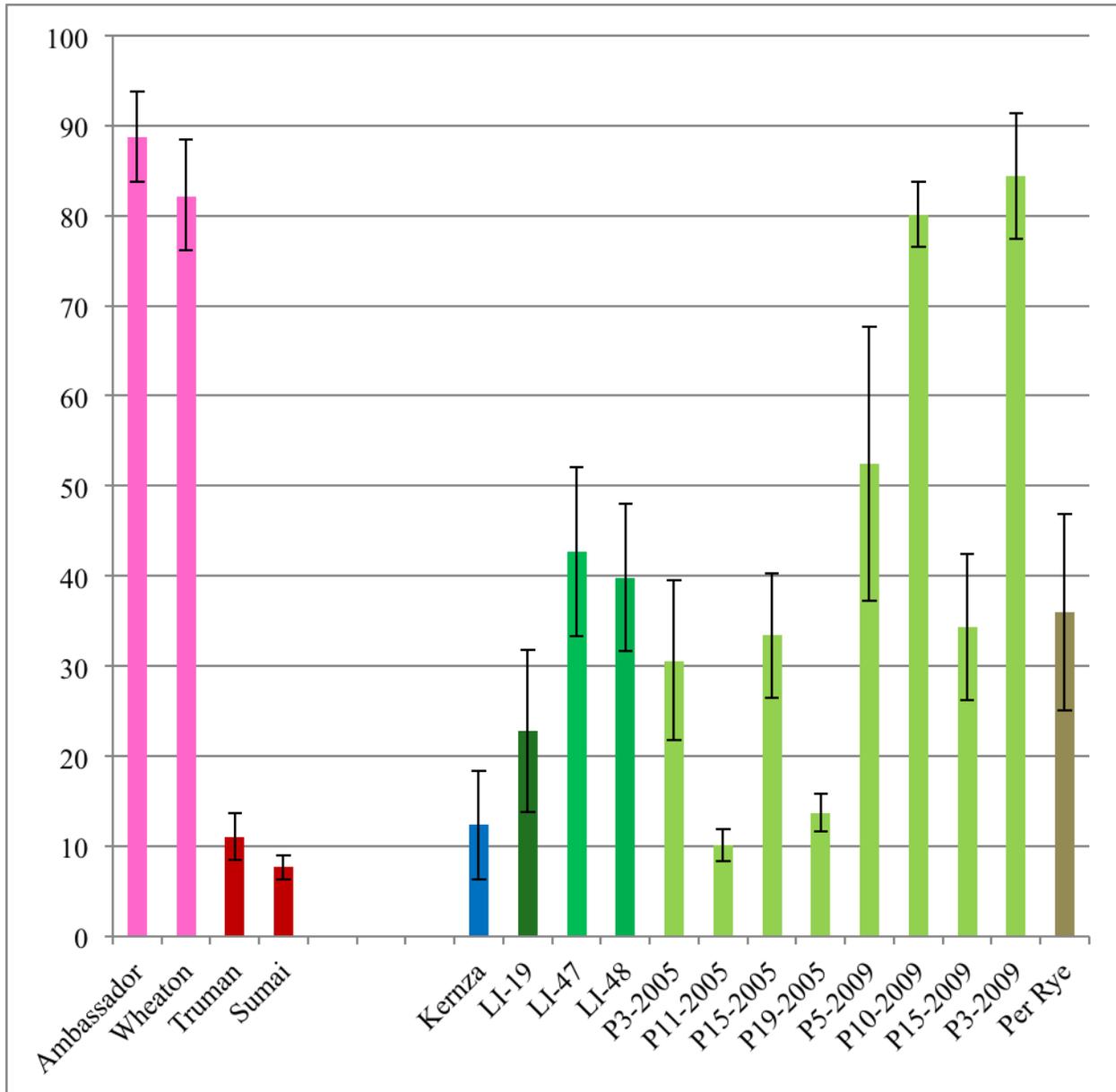
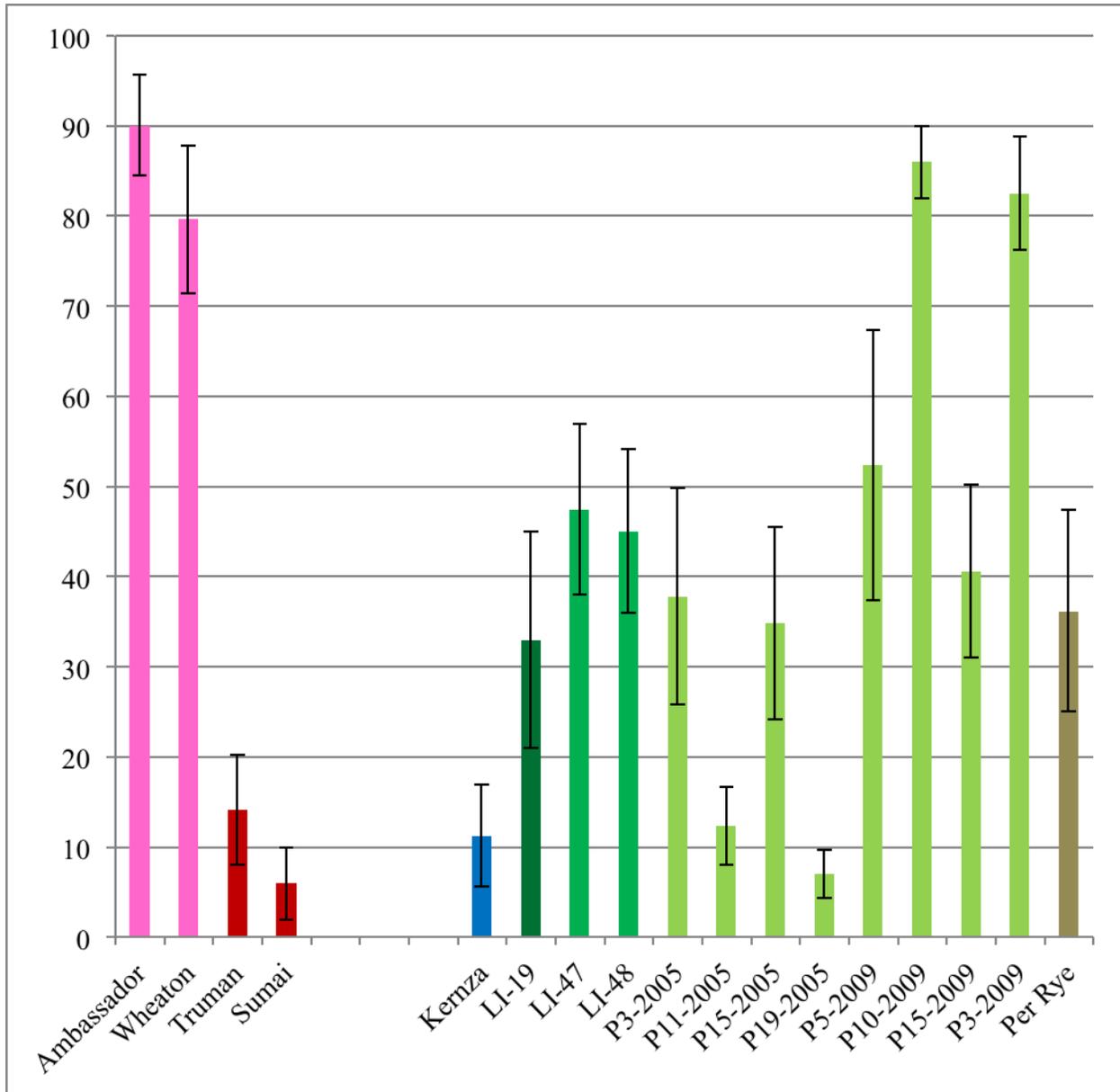


Figure 27. Percent of *Fusarium*-infected rachis segments in 13 perennial grass and grass-cereal amphiploid lines, compared against susceptible and resistant annual wheat controls. Data were taken from a 2010-2011 greenhouse study of susceptibility to *Fusarium* head blight (FHB) in perennial cereals, conducted in East Lansing, MI. Pink = susceptible annual wheat lines, red = resistant annual wheat, blue = *Thinopyrum intermedium*, dark green = wheat x *Th. junceiforme*, green = wheat x *Th. intermedium*, light green = wheat x *Th. elongatum*, brown = perennial rye.



REFERENCES

REFERENCES

- Acharya SN, Mir Z, Moyer JR, Orshinsky BR, Thomas JE. 2003. Effect of row spacing and seeding date on forage yield and quality of perennial cereal rye (*Secale cereale* L.). *Canadian Journal of Plant Science* 83: 363-369.
- Acharya SN, Mir Z, Moyer JR. 2004. ACE-1 perennial rye. *Canadian Journal of Plant Science* 84: 819-821.
- Bai G, Shaner G. 2004. Management and resistance in wheat and barley to *Fusarium* head blight. *Annual Review of Phytopathology* 42: 135-161.
- Ban T, Suenaga K. 2000. Genetic analysis of resistance to *Fusarium* head blight caused by *Fusarium graminearum* in Chinese wheat cultivar 'Sumai 3' and the Japanese cultivar 'Saikai 16S'. *Euphytica* 113: 87-99.
- Busch R, McVey D, Rauch T, Baumer J, Elsayed F. 1984. Registration of 'Wheaton' wheat. *Crop Science* 24: 622.
- Buerstmayr H, Ban T, Anderson, JA. 2009. QTL mapping and marker-assisted selection for *Fusarium* head blight resistance in wheat: a review. *Plant Breeding* 128: 1-26.
- Cai X, Chen PD, Xu SS, Oliver RE, Chen X. 2005. Utilization of alien genes to enhance *Fusarium* head blight resistance in wheat- A review. *Euphytica* 142: 309-318.
- Capelli RA, Peterson JL. 1965. Macroconidium formation in submerged cultures by a non-sporulating strain of *Gibberella zeae*. *Mycologia* 57: 962-966.
- Cox TS, Bender M, Picone C, Van Tassel DL, Holland JB, Brummer EC, Zoeller BE, Paterson, AH, Jackson W. 2002. Breeding perennial grain crops. *Critical Reviews in Plant Science* 21: 59-91.
- Cox CM, Murray TD, Jones SS. 2002. Perennial wheat germ plasm lines resistant to eyespot, *Cephalosporium* stripe, and wheat streak mosaic. *Plant Disease* 86: 1043-1048.
- Cox CM, Garrett KA, Cox TS, Bockus WW, Peters T. 2005. Reactions of perennial grain accessions to four major cereal pathogens of the Great Plains. *Plant Disease* 89: 1235-1240.
- Cox TS., Glover JD., Van Tassel DL., Cox CM., DeHaan, LR. 2006. Prospects for developing perennial grain crops. *BioScience* 56: 649-659.
- DeHaan LR, Van Tassel DL, Cox TS. 2005. Perennial grain crops: a synthesis of ecology and plant breeding. *Renewable Agriculture and Food Systems* 20: 5-14.

- Fedak G, Han F. 2005. Characterization of derivatives from wheat-*Thinopyrum* wide crosses. *Cytogenetic and Genome Research* 109: 360-367.
- Fu S, Lu Z, Qi B, Guo X, Li J, Liu B, Han F. 2011. Molecular cytogenetic characterization of wheat-*Thinopyrum elongatum* addition, substitution and translocation lines with a novel source of resistance to wheat Fusarium Head Blight (FHB). *Journal of Genetics and Genomics* 39: 103-110.
- Gilbert J, Fedak G, Procunier JD, Aung T, Tekauz A. 1996. "Strategies for Breeding for Resistanceto FHB in Canadian Spring Wheat." In "Fusarium Head Scab: Global Status and Future Prospects." Proceedings of a Workshop Held at CIMMYT, El Batan, Mexico, 13-17 Oct 1996.
- Han FP, Fedak G, Benabdelmouna A, Armstrong K, Oullet T. 2003. Characterization of six wheat x *Thinopyrum intermedium* derivatives by GISH, RFLP, and multicolor GISH. *Genome* 46: 490-495.
- Khavkin EE, Zabrodina MV, and Sillis DY. 1996. Isozymes of aspartate aminotransferase in perennial and annual rye and their hybrids. *Genome* 39: 513-519.
- Lammer D, Cai X, Arterburn M, Chatelain J, Murray T and Jones S. 2004. A single chromosome addition from *Thinopyrum elongatum* confers a polycarpic, perennial habit to annual wheat. *Journal of Experimental Botany* 55: 1715-1720.
- Lewis JM, Siler L, Souza E, Ng, PKW, Dong Y, Brown-Guedira G, Jiang G-L, Ward RW. 2010. Registration of 'Ambassador' wheat. *Journal of Plant Registrations* 4: 195-204.
- Li H, Wang X. 2009. *Thinopyrum ponticum* and *Th. intermedium*: promising sources of resistance to fungal and viral diseases of wheat. *Journal of Genetics and Genomics* 36: 557-565.
- Liu S, Hall M.D., Griffey, C. A., and McKendry, A. L. 2009. Meta-analysis of QTL associated with *Fusarium* head blight resistance in wheat. *Crop Science* 49: 1955-1968.
- Mackintosh CA, Lewis J, Radmer LE, Shin S, Heinen SJ, Smith LA, Wycoff MN, Dill-Macky R, Evans CK, Kravchenko S, Baldrige GD, Zeyen RJ, Muehlbauer GJ. 2007. Overexpression of defense response genes in transgenic wheat enhances resistance to *Fusarium* head blight. *Plant Cell Reports* 26: 479-488.
- McKendry AL, Tague DN, Wright RL, Tremain JA, Conley SP. 2005. Registration of 'Truman' wheat. *Crop Science* 45: 421-423.
- Mesterházy A. 1995. Types and components of resistance to *Fusarium* head blight of wheat. *Plant Breeding* 114: 377-386.
- Mesterházy A, Bartók T, Lamper, C. 2003. Influence of wheat cultivar, species of *Fusarium*, and isolate aggressiveness on the efficacy of fungicides for control of *Fusarium* head blight. *Plant*

Disease 87: 1107-1115.

Okubara PA, Jones SS. 2011. Seedling resistance to *Rhizoctonia* and *Pythium* spp. in wheat chromosome group 4 addition lines from *Thinopyrum* spp. *Canadian Journal of Plant Pathology* 33: 416-423.

Oliver RE, Cai X, Xu SS, Chen X, Stack R. 2005. Wheat-alien species derivatives: a novel source of resistance to *Fusarium* head blight in wheat. *Crop Science* 45: 1353-1360.

Oliver RE, Xu SS, Stack RW, Friesen TL, Jin Y and Cai X. 2006. Molecular cytogenetic characterization of four partial wheat- *Thinopyrum ponticum* amphiploids and their reactions to *Fusarium* head blight, tan spot, and *Stagonospora nodorum* blotch. *Theoretical and Applied Genetics* 112: 1473-1479.

Oliver RE, Cai X, Wang R-C, Xu SS, Friesen TL. 2008. Resistance to tan spot and *Stagonospora nodorum* blotch in wheat-alien species derivatives. *Plant Disease* 92:150-157.

Pirgozliev SR, Ray RV, Edwards SG, Hare MC, and Jenkinson P. (2010). Effect of timing of fungicide application on the development of *Fusarium* head blight and the accumulation of deoxynivalenol (DON) in winter wheat grain. *Cereal Research Communications* 36: 289-299

Pritsch C, Muehlbauer GJ, Bushnell WR, Somers DA, Vance CP. 2000. Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. *Molecular Plant-Microbe Interactions* 13: 159-169.

Reimann-Philipp, R. 1986. Perennial spring rye as a crop alternative. *Journal of Agronomy and Crop Science* 157: 281-285.

Scheinost PL, Lammer DL, Cai X, Murray TD, Jones, SS. 2001. Perennial wheat: the development of a sustainable cropping system for the U.S., Pacific Northwest. *American Journal of Alternative Agriculture* 16: 147-151.

Schroeder HW, Christensen JJ. 1963. Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. *Phytopathology* 53: 831-838.

Shen X, Kong L, Ohm H. 2004. *Fusarium* head blight resistance in hexaploid wheat (*Triticum aestivum*)-*Lophopyrum* genetic lines and tagging of the alien chromatin by PCR markers. *Theoretical and Applied Genetics* 108: 808-813.

Shen X, Ohm H. 2007. Molecular mapping of *Thinopyrum*-derived *Fusarium* head blight resistance in common wheat. *Molecular Breeding* 20: 131-140.

Van Tassel DL, DeHaan LR, Cox TS. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications* 3: 434-452.

Wan Y-F, Yen C, Yang J-L. 1997. The diversity of head scab resistance in Triticeae and their

relation to ecological conditions. *Euphytica* 97: 277-281

Wang JI-R, Wang L, Gulden S, Rocheleau H, Balcerzak M, Hattori J, Cao W, Han F, Zheng Y-L, Fedak G, Ouellet T. 2010. RNA profiling of fusarium head blight resistant wheat addition lines containing the *Thinopyrum elongatum* chromosome 7E. *Canadian Journal of Plant Pathology* 32: 188-214.

Weik L, Kaul H-P, Kübler E, and Aufhammer W. 2002. Grain yields of perennial grain crops in pure and mixed stands. *Journal of Agronomy and Crop Science* 188: 342-349.

Xue AG, Voldeng HD, Savard ME, Fedak G. 2009. Biological management of *Fusarium* head blight and mycotoxin contamination in wheat. *World Mycotoxin Journal* 2: 193-201.

Zhang L, Luo P, Ren Z, Zhang H. 2011. Controlling *Fusarium* head blight of wheat (*Triticum aestivum* L.) with genetics. *Advances in Bioscience and Biotechnology* 2: 263-270.

Zhang X, Shen X, Hao Y, Cai J, Ohm H, Kong L. 2011. A genetic map of *Lophopyrum ponticum* chromosome 7E, harboring resistance genes to *Fusarium* head blight and leaf rust. *Theoretical and Applied Genetics* 122: 263-270.

Zhou MP, Hayden MJ, Zhang ZY, Lu WZ and Ma, X-H. 2010. Saturation and mapping of a major *Fusarium* head blight resistance QTL on chromosome 3BS of Sumai 3 wheat. *Journal of Applied Genetics* 51: 19-25.

CONCLUSION. BROADER IMPACTS OF PERENNIAL CEREALS

In Chapter 1 of this dissertation, we considered environmental implications of perennial cereals within agricultural landscapes. Some of the specific ecosystem services which perennial cereals could be expected to contribute, include the following:

- Edible grain yield (as this comprises an ecosystem service if humans and domesticated animals are considered part of the ecosystem)
- Reduced nutrient leaching
- Increased carbon sequestration
- Reduced soil erosion
- Increased tolerance/resistance to insect herbivores and pathogens, due to structural, physiological and biochemical traits inherited from perennial ancestors
- Increased populations of pollinator and predatory insects.

In contrast, perennial cereals might also contribute certain ecosystem disservices. For example, perennial plants which persisted year round in the field could serve as hosts for pathogens and insect herbivores during periods of the year when annual stands were not present or were very sparse (e.g. winter for the fungal pathogen *Fusarium graminearum*, late summer for the Hessian fly *Mayetiola destructor*). These herbivores or pathogens might be able to maintain or increase their populations during the off-season, due to the presence of perennial cereal hosts, and could thus spread to adjacent agricultural fields during the late fall or spring. By serving as an alternate host and reservoir for pathogens and insects, perennial cereals could contribute ecosystem disservices in addition to the hypothesized ecosystem services.

Our research allows several of these questions to be addressed. In Chapter 1 of this

dissertation, we found that perennial wheat and perennial rye achieved approximately 50% and 70% of annual wheat and annual rye grain yields, respectively, and that these yields were maintained over at least two years. No additional benefits in terms of extra forage production were achieved. Thus perennial wheat and perennial rye are at this point still greatly inferior to their annual counterparts in terms of one important ecosystem service (grain yield) and further breeding efforts must strive to increase grain and forage yields.

How could breeding efforts most effectively increase seed production? One approach might be to increase capacity to acquire energy and nutrient resources, while another approach would focus on breeding directly for increased reproductive sinks., Currently, as demonstrated in Chapter 4, perennial cereal species do not appear to be source limited, and have excess capacity to photosynthesize and mobilize stored carbohydrates. Changes in sink / source ratio, especially in perennial wheat, do not appear to strongly affect seed size. Thus breeding efforts should most likely focus on breeding for greater spikelet number, lower sterility, and increased germination percentage. Preliminary observations (data not published) suggest that perennial wheat currently has longer spikes and more spikelets than annual wheat, but is markedly inferior in terms of germination ability and sterility, with many spikelets never being fertilized or filled. Thus decreased sterility and increased ability to germinate seem to be important pathways to achieving higher grain yield in perennial cereals. Preliminary research (Culman S. et al., 2012) indicates that intermediate wheatgrass yields far below annual wheat, although yields improve in the second year. Thus further wheatgrass breeding efforts must also focus intensively on improving sink strength, reproductive allocation and seed size.

Our research did not directly address the question of whether perennial cereals do in fact show less nutrient leaching than annual relatives. However, ongoing research at Kellogg

Biological Station (Sprunger et al., unpublished data) does indicate that intermediate wheatgrass shows much lower nitrogen leaching, and much lower concentrations of nitrogen in groundwater, than annual wheat. This could be related to higher rates of nutrient uptake by larger and more persistent root networks. Further research should investigate whether other perennial cereal species show lower nutrient leaching rates than annual relatives as well.

Our study did not directly investigate insect pressure or composition of insect communities in perennial cereal plots. However, with respect to pathogen pressure, we did find that perennial rye was generally fairly susceptible to *Fusarium graminearum*, while intermediate wheatgrass was extremely resistant, and perennial wheat lines varied in resistance.

With respect to this particular pathogen, therefore, the evidence suggests that perennial rye does present risks of contaminating adjacent annual rye fields with a ready source of inoculum in the spring. This may be less of a risk during the first year of a perennial rye crop, as this species matures later than annual rye, so that by the time *F. graminearum* has infected perennial rye spikes and been able to multiply, annual rye would already be past the point of maximal susceptibility. In later years, however, as perennial rye and annual rye appear to mature at the same time, the danger would be greater. Another pathogen to which perennial rye appears to be particularly vulnerable is the fungal pathogen ergot (*Claviceps purpurea*): this pathogen may be particularly prevalent in small plots, as it preferentially attacks unfertilized heads, and perennial rye is a cross pollinated species which depends on nearby conspecific plants to be pollinated. Observations in Part 2 of this dissertation suggest that perennial rye and annual rye were both highly susceptible to *C. purpurea*, and thus perennial rye could serve as a year round host and source of inoculums for ergot infection of annual rye fields. Future selection and cultivation practices, therefore, should focus on breeding more disease resistant perennial rye

lines and on optimizing cultivation practices to minimize ergot infection (e.g. larger fields, deeper planting).

Intermediate wheatgrass, by contrast, was highly resistant to *F. graminearum*, as were some of our perennial wheat lines. Our study suggests that the likelihood of this particular ecosystem disservice (serving as a reservoir for *Fusarium* infection) is low in intermediate wheatgrass. Casual observation of the intermediate wheatgrass lines growing in the field in Part 3 of this dissertation suggest that they had very low susceptibility to other common diseases in the region as well, as very few disease symptoms were seen. This corroborates reports of very low disease pressure on intermediate wheatgrass lines in Kansas (DeHaan LR, [personal communication](#)). Intermediate wheatgrass appears, in general, to be a species that would be highly disease resistant on its own, and would probably not serve as a source of inoculum for nearby annual crops. It might thus be a net contributor of ecosystem services with respect to disease prevalence, inasmuch as intermediate wheatgrass could potentially be grown with less reliance on fungicides. Perennial wheat, by contrast, showed more *Fusarium* head blight susceptibility, and field observations of other disease symptoms indicated that some lines showed generally less foliar and spike disease symptoms than annual wheat, but more than intermediate wheatgrass. Other lines, by contrast, showed as much or more disease pressure (from pathogens like barley yellow dwarf virus, leaf rust, and *Septoria* leaf blotch) as annual wheat. Further breeding efforts in perennial wheat should be based on the lines identified as showing greatest disease resistance, and should focus on selection for adequate levels of disease resistance simultaneous with strong regrowth.

The one ecosystem service which our research addresses most directly, is carbon sequestration. While we did not directly measure carbon sequestration in terms of inputs of fixed

carbon into the soil, we do have indirect evidence from Chapter 3 of this dissertation that suggests perennial cereals may fix more carbon from the atmosphere through photosynthesis. Photosynthetic rates during mid-season were found to be approximately 29% higher in perennial wheat and 45% higher in intermediate wheatgrass compared to annual wheat, and 29% higher in perennial rye compared to annual rye (averaging across measurement dates). These measurements are based on snapshots at only two or three time points during the season, and do not represent an integrated measure of photosynthesis over the season: nevertheless they are at least indicative that at a key time point during the season, photosynthetic carbon fixation in the perennial cereals studied is greater than in their annual relatives. As carbon export belowground, and subsequent carbon sequestration is dependent on the amount of carbon fixed from the atmosphere, higher photosynthetic rates could in the long term contribute to greater carbon sequestration within perennial cereal agroecosystems.

As yet, our measures of photosynthesis are taken on a per leaf area basis, and do not represent measurements of carbon fixation for a whole stand. Nevertheless, by making some broad assumptions we can at least make a rough estimate of carbon fixation on a per-plot level. To do this, we assume that photosynthetic rates are constant over the growing season. While this is certainly not true, we can use this broad assumption to help formulate some initial estimates, which can later be adjusted on the basis of more accurate models of how photosynthetic rate varies seasonally. We further assume, on the basis of observations over four summers between 2009 and 2012:

- that annual wheat, annual rye and first year perennial cereals are planted around Oct 15,
- that annual rye senesces around June 15 and annual wheat around June 25,

- that perennial rye senesces 14 days after annual rye (based on observations of flowering date in Chapter 2, plus the observation that perennial cereals senesce somewhat later after flowering than annuals)
- that perennial wheat senesces 14 days after annual wheat (based, again, on data from Chapter 2 plus the same assumption of later senescence after flowering)
- that intermediate wheatgrass flowers one month after annual wheat and takes one week longer for leaves to senesce after flowering (based on unreported observational data in 2011 and 2012),
- that all plants are inactive between November 15 and March 15, due to cold and snow cover
- that perennial cereals begin regrowing again on August 15th.

We then use the data collected in Chapter 3 of this indication, that find that leaf mass ratio does not vary between annual and perennial cereal species, and that leaf mass per area (LMA) is 28% and 26% higher in perennial wheat and intermediate wheatgrass, respectively, compared to annual wheat. Finally, we use data on plant biomass per plot from first-year perennial and annual cereal species in 2011 (data not reported here) that indicate perennial wheat and intermediate wheatgrass do not produce significantly different quantities of biomass on a per-plot basis than annual wheat, while perennial rye produces 49% less than annual rye ($t = 3.55$, $p = 0.016$, $n = 5$). Our estimate of total net primary production per plot is thus:

$$\text{NPP} = \text{Plant Biomass} / \text{Area} \times (\text{LMR}) / (\text{LMA}) \times (A_{\text{max}}) \times (\text{Length of Growing Season}),$$

with all parameters standardized such that the annual species (annual rye or annual wheat, respectively) has a value of 1 for each.

This gives us as an initial estimate that intermediate wheatgrass, in its first year, has a net fixation of 102% more CO₂ than annual wheat, while perennial wheat fixes 58% more CO₂ than annual wheat. Perennial rye fixes approximately 101% as much CO₂ as annual rye. These estimates are due to the existence of a substantial time period in the fall for additional carbon accumulation, later flowering, and higher photosynthetic rates per unit leaf area, and are subject to the limitations mentioned above. Nevertheless, if these higher rates of carbon accumulation are reflected in higher rates of carbon export and sequestration belowground, perennial cereals could represent a substantially superior sink for atmospheric CO₂, compared with annual wheat. Preliminary work at Kellogg Biological Station (Sprunger C., unpublished data) does indicate that intermediate wheatgrass does, even over a short time period of three years, show greater carbon sequestration than annual wheat. As dealing with elevated atmospheric carbon dioxide is one of the great environmental challenges of the near future, the suggestive indications that perennial cereals could be a carbon sink, point to what might be their most important and reliable ecosystem service.

In conclusion, our research has found that these three perennial cereals, in spite of their as yet low grain yields compared to annual equivalents, do have some interesting physiological traits (disease resistance and high photosynthetic rates) that could allow them, in future, to form part of more environmentally sustainable agroecosystems. Two species in particular, perennial wheat and perennial rye, are already approaching the yield thresholds at which they may be

economically feasible to introduce as crops to marginal areas, and their greater degree of sink limitation compared to annual equivalents suggests that they could be successfully bred for higher grain yields in future without compromising perennial growth habit. We thus conclude our study of these novel, high yielding herbaceous perennials with a suggestion that future breeding efforts could develop these species into agronomically important and economically productive crops, as well as physiologically interesting representatives of an unusual growth form.