PARTICIPATORY BREEDING OF WHEAT FOR ORGANIC PRODUCTION

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Future generations require food systems that sustain functional natural resources and nourish human communities. Scientific researchers, farmers, processors, and consumers are all integral partners in identifying crop genotypes for sustainable food systems. We engaged diverse stakeholders in plant breeding and variety testing for organic wheat. To inform the structure of a breeding program for organic wheat, we assessed genotype by environment interactions and the potential for locally-adapted varieties. After ten regional farmers selected breeding populations on their farms, we evaluated the effectiveness of participatory breeding for traits of interest.

In aggregate, this research stresses engagement and diversity in organic wheat breeding. In contrast to the priorities of most conventional wheat breeding programs, clients of the organic breeding program identified distinct priorities, including weed-competitive ability, artisanal baking quality, flavor, and low reactivity for those with wheat sensitivity. Trials distinguished varieties that induce less wheat sensitivity, exhibit high quality artisanal processing and desirable sensory attributes, and perform well under organic management. However, no one genotype performed best for all the farmer priorities, environments, processing applications, and types of wheat sensitivity. Our results confirm that many genotypes are needed to meet the diverse needs and environments of local and organic food systems.

Furthermore, decentralized and participatory selection were proven to be effective methods for improving organic wheat genetics. Genotype by environment interactions revealed

that decentralized selection in the northeastern and northcentral United States can optimize genetic gains for yield, test weight, weed-competitive ability, and early vigor in organic wheat. Evaluation of a participatory breeding program indicated that organic farmers were effective at selecting improved genotypes for their farms. Lines selected by farmers demonstrated gains in selection for the most important trait to organic wheat farmers: weed-competitive ability. Notably, optimal performance was seen on the actual farms where selections took place. We conclude that maximizing gains in organic wheat breeding requires many selection and testing environments. To reveal the true potential of breeding lines, testing environments must have similar genetic correlation to regional farmers' fields.

BIOGRAPHICAL SKETCH

"I live my life in widening circles" -Rainer Maria Rilke

Grounded in family, jovial company, and faith, Lisa ventured from her native Missouri to seek truth and make lasting contributions from Idaho to Ecuador, Minnesota to Chiapas, Cuba to Washington, and New York to Wisconsin. Her various initiatives center on the same goal: helping humanity have fun while living lightly on this Earth. Agriculture is her chosen tool for growing delight and responsibility.

After digging and planting throughout childhood, Lisa studied Environmental Science and Agroecology at the University of Minnesota. She was awarded a fellowship year to study farmer innovation with The National Institute for Agrarian Sciences (INCA) in Cuba and The Tropical Agricultural Research and Higher Education Center (CATIE) in southern Mexico. After four years of boots-on-the-ground agricultural conservation in the Yakima Valley with the United States Department of Agriculture (USDA) - Natural Resources Conservation Service, Lisa joined Cornell University to become a plant breeder.

Together with farms and science, Lisa enjoys dance, conversation, food, and Hero Stories. She and the love of her life, Leo, have found home at their farm in Aztalan, Wisconsin.

DEDICATION

I dedicate this work to those who teach compassion, selflessness, and abundance; to those who persevere in building a better world, despite fear; and to those thoughtful conservatives who refused to vote for Donald Trump.

ACKNOWLEDGEMENTS

I would like to thank all my teachers for their boundless generosity: my parents, grandparents, spouse, and sister; professors at the University of Minnesota; open-handed farmers throughout the Americas; mentors at the USDA; and classmates and educators at Cornell University. I am grateful for the steadfast support and trust of my advisor, Dr. Mark Sorrells, whose exceptional breeding program ventured into uncharted territory with this research. I thank each of my committee members: Dr. Julie Dawson for selflessly allowing me to be her apprentice in participatory breeding; Dr. Matt Ryan for enthusiastically advising in organic agriculture, weed science, and my career; and Dr. Margaret Smith for exemplifying patience and respect in extension service, and for her keen plant breeding guidance.

This research took true teamwork, and it would not exist without the help of collaborators Dr. Julie Dawson, June Russell, Dr. Elizabeth Dyck, Dr. Ellen Mallory, Dr. Heather Darby, Nicholas Santantonio, Dr. Plaimein Amnuaycheewa, Lynn Veenstra and Dr. Hugh Gauch; hard-working co-workers in the field, notably the magnificent David Benscher, Dr. Michael Davis, Tom Molloy, Erica Cummings, Steve Zwinger, and Dr. Greg Roth; collaborating farmers who took the road less travelled to build better wheat varieties for our region (Oechsner Farms, Threshold Farm, Essex Farm, Rusted Rooster Farm, Grange Corner Farm, Butterworks Farm, Gleason Grains, White Frost Farm, Adirondack Organic Grains, and Lakeview Organic Grain); the many bakers, pasta makers, and tasters who conducted variety evaluations; and Elizabeth Johnson, a resolute volunteer who provided needed inspiration.

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TABLE OF CONTENTS

CHAPTER 1

PARTICIPATORY BREEDING OF WHEAT TO ENHANCE LOCAL AND ORGANIC FOOD SYSTEMS

Abstract

Despite its benefits, wheat is underrepresented in local and organic food systems. Improved wheat genetics can boost sustainability and meet product demand. This chapter outlines a wheat breeding program to develop improved varieties for organic production. Participatory plant breeding was used to enhance all steps of the breeding program, including (1) clarifying needs in crop improvement, (2) identifying promising parental varieties, (3) generating improved genotypes, and (4) achieving adoption of developed varieties. Clients of the breeding program revealed priorities for selection, including weed-competitive ability, Fusarium head blight resistance, straw production/tall height, lodging resistance, artisanal bread quality, flavor, and low wheat sensitivity. A participatory wheat breeding program enabled the identification, selection, and adoption of superior genotypes for organic production.

1.1 Introduction

1.1.1 Benefits of organic agriculture

Organic agriculture can reduce many negative environmental and economic impacts that are characteristic of conventional production. Organic agriculture reduces non-point source pollution from nitrogen fertilizers (Drinkwater et al. 1998) and pesticides (Pimentel et al. 2005) when compared with conventional agriculture. With fewer energy inputs and higher carbon sequestration in soil, organic systems also have a lower global warming potential compared with

conventional systems (Meisterling et al. 2009; Mäder et al. 2002; Pimentel et al. 2005; Teasdale et al. 2007). Organic systems also show yield stability over variable weather conditions, particularly during drought (Pimentel et al. 2005; Letter et al. 2003). A meta-analysis of 44 studies showed that on average, organic systems were 22-35% more profitable than conventional operations, allowing more small and mid-sized farmers to stay in business (Crowder & Reganold 2015). The benefits of organic agriculture are enhanced with local sales, as global warming potential of agricultural products are strongly tied to the transport distance from farm to marketplace (Meisterling et al. 2009). Local food systems also improve farmer profitability, support smaller farms, and increase employment and income of rural communities (Martinez et al. 2010).

1.1.2 Small grains for organic systems

Small grains are important components of organic and local food systems. The fibrous root systems and high carbon plant residues of small grains can improve soil health by rapidly building organic matter (Snapp et al. 2005). Diversifying rotations with small grains mitigates two primary challenges of organic agriculture identified by Cavigelli et al. (2008): weed competition and nitrogen supply. Incorporating wheat into rotation reduced weed pressure in comparison with monocultures of other crops (see review by Liebman & Dyck 1993). Additionally, the relatively early harvest of small grains allows the introduction of semi-perennial forages and legumes into rotations, providing nitrogen that tends to increase yields in organic systems (Seufert et al. 2012). Beyond agronomic benefits, small grains bolster the economic stability of farms and rural communities. In a meta-analysis by Crowder and Reganold (2015), organic cereals (along with oil and fiber crops) provided the greatest financial benefit-to-

cost ratio when compared with conventional production. The long processing chains required for small grains also invigorate regional economies, providing entrepreneurial opportunities for millers, bakers, pasta makers, and restaurants to sell locally produced grain (Halloran 2015).

Despite such benefits, small grains are underrepresented in local and organic food systems. In the United States, consumers increasingly purchase organic (ERS 2014) and local foods (Elbehri 2007; Low et al. 2015). However, only 0.6% of US wheat hectacres are organic (ERS 2013), a small proportion compared to acres under vegetable production (*e.g.,* carrots: 14.4%, lettuce: 11.6%), acres in fruit production (*e.g.,* apples 4.9%), and number of dairy cows (2.8%). While 45.2% of farmers' market vendors in the United States sold fresh fruits or vegetables, grains were not even listed as a category of possible products in the 2006 Farmers Market Survey (Ragland & Tropp 2009). Although wheat remains one of the most industrially-consolidated food products (Hendrickson & Heffernan 2007), local food systems are increasingly demanding organic wheat produced by nearby farms, reincorporating small-scale flour mills, and sprouting bakeries that have been absent for decades (Brannen 2013; Hergesheimer & Wittman 2012; Hills 2012).

Significant barriers remain to satisfy growing demand for local and organic wheat. Worldwide meta-analyses show that wheat (along with barley and potato) has the lowest organic-to-conventional yield ratios in comparison with other crops (Ponisio et al. 2014; Seufert et al. 2012). Wheat genetics that underperform in organic systems may be reducing the crop's potential. Consequently, improved varieties would likely strengthen organic small grain production.

1.1.3 Designing a genetic improvement program for organic wheat[1]

Creating an effective breeding program for organic wheat requires (1) clarifying needs in crop improvement, (2) identifying promising parental varieties, (3) selecting better genotypes, and (4) achieving field adoption of developed varieties. Participatory plant breeding (PPB) methods can help accomplish all four of these stages. PPB incorporates the involvement of "clients" in the breeding process (Witcombe et al. 2005), and decentralization of selection sites into farmers' fields (Ceccarelli 2015). This section reviews whether participatory plant breeding is a good fit for organic wheat breeding.

1.1.3.1 Client needs

At the beginning of a breeding program, client participation pinpoints what problems need to be solved by genetic improvement. Across many crop species and environments, clients have consistently identified priority traits for selection that are different from plant breeders (see review by Ashby 2009). Participation also allows flexibility in the selection program, so that if needs change during the lengthy process of plant breeding, clients can help reorient the objectives to ensure relevant end products. Surveys, transect walks, focus group discussions and other basic tools of social science can assess the needs of clients (Pretty and Vodouhê 1997; Soleri and Cleveland 2009; OSA 2012).

Clients of organic and local wheat, including organic farmers, artisan processors, and consumers, have distinct needs from the clients of conventional wheat breeding programs.

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[1]*Content adapted with permission from its originally published form:* Kissing Kucek L, Darby H, Mallory E, Dawson J, Davis M, Dyck E, Lazor J, O'Donnell S, Mudge S, Kimball M, Molloy T, Benscher D, Tanaka J, Cummings E, Sorrells ME. 2015. *Participatory Breeding of Wheat for Organic Production*. Proceedings from the Organic Agriculture Research Symposium, La Crosse, WI.

Organic farmers in Minnesota defined different priorities for agronomic and quality traits than conventional breeding programs (Kandel et al. 2008). Consumers also increasingly demand food with complex flavor (Codron et al. 2005) and fewer additives (Kaptan & Kayisoglu 2015). Thirty percent of U.S. consumers also reduce gluten consumption in their diets (Balzer 2013). Such consumers seek heritage and ancient wheats, which are perceived to cause fewer problems for those with wheat sensitivity, such as celiac disease, allergies and nonceliac wheat sensitivity. Heritage wheat refers to cultivars that were developed before the use of dwarfing genes in the 1950s, and ancient wheats are the hulled relatives of wheat, including spelt (*Triticum aestivum, ssp. spelta*), emmer (*Triticum turgidum ssp. dicoccum* Schrank) and einkorn (*Triticum monococcum* L.). The focus on heritage and ancient wheat is rare in conventional breeding programs.

1.1.3.2 Identifying top-performing parents for breeding

Once prioirites for breeding are established, parental selection is key to developing superior genotypes (Virk et al. 2005; Weber 1979; Bernardo 2003). Engaging stakeholders in variety evaluation can ensure that parents are chosen that meet priority traits.

1.1.3.3 Decentralized breeding for genetic improvement

Understanding genotype performance across environments is necessary to structure a new breeding program. To maximize genetic gain (R), most breeding programs seek high narrow-sense heritability (h^2), phenotypic standard deviation of the breeding population (σ_P), and intensity of selection (i) (Equation 1.1). However, breeders make selections in environments that differ from the array of farms that will eventually grow developed varieties (*i.e.*, the target

environment). By making selections outside of the target environment, a selected trait is a correlated trait merely associated with performance in the target environment. The correlated response (CR) tracks the actual genetic gains that a breeding program will realize for the target environment (Equation 1.2). Primary determinants of the correlation coefficient include: heritability for the trait in the selection environment (h_y^2), and in the target environment (h_x^2); and the genetic correlation coefficient between the selection and target environments (r_g). Many breeding programs focus on homogeneous high-input environments that can increase trait heritability (Hammond 1947). However, if genotypic performance between selection and target environments is inconsistent, large genotype by environment variance (σ^2_{GE}) relative to genetic variance (σ^2_G) will generate small or negative r_g values (Equation 1.3). In such cases, gains made in a breeding program will be inefficient or irrelevant to farmers' fields. Studies have documented significant genotype by environment interactions between organic and conventional management systems (Kirk, Fox, and Entz 2012; Hoagland 2009; Reid et al. 2011; Murphy et al. 2007), indicating that gains made in conventional breeding programs may be irrelevant for organic farmers.

Equation 1.1	$R=\sigma_P i h^2$	(Falconer 1981)
Equation 1.2	$CR=R_y h_x^2/h_y^2 r_g$	(modified from Falconer 1981)
Equation 1.3	$r_g=\sigma^2_G/(\sigma^2_G+\sigma^2_{GE})$	(Dickerson 1962)

Decentralized selection is a tool that moves selection closer to the target environment. In situations with high genotype by environment interactions (GxE), decentralization can increase genetic correlation coefficients by moving selection into the target environment, and

consequently, increasing the response to selection (Ceccarelli 2015). PPB takes decentralization to the extreme by making selections directly in the target environment of farmers' fields. With high GxE in breeding contexts, lines selected under PPB have performed better for client priority traits than materials selected under formal plant breeding methods (Joshi et al. 2007; Ceccarelli et al. 2001; Goldringer 2014; Kirk et al. 2015). Little is known about the magnitude and structure of GxE among organically managed environments, and whether decentralization will be useful in organic wheat breeding.

1.1.3.4 Variety adoption

Participation facilitates one of the most difficult stages of plant breeding: variety adoption. PPB varieties are more likely to be adopted because participants developed materials that are relevant to their needs (Ashby 2009). In addition to adopting more varieties, farmers involved in PPB projects also adopt varieties earlier (Ashby 2009; Ortiz-Perez et al. 2006; Mustafa et al. 2006). Among clients of organic wheat breeding, including farmers, bakers, and consumers, there is widespread mistrust of modern varieties and conventional breeding (Davis 2011; Kissing Kucek et al. 2015). Consequently, participatory plant breeding is a great fit for rebuilding client trust of improved varieties.

PPB programs can also reduce the costs of breeding programs. Cost savings are primarily derived from less trialing of advanced lines (Mangione et al. 2006). Since decentralized selection takes place over multiple years in the target environment, lines can be tested for fewer years in multi-environment trials prior to release. Several factors limit the market incentive to breed organic wheat varieties, such as: less than one percent of farms in the United States are organic, a small fraction of those organic farms grow wheat, and many farmers save their own seed (USDA

2014; ERS 2013). To overcome these financial barriers, the PPB structure for our breeding project was chosen as a minimal investment strategy with potentially large benefits for farmers, millers, bakers, and consumers.

Based on its the potential benefits, we implemented a participatory plant breeding program for organic wheat in the northeast and northcentral United States. After identifying client priorities, we crossed promising parents, used decentralized selection to advance superior progeny, and evaluated the potential for adoption of varieties developed in the breeding program.

1.2 Methods

To understand client needs, we conducted semi-structured interviews with regional organic wheat farmers (Table 1.1). Research and extension collaborators used purposotive sampling to nominate ten organic farmers to participate in wheat breeding. After learning about the project objectives, all farmers agreed to participate. Farms included a diversity of sizes, production systems, and climates of the northeastern United States (Figure 1.1).

Table 1.1. Semi-structured interview questions.

1. What crops and/or livestock do you farm? What is your typical rotation?
2. How many acres do you farm? How many acres are in wheat? Is some or all your land certified organic?
3. For how many years have you been farming? For how many years have you grown wheat?
4. What products do you market, and where do you market them?
5. What are your short and long term goals for your farm?
6. Why are you interested in growing wheat on your farm? What do you hope to achieve by growing these crops?
7. What barriers do you see to meeting your objectives in growing organic wheat?
8. Describe your ideal wheat. What specific characteristics do you seek in this ideal wheat? Please rank them in order of importance to you.

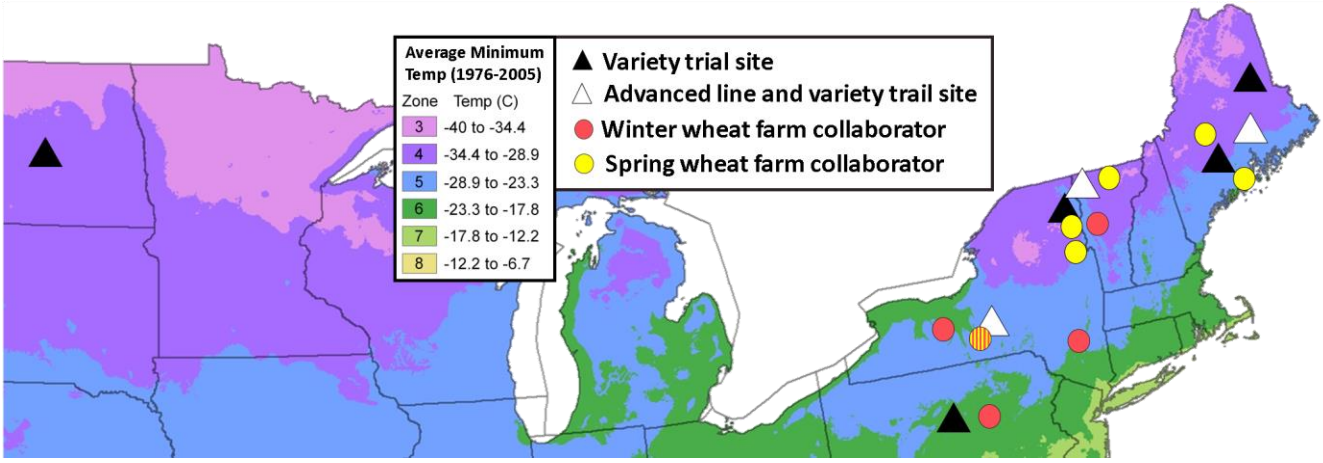

Figure 1.1. Locations of participating farms, variety trials, and advanced line testing. Locations of farms participating in needs assessment and decentralized selection for winter (red circles) and spring (yellow circles) wheat, variety trial sites (black and white triangles) for identification of parental lines. Background map modified from USDA (ARS 2012).

1.3 Results and discussion

Seven of ten barriers identified by organic wheat farmer project collaborators (winter kill, dehulling, weed control, Fusarium head blight (FHB), lodging, late maturity, and protein) were related to crop genetics (Figure 1.2). These results confirm that a breeding program can address challenges in organic wheat systems.

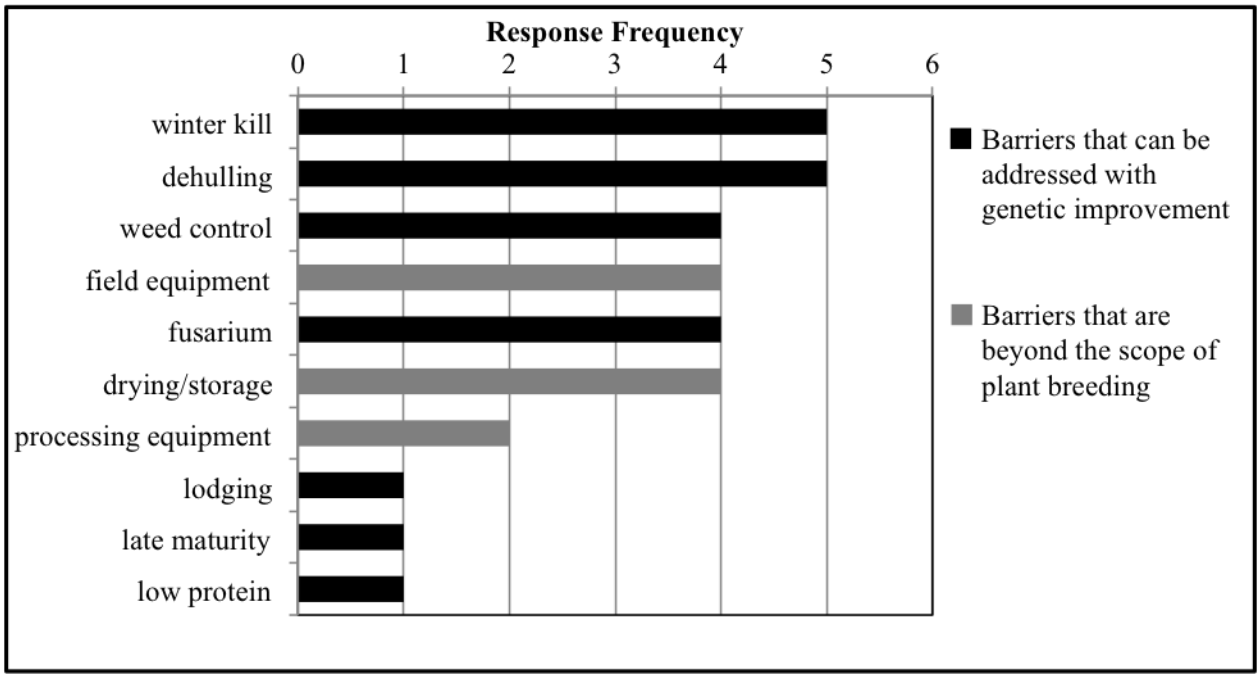

Figure 1.2. Barriers that prevent organic wheat farmer respondents from meeting their objectives in growing wheat. 70% of barriers can be addressed through genetic improvement.

1.3.1 Client needs

Demographics indicate that participating organic farms differ from clients of conventional wheat breeding programs. Ten participants farmed a mean of 255 hectacres (range 2.83-607). The average number of wheat acres per farm, 16.6 (range 0.405-80.9), sharply contrasted with the nationwide average of 134 (2012 Ag Census). Years of farming experience revealed the newness of organic wheat farming in the region. Although farming experience averaged 23 years per farmer (range 3-40), experience farming wheat was only half that, at 12 years (range 3-40). Farms exhibited a high level of temporal and spatial crop diversity. Over a mean rotation length of 5.5 years (range 2-8), 17 crops were grown per farm (range 5 to >30). Ninety percent (9 of 10) of participants also raised livestock on their farm. Crop and livestock diversity contrasts with commodity wheat farms that focus on a small number of crops. Diversity impacted farmer priorities for breeding. Participants who farmed livestock identified straw

quantity as an important target for wheat breeding (see Figure 1.3), a priority not met in conventional programs that prioritize short height and high harvest index. Nine of ten farmers milled their own flour or were partners with a local miller. As markets for their wheat were focused on local, direct-to-market sales, artisanal baking quality and flavor were traits of interest to local farms- traits that are rarely addressed in conventional wheat breeding.

Through interviews, farmers ranked the traits that they found to be most important in a wheat variety. Figure 1.3 depicts the relative importance of wheat traits to farmer participants. Most farmers valued several traits in common (weed-competitive ability, straw production/tall height, and lodging resistance for spring wheat; FHB tolerance, protein content, baking quality, and flavor for winter wheat), although many farmers emphasized traits that were uniquely important to their operation (white grain color, resistance to leaf diseases, high number of seeds per head, and performance under low nitrogen conditions). Some traits of importance identified by organic wheat farmers – such as tall height, weed-competitive ability, and the ability to produce under low nitrogen conditions – are negatively correlated with the targets of most conventional wheat breeding programs (Figure 1.3). Other priority traits, such as protein and yield, demonstrate inconsistent genotype performance between organic and conventional selection environments (Kirk, Fox, and Entz 2012; Hoagland 2009; and Murphy et al. 2007). When grown under organic conditions, wheat populations selected under organic management produced higher yield and protein content than genotypes selected under conventional environments (Kirk, Fox, and Entz 2012; Reid et al. 2011; Murphy et al. 2007; Brancourt-Hulmel et al. 2005). Consequently, the estimated 95% of plant breeding environments that are not organic are failing to produce optimal genotypes for organic systems (Lammerts van Bueren et al. 2011). Farmers also prioritized traits that are rarely screened in conventional wheat

breeding programs, such as the ability of a genotype to make a great loaf of artisanal bread. Our results suggest that a distinct breeding program is needed to meet the unique needs of organic and local wheat stakeholders.

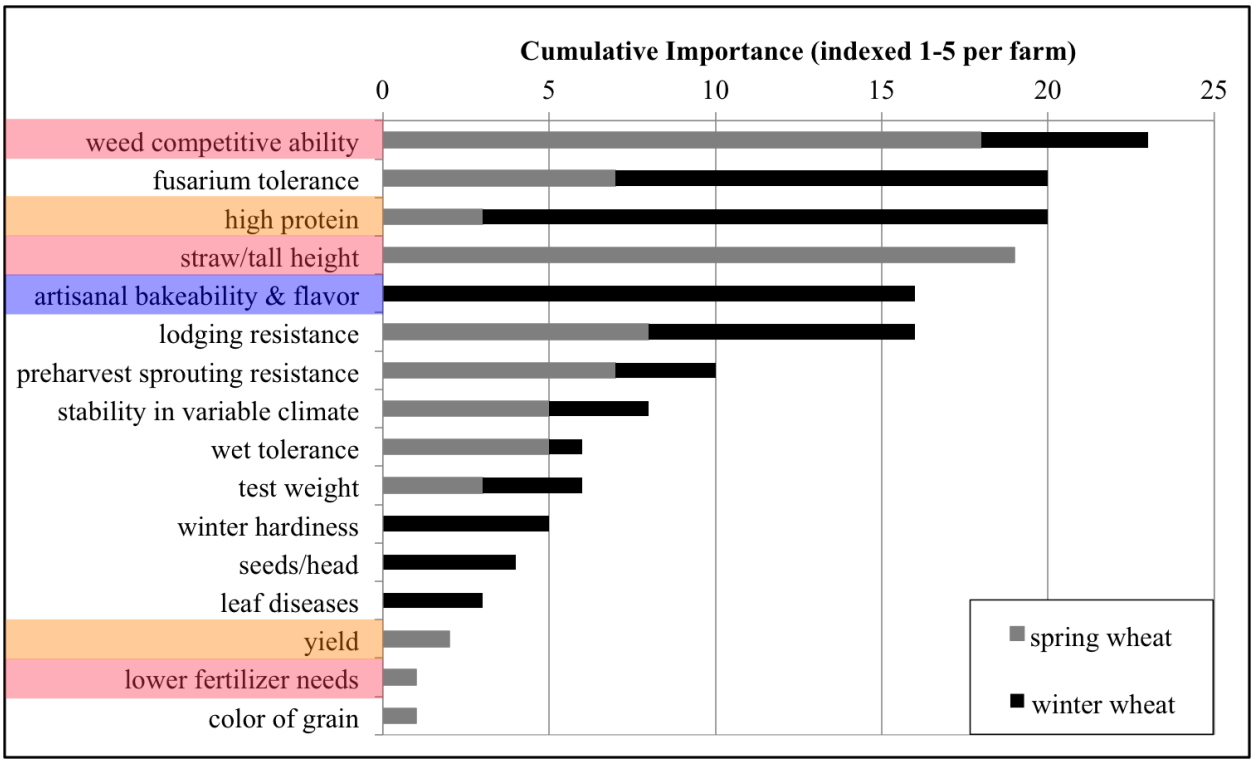

Figure 1.3. Farmer-identified priority traits for selection, weighted by rank of importance. Each of the ten farmers identified up to five traits and ranked them in order of importance. Traits highlighted in pink are negatively correlated with traits targeted in most conventional wheat breeding program. Traits highlighted in orange have evidence of high genetic by environment interactions between organic and conventional selection environments (Kirk, Fox, and Entz 2012; Reid et al. 2011; Murphy et al. 2007; Brancourt-Hulmel et al. 2005). Traits highlighted in purple are rarely screened in conventional wheat breeding programs.

1.3.2 Identifying top-performing parents for breeding

Clients of the breeding program – including bakers, chefs, and consumers – actively participated in variety evaluations for processing and sensory quality. Chapter 2 presents the winning genotypes for low-additive artisanal baking and taste. To further explore varieties suited

to consumer needs, Chapter 3 explains how genotypes and species of wheat impact individuals with celiac disease.

Chapters 4 and 5 review variety performance among diverse organic environments of the northeastern and northcentral United States. Forty-three site-years of field trials identified the best winter and spring wheat varieties for traits prioritized by farmers, including weed-competitive ability, height, lodging, FHB tolerance, protein, yield, test weight, and pre-harvest sprouting resistance (low falling number). Field trials were organically managed, as grain yield and stability rankings have differed between conventional and organic trials (Reid et al. 2011), and because surveyed organic farmers have unanimously valued variety trials conducted on certified organic land (Kandel et al. 2008).

1.3.3 Decentralized breeding for genetic improvement

To assess the need for decentralization in regional organic wheat breeding, we evaluated the performance of varieties over 35 environments of the target region. Chapters 4 and 5 explore the magnitude of GxE effects for yield, test weight, protein, falling number, weed-competitive ability, and early vigor in organic wheat. These chapters also assess which locations have distinct variety performance (mega-environments). To test the effectiveness of decentralized selection in practice, we collaborated with ten organic farmers (Figure 1.2), who selected wheat populations for traits of interest to their farm. Chapter 5 evaluates whether decentralized selection improved traits of interest. Moreover, Chapter 5 quantifies the local adaptation of lines selected at many farms throughout the region.

1.3.4 Variety adoption

In Chapter 5, we tested whether populations developed through PPB met the needs defined by organic wheat farmers. An evaluation of adaptation of selected lines to diverse environments of the northeast United States also assessed if PPB could reduce the costs of advanced line testing throughout a region.

1.4 Conclusions

Wheat provides benefits to organic rotations, but is underutilized partially due to suboptimal genetics. A participatory breeding program was designed to understand the needs of organic farmers, millers, bakers, and consumers. Semi-strucutured interviews confirmed that client needs are not met through conventional wheat breeding programs. To develop improved genotypes of wheat for organic systems, genotypes were screened and selected for traits of interest to clients of the organic breeding program. Selection was decentralized to maximize gains in priority traits for the diverse needs and environments of organic farmers.

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CHAPTER 2

EVALUATION OF WHEAT AND EMMER VARIETIES FOR ARTISANAL BAKING, PASTA MAKING, AND SENSORY QUALITY[2]

Abstract

Identifying varieties best suited to local food systems requires a comprehensive understanding of varietal performance from field to fork. After conducting four years of field trials to test which varieties of ancient, heritage, and modern wheat grow best on organically managed land, we screened a subset of varieties for bread, pastry, pasta, and cooked grain quality. The varieties evaluated were three lines of emmer (*T. turgidum* L. ssp. *dicoccum* Schrank ex Schübl) and eleven lines of common wheat (*Triticum aestivum* L.), including two modern soft wheat varieties, four soft heritage wheat varieties, four hard modern wheat varieties, and one hard heritage wheat variety. A diverse group of bakers, chefs, researchers, and consumers compared varieties for qualities of interest to regional markets. Participants assessed differences in sensory profiles, pasta making ability, and baking quality for sourdough, matzah crackers, yeast bread, and shortbread cookies. In addition to detecting significant differences among varieties for pasta, sourdough, and pastry quality, participants documented variation in texture and flavor for the evaluated products. By demonstrating which varieties perform best in the field, in the bakery, and on our taste buds, these results can support recommendations that strengthen the revival of local grain economies.

[2] Kissing Kucek L, Dyck E, Russell J, Clark L, Hamelman J, Burns-Leader S, Senders S, Jones J, Benscher D, Davis M, Roth G, Zwinger S, Sorrells ME, Dawson J. Evaluation of wheat and emmer varieties for artisanal baking, pasta making, and sensory quality. Journal of Cereal Science. *In press*.

2.1 Introduction

Global consumers increasingly demand food that is organic (ERS 2014) and local (Elbehri 2007; Low et al. 2015), with fewer additives (Kaptan and Kayisoglu 2015) and excellent sensory quality (Codron et al. 2005). Bread is a key component of changing consumer demand. Although wheat remains one of the most industrially consolidated food products (Hendrickson and Heffernan 2007), local food systems are increasingly purchasing wheat produced on nearby farms and sprouting small-scale flour mills and bakeries that have been absent for decades (Halloran 2015; Hills 2012). This transition requires the identification of varieties that best support local grain economies.

Previous research has not identified the wheat varieties that are best suited to the local grain markets of the United States. Local markets focus on organic production, low-extraction stone milling, artisanal sourdough baking, and consumer demand for unique taste. Consumers, bakers, and farmers involved in local and organic grain economies of the United States have also expressed interest in heritage and ancient wheat varieties (Packaged Facts 2015), in part because some genotypes have demonstrated distinctive flavors and reduced impacts in individuals with wheat sensitivity (Kissing Kucek et al. 2015). The term heritage describes varieties of common wheat (*Triticum aestivum* L.) developed before the use of dwarfing genes in the 1950's, while modern wheat refers to varieties of common wheat developed after that time. Ancient wheat describes hulled relatives of wheat, such as emmer (*T. turgidum* L. ssp. *dicoccum* Schrank ex Schubl). The baking quality of heritage wheat varieties, however, are poorly documented. Moreover, few scientific studies have compared the sensory attributes of different varieties of heritage, ancient, and modern wheat.

Vindras-Fouillet et al. (2014) found significant differences in artisanal baking and sensory quality among eight farmer-selected wheat populations and one modern variety in France. Similarly, four varieties demonstrated different texture and appearance when baked into wholemeal bread in Germany (Ploeger et al. 2008). Starr et al. (2013) also documented significant differences in texture, appearance, aroma, and flavor of cooked grain from 20 wheat varieties grown in Northern Europe. None of the varieties assessed in these studies, however, are commonly grown in the United States. To inform local markets of the United States, this study compared varieties of organically grown heritage, modern, and ancient wheat for whole-grain technical parameters, artisanal bread baking, pasta making, pastry quality, and sensory attributes.

2.2 Materials and methods

2.2.1 Field methods

To identify varieties that may be best suited to organic production in the northeastern and northcentral United States, we evaluated 40 winter wheat, 24 spring wheat, and 16 spring emmer entries over four years (2012-2015) at three organically certified locations in Willsboro, NY, Freeville, NY, and Rock Springs, PA. Spring wheat and emmer entries were also tested on certified organic land in Carrington, ND. All entries were replicated three times and plot sizes varied from 3.78 to 8.91 m^2, depending on location. Agronomic results of these variety trials are published elsewhere (Sorrells 2015).

2.2.2 Variety selection

A subset of varieties entered each of three quality evaluations: bread wheat varieties for sourdough baking and cooked grain; soft wheat varieties for matzah crackers [plural matzot], yeast bread, shortbread cookies, and cooked grain; and emmer varieties for pasta and cooked

grain. Table 2.1 provides an overview of which varieties were included in each evaluation, and their technical parameters. During all baking, pasta making, and sensory evaluations, a randomly generated three-letter code masked the identity of each variety.

2.2.2.1 Sourdough bread and cooked grain evaluation

For the sourdough baking and cooked grain evaluation, principal component analysis was used to select wheat varieties with a broad range of technical quality parameters (Figure 2.1). The seven selected varieties included heritage varieties ('Fulcaster' and 'Red Fife'), modern cultivars that were widely grown by organic farmers in the northeastern United States ('Warthog,' 'Fredrick,' and 'Glenn'), and other modern cultivars that had performed well in variety trials ('Appalachian White' and 'Tom'). A blend of 2012 (21%) and 2013 (79%) grain harvested at the Freeville, NY site was used for the sourdough evaluation.

2.2.2.2 Matzah cracker, yeast bread, shortbread cookie, and cooked soft wheat grain evaluation

To evaluate soft wheat varieties for matzah crackers, yeast bread, shortbread cookies, and cooked grain, five soft wheat varieties were selected: the heritage varieties 'Forward,' 'Pride of Genesee,' and 'Yorkwin' and two high-yielding modern varieties, 'Susquehanna' and 'Fredrick.' Grain for the soft wheat evaluation originated from the 2014 Freeville, NY harvest.

2.2.2.3 Pasta and cooked emmer grain evaluation

The pasta and cooked grain evaluation included the three emmer varieties 'Lucille,' 'North Dakota Common,' and 'Red Vernal,' all of which were high-yielding in field trials. Emmer grain was a blend of 45% grain from 2012 and 55% grain from 2014 Freeville, NY trial harvests.

Table 2.1. Technical parameters of varieties included in the grain quality evaluations.

Evaluation[±]	Processing Evaluators	Sensory Evaluators	Habit/ Species	Variety type	Variety name	Class hardness and color	Yield[§] kg/ha	Test weight[§] kg/hl	Flour moisture %	Grain protein at 12% moisture	Falling number sec	DON[+] ppm
Sourdough Bread and Cooked Grain	8 sourdough bakers	30 trained panelists	Winter Wheat	Modern	Appal-achian White	Hard white	3071	72.8	10.7	9.8	459.3	<0.5
				Modern	Fredrick	Soft white	3233	71.7	11.0	9.5	335.5	0.7
				Heritage	Fulcaster	Soft red	2766	72.9	10.6	10.5	393.5	<0.5
				Modern	Warthog	Hard red	3393	74.2	10.5	9.9	434.4	<0.5
			Spring Wheat	Modern	Glenn	Hard red	2277	71.1	10.3	15	406.8	0.7
				Heritage	Red Fife	Hard red	1798	66.7	10.3	14.8	370	<0.5
				Modern	Tom	Hard red	2384	69.9	8.3	14.7	513.4	0.7
Matzah Cracker, Yeast Bread, Shortbread, and Cooked Grain	9 yeast-based bread bakers	11 trained panelists, 24 public preference tasters	Winter Wheat	Heritage	Forward	Soft red	3040	72.5	9.1	13.0	403	0.8
				Modern	Fredrick	Soft white	3233	71.7	9.7	11.5	233	1.3
				Heritage	Pride of Genesee	Soft white	2801	72.8	9.2	13.3	311	0.7
				Modern	Susque-hanna	Soft red	3307	69.3	9.6	11.1	301	<0.5
				Heritage	Yorkwin	Soft white	3078	71.6	8.9	12.8	308	1.0
Pasta and Cooked Grain	3 pasta makers	12 trained panelists, 26 public preference tasters	Spring Emmer	Ancient	Lucille	Hard red	2494*	46.4*	12.1	14.2	545.4	0.7
				Ancient	North Dakota Common	Hard red	2499*	47.4*	11.9	14.6	492.8	0.6
				Ancient	Red Vernal	Hard red	2478*	46.8*	11.9	15.0	594.5	0.7

[±]Tested flour for the sourdough bread and cooked grain evaluation was a blend of 21% 2012 and 79% 2013 harvests from Freeville, NY; flour for the matzah cracker, yeast bread, shortbread cookie, and cooked soft wheat grain evaluations was harvested from Freeville, NY in 2014; flour for the pasta and cooked emmer grain evaluation was a blend of 45% 2012 and 55% 2014 harvests from Freeville, NY

[§]Yield and test weight values are a mean of three sites over four years (2012-2015)

*Yield and test weight values for emmer are reported in the hull.

[+] Deoxynivalenol (DON) had a minimum detectable value of 0.5 ppm.

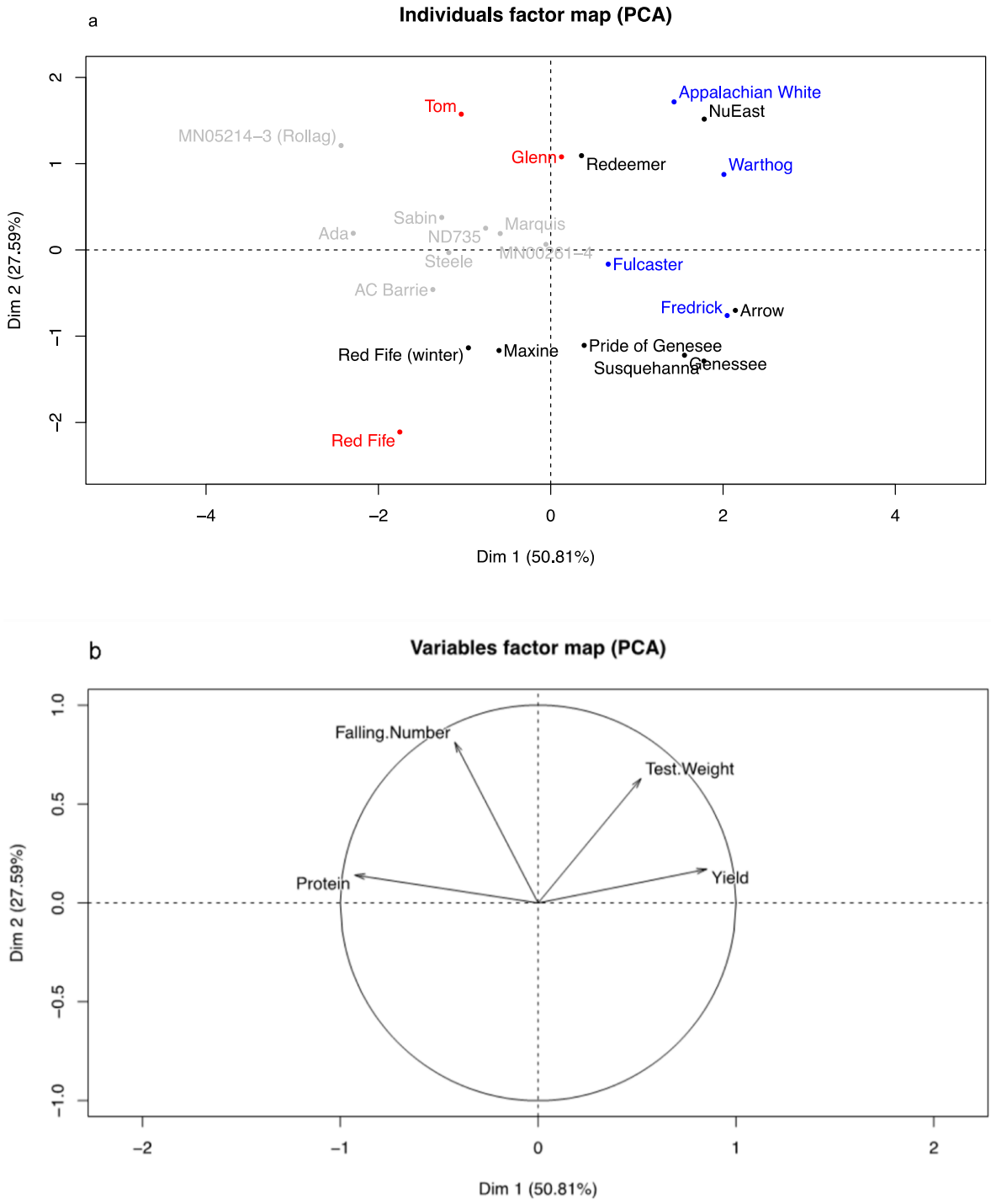

Figure 2.1. Selection of divergent varieties for sourdough evaluation. (a) Principal components analysis of candidate varieties for the sourdough bread and cooked grain evaluation. All wheat varieties had deoxynivalenol less than one. Selected spring varieties are shown in red and selected winter varieties in blue. Unselected spring varieties are shown in grey and unselected winter types in black. (b) Variable factor map of principal components.

2.2.3 Baking and pasta making evaluations

2.2.3.1 Sourdough bread evaluation

For the sourdough baking evaluation, grain was milled on an Osttiroler Getreidemuehlen tabletop stone mill (Rondella model), which has similar properties to stone mills commonly used by artisan millers in the Northeast. The unsifted flour rested at room temperature for 31 days before baking. A panel of eight artisan bakers from the northeastern and northcentral United States prepared and evaluated loaves of bread made from individual varietal flours. Baking methods followed a typical traditional sourdough recipe for the region (Table A.1). After developing a common ranking scale and vocabulary, bakers scored doughs individually throughout the baking process. Bakers varied levels of hydration, rest time, and mix time to allow each varietal flour to reach its full potential in bread making (Table A.1). Researchers measured circumference and weight of all baked loaves. For height, a subsample of five varietal loaves was cut in half and measured from the lowest to highest point. To calculate density, researchers determined loaf volume of three representative varietal loaves by displacement in flaxseed.

2.2.3.2 Matzah cracker, yeast bread, and shortbread cookie evaluation

For evaluations of soft wheat, grain was ground using the Osttiroler Getreidemuehlen tabletop stone mill (Rondella model) three days before the baking evaluation. Two regional millers sifted flour with a coarse mesh, obtaining 90 to 97% extraction rates. To test the yeast bread-baking quality of soft wheat varieties, a panel of nine bakers compared four soft wheat varieties (‘Forward,’ ‘Pride of Genesee,’ ‘Yorkwin,’ and ‘Fredrick’) to a hard spring wheat check with high baking quality, ‘Red Fife.’ Bakers used a yeast-based bread recipe typical for

the region (Table A.1). The bakers changed mixing time, hydration, autolyse time, and number of folds as needed to optimize bread quality for each variety (Table A.1). To make matzah crackers, bakers followed the formula in Table A.2. Bakers also prepared shortbread cookies following the formula in Table A.3. After a consensus was developed on vocabulary, bakers individually scored doughs for all products.

2.2.3.3 Emmer pasta evaluation

For emmer pasta evaluations, grain was dehulled using a Codema lab-scale oat dehuller and ground four days before the pasta making evaluation using a KoMo Fidibus 21 tabletop mill with a ceramic/corundum millstone that achieved a fineness of grind similar to commercially available emmer flour. The flour was not sifted. Three pasta makers evaluated varieties of emmer for pasta quality. Evaluators chose a 64% emmer-based pasta formula (Table A.4), which, in their experience, was the highest concentration of local emmer flour that could produce a functional dough. Pasta makers treated each varietal dough with additional quantities of Antico Molino Caputo 00 flour during rolling to create an ideal pasta feel (Table A.4). Since pasta makers evaluated pastas as a group, only one overall score was recorded per variety, and statistical analyses were not possible.

2.2.4 Sensory evaluations

Trained panels conducted descriptive analysis of sourdough bread, matzot, pasta, and cooked grains of the test varieties. To screen out nontasters, *i.e.*, those who lack taste receptor(s) for one or more basic tastes, all prospective panel members took a blind taste test of sour, sweet, and salty solutions, each at low, medium, and high concentrations. For the soft wheat, baked

goods and pasta evaluations, prospective panelists were also tested on bitter solutions. To qualify as a panelist, each taster needed to accurately identify all taste groups and correctly label at least 78% of concentrations.

2.2.4.1 Sourdough bread and cooked grain evaluation

The panel for the sourdough bread and cooked grain tasting consisted of six professional bakers who participated in the baking evaluation and 24 consumers in the Ithaca, NY area who regularly purchase local sourdough bread. Training on flavor attributes (Table A.5) and on visual and texture characteristics (Table A.6) was held for six hours over two days. For the evaluation, bread made from each variety was cut into 7.62 cm diameter slices that included both crust and crumb. Slices were kept under cellophane until consumed. Whole grains of each variety were cooked using a 2:1 ratio of water to grain until *al dente*, drained, and refrigerated until served in 30 mL portions. Panelists tasted two replicates of the bread samples and one replicate of the cooked grains. Using a randomized complete block design, each panelist received one sample at a time. The tasting of both bread and cooked grain samples was completed in four and a half hours.

2.2.4.2 Matzah cracker and cooked soft wheat grain evaluation

The matzah cracker and cooked grain sensory panel consisted of seven students and two faculty members of the Culinary Institute of America and two research team members. Training in distinguishing ten flavors (Table A.7) and visual and mouthfeel characteristics (Table A.8) was conducted in nine hours over three days. For the matzah evaluation, each panelist was simultaneously given four, 11 cm diameter matzot. Cooked grain was prepared as stated in

Section 2.2.4.1. Each panelist was simultaneously presented with four 30 mL containers filled with cooked grain. Two replications of each evaluation were completed, with panelists alternating between evaluating matzot and cooked grain. No time limit was given for the evaluations, but all panelists completed the evaluations within three hours.

A preference tasting of cooked grain samples of the four soft wheat varieties was also held during an event on local grains that was open to the public. No training was held. Instead, 24 participants were each simultaneously presented with four cooked grain samples and were given written instructions that asked them to rank the samples according to preference and then answer questions on flavor attributes and their willingness to purchase.

2.2.4.3 Pasta and cooked emmer grain evaluation

Five instructors at The Natural Gourmet Institute, two food journalists, and five members of the research team completed a descriptive sensory analysis of varietal pasta and cooked emmer grain. Training in distinguishing ten flavors (Table A.7) and visual and mouthfeel characteristics (Table A.9) was conducted in six hours over one day. For the pasta evaluation, each panelist was simultaneously given three 30 mL cups filled with varietal pastas. Cooked emmer grain was prepared and evaluated as stated in Sections 2.2.4.1 and 2.2.4.2, respectively. Two replications of each evaluation were completed within three hours.

A preference tasting of cooked emmer grain samples of four emmer varieties was also held during a by-invitation only event. Twenty-six participants evaluated samples in a manner similar to the public preference tasting described in Section 2.2.4.2. The emmer tasted in this evaluation was grown in a different environment (Rock Springs, PA 2014) than the grains tasted by the trained sensory panel.

2.2.5 Statistical analysis

Statistical analyses were completed in R [version 3.2.2] (R Core Team 2015), package 'lme4' [version1.1-10] (Bates et al. 2015). Equation 2.1, similar to that used by Vindras-Fouillet et al. (2014), incorporated the effects of variety, panelist, order and their subsequent interactions. A reduced model was used if there was not a second replicate (*e.g.*, sensory evaluation of cooked grain) or an order term (*e.g.*, baking trials). For continuous responses, an analysis of variance (ANOVA) allowed the detection of differences among varieties, using a significance threshold of $p<0.05$. A Satterthwaite approximation facilitated the analysis of unequal variances (Satterthwaite, 1946). As a consequence of unbalanced data, package 'lmerTest' [version 2.0-32] (Kuznetsova et al. 2016) calculated either Type III ANOVA when interactions were significant, or Type II ANOVA to increase power when interactions were not significant. Tukey's honestly significant difference (HSD) made pairwise comparisons of varieties through the package 'multcomp' [version1.4-2] (Hothorn et al. 2008). To validate model assumptions, errors and random effects were checked for normal distribution, homogeneous variance, and independence.

Equation 2.1 $$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \alpha_i{:}\beta_j + \alpha_i{:}\gamma_k + \alpha_i{:}\delta_l + \beta_j{:}\gamma_k + \varepsilon_{ijkl}$$

Y_{ijkl}: response for variety i, panelist j, replicate k, and order l

μ: overall mean response
α_i: fixed effect of variety i
β_j: random effect of panelist j
γ_k: fixed effect of replicate k
δ_l: fixed effect of order l
$\alpha_i{:}\beta_j$: random interaction of variety i by panelist j
$\alpha_i{:}\gamma_k$: fixed interaction of variety i by replicate k
$\alpha_i{:}\delta_l$: fixed interaction of variety i by order l
$\beta_j{:}\gamma_k$: random interaction of panelist k by replicate k
ε_{ijkl}: experimental error associated with response i,j,k,l

For binomial responses, logistic regression models evaluated whether variety was significantly associated with the log odds of preference or flavor presence in a sample. A likelihood ratio test compared models with and without variety used in the model below. Varieties were determined to be significantly different using least-square means at a significance level of $p<0.05$. To validate model assumptions, the number of observations multiplied by the sample probability mean for each response needed to be greater than five. Results were graphed using the R package 'plotrix' [version 3.6-1] (Lemon 2006).

Equation 2.2 $$Y_{ijkl} = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{j2} + \beta_3 x_{k3} + \beta_4 x_{l4}$$

Y_{ijkl}: log odds of success (e.g. a flavor used to describe a sample, or selection of preference)

B_0: intercept log odds of reference sample and replicate
β_1: partial slope associated with variety
x_{i1}: fixed variable of variety i
β_2: partial slope associated with panelist
x_{j2}: random variable of panelist j
β_3: partial slope associated with replicate
x_{k3}: fixed variable of replicate k
β_4: partial slope associated with order
x_{l4}: fixed variable of order l

2.3 Results

2.3.1 Baking and pasta evaluations

2.3.1.1 Sourdough baking evaluation

There were significant differences among scores for varietal performance throughout the sourdough baking process, including mixing, floor time, make-up, proofing, loaf, and crumb quality (Figure 2.2). The three spring wheat varieties ('Glenn,' 'Tom,' and 'Red Fife') received the highest overall baking performance scores. Although the winter wheat varieties had lower protein content than the spring varieties, the overall baking score for 'Warthog' was not significantly different than for 'Red Fife' (p=0.1730). Bakers thought that all varieties made satisfactory loaves, except for the soft wheat 'Fredrick,' which was difficult to manage. Bakers recognized early in the process that 'Fredrick' was a soft wheat and even considered using a loaf pan for baking since the preshape did not look viable. There were also significant differences in loaf measurements among varieties (Table 2.2). In terms of loaf height, 'Glenn,' 'Tom,' and 'Warthog' made the highest loaves, and 'Fredrick' the lowest (p<0.0001). The circumferences of 'Glenn' and 'Appalachian White' loaves were smaller than 'Fulcaster,' 'Tom,' and 'Red Fife' (p=0.0088). The loaves made of 'Glenn,' 'Red Fife,' 'Tom,' and 'Warthog' were heavier than those of 'Appalachian White' and 'Fredrick' (p<0.001). Loaf volume and density did not differ significantly among varieties (p=0.1085 and 0.3367, respectively).

Table 2.2. Results of processing and sensory evaluations. Black and grey cells indicate values that tend to be more and less preferred, respectively. Letters show Tukey's significant difference at $P<0.05$. NE indicates not evaluated, *** indicates a significant difference at $P<0.0001$, ** at $P<0.001$, * at $P<0.05$, and '.' at $P<0.10$.

Evaluation	Variety Details		Processing and Sensory Results											
Sourdough Bread and Cooked Grain	**Growth Habit**	**Variety** name	**Sourdough Baking Score** 10=ideal	**Bread Height** cm	**Bread Weight** grams	**Bread Taste Intensity** 10=intense	**Average Air Bubble Size** cm	**Surface Texture** 10=rough	**Crumb Texture** 10=hearty	**Bread Graininess** 10=grainy	**Bread Dryness** 10=moist	**Bread Cohesion** seconds to dissolve	**Cooked grain Flavor** 10=intense	**Cooked grain Dryness** 10=moist
			***	***	***	P=0.0798.	***	***	***	***	***	***	***	P=0.9595
	Winter Wheat	Appalachian White	5.5d	6.5b	599de	5.2a	2.0ab	5.6a	6.7b	5.1ab	4.5ab	20.3b	3.3c	4.8a
		Fredrick	3.9e	5.1c	589e	5.5a	2.1b	6.7a	7.9a	5.6a	3.8b	20.7b	4.7ab	4.9a
		Fulcaster	6.2c	5.9b	612cd	5.1a	2.5ab	5.0ab	6.9b	5.3a	4.0ab	19.5b	4.1bc	4.8a
		Warthog	6.5bc	7.5a	615c	4.8a	2.4ab	5.6ab	6.6b	5.4ab	4.0ab	20.3b	5.4a	4.9a
	Spring Wheat	Glenn	7.7a	8.0a	652a	5.3a	2.3ab	3.7b	5.4c	3.9b	5.6a	27.8a	3.7c	4.6a
		Red Fife	6.8b	6.3b	622bc	5.7a	2.0ab	4.8ab	6.9ab	4.7ab	4.8ab	21.9ab	4bc	4.8a
		Tom	7.6a	7.4a	631b	5.4a	3.0a	3.9ab	6.5b	4.7ab	4.6ab	23.5ab	4.2abc	4.7a
Matzah Cracker, Yeast Bread, Shortbread, and Cooked Grain	**Growth Habit**	**Variety** name	**Matzah Making Score** 10=ideal	**Short-bread Score** 10=ideal	**Yeast Bread Baking Score** 10=ideal	**Matzah Visual Texture** 1=smooth	**Matzah Visual Shape** 10=jagged	**Matzah Roughness** 10=rough	**Matzah Graininess** 10=grainy	**Matzah Firmness** 10=hard	**Matzah Cohesion** seconds to dissolve	**Cooked grain Preferred** 1=best	**Cooked grain Texture** 10=chewy	**Cooked grain Dryness** 10=moist
			P=0.0166*	***	P=0.0327*	P=0.1131	P=0.4487	P=0.5123	P=0.0834.	P=0.1924	P=0.5456	***	***	***
	Winter Wheat	Forward	7.1a	6.4b	7.2ab	5.6a	5.0a	5.0a	5.6a	5.6a	16.0a	2.4ab	5.0ab	4.2b
		Fredrick	NE	NE	7.7ab	NE	NE	NE	NE	NE	NE	NE	NE	NE
		Pride of Genesee	5.2b	6.9b	6.0b	4.6a	4.7a	4.7a	5.9a	5.4a	15.2a	2.0b	6.5a	3.9b
		Susquehanna	6.4ab	5.6b	NE	4.7a	4.7a	4.7a	5.1a	5.4a	15.5a	2.4b	4.8b	5.2a
		Yorkwin	6.4ab	8.9a	7.2ab	5.2a	5.2a	4.2a	5.6a	6.2a	16.3a	3.0a	6.5a	3.6b
	Spring	Red Fife	NE	NE	7.9a	NE	NE	NE	NE	NE	NE	NE	NE	NE
Pasta and Cooked Grain	**Growth Habit**	**Variety** name	**Pasta Making Score** 10=ideal	**Pasta Preferred** probability	**Pasta Sheen** 10=shiny	**Pasta Surface Stickiness** 10=sticky	**Pasta Texture Starchiness** 1=starchy	**Pasta Roughness** 10=rough	**Pasta Graininess** 10=grainy	**Pasta Firmness** 10=chewy	**Pasta Cohesion** seconds to dissolve	**Cooked grain Preferred** probability	**Cooked grain Texture** 10=chewy	**Cooked grain Dryness** 10=moist
			NA	P=0.0888.	P=0.1284	P=0.7818	P=0.4005	P=0.0157*	***	***	P=0.0003**	P=0915.	***	P=0.4076
	Spring Emmer	Lucille	7	0.42a	5.2a	4.5a	5.1a	4.6a	3.9b	4.5b	11.1ab	0.19a	5.4b	4.7a
		North Dakota Common	6	0.19a	5.9a	4.6a	5.6a	3.5b	3.6b	3.6c	10.2b	0.42a	6.3a	4.6a
		Red Vernal	7	0.27a	4.8a	4.2a	4.9a	5.0a	5.7a	6.2a	13.5a	0.15a	6.2ab	4.3a

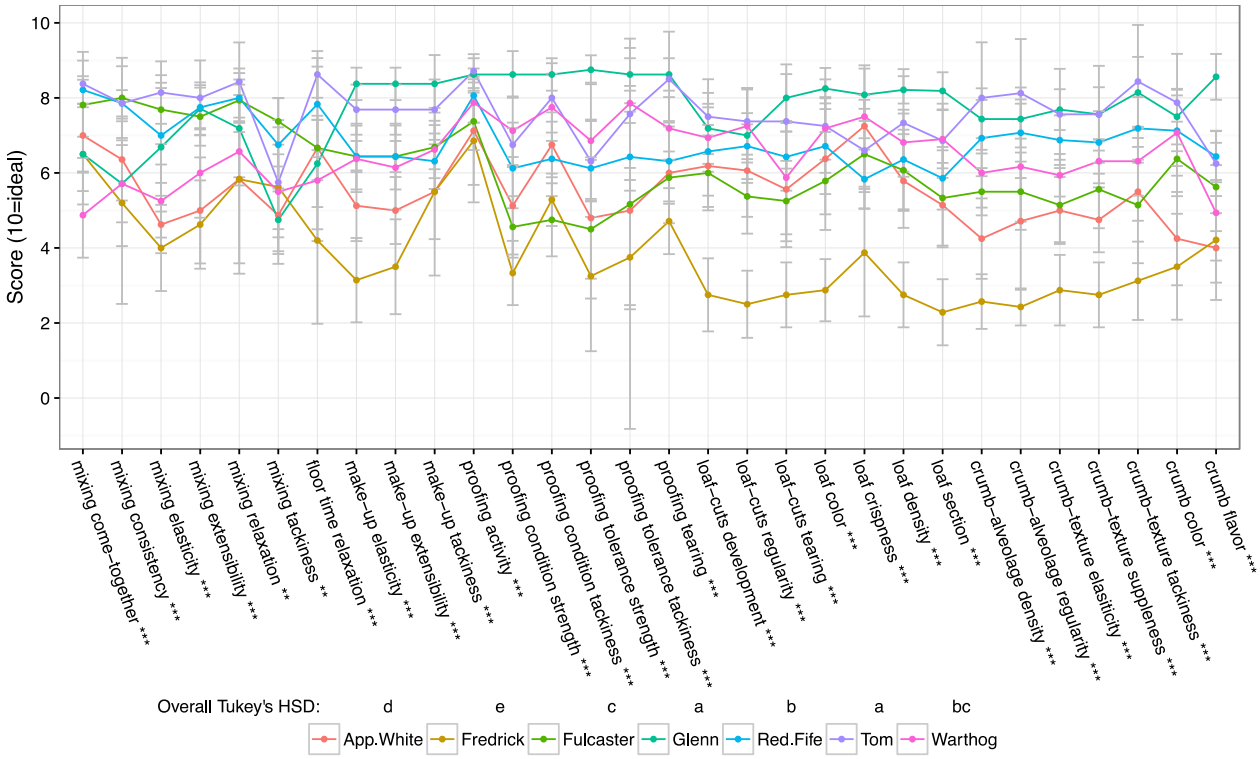

Figure 2.2. Varietal performance as rated by bakers in the sourdough bread baking evaluation. Significant differences are indicated by ** (p<0.01) and *** (p<0.001). Error bars show 95% confidence intervals.

2.3.1.2 Matzah cracker, yeast bread, and shortbread cookie soft wheat evaluation

In the evaluation of soft wheat varieties for yeast bread baking, the hard wheat check included in the evaluation, 'Red Fife,' received a significantly higher overall baking performance score than 'Pride of Genesee' (p=0.0396) (Figure 2.3). 'Fredrick,' which scored lowest in overall baking performance in the sourdough trial, did not score significantly lower than 'Red Fife' when made into yeast-based bread (p=0.9968). Both varieties tore less during proofing than the soft heritage wheat varieties (p=0.0077).

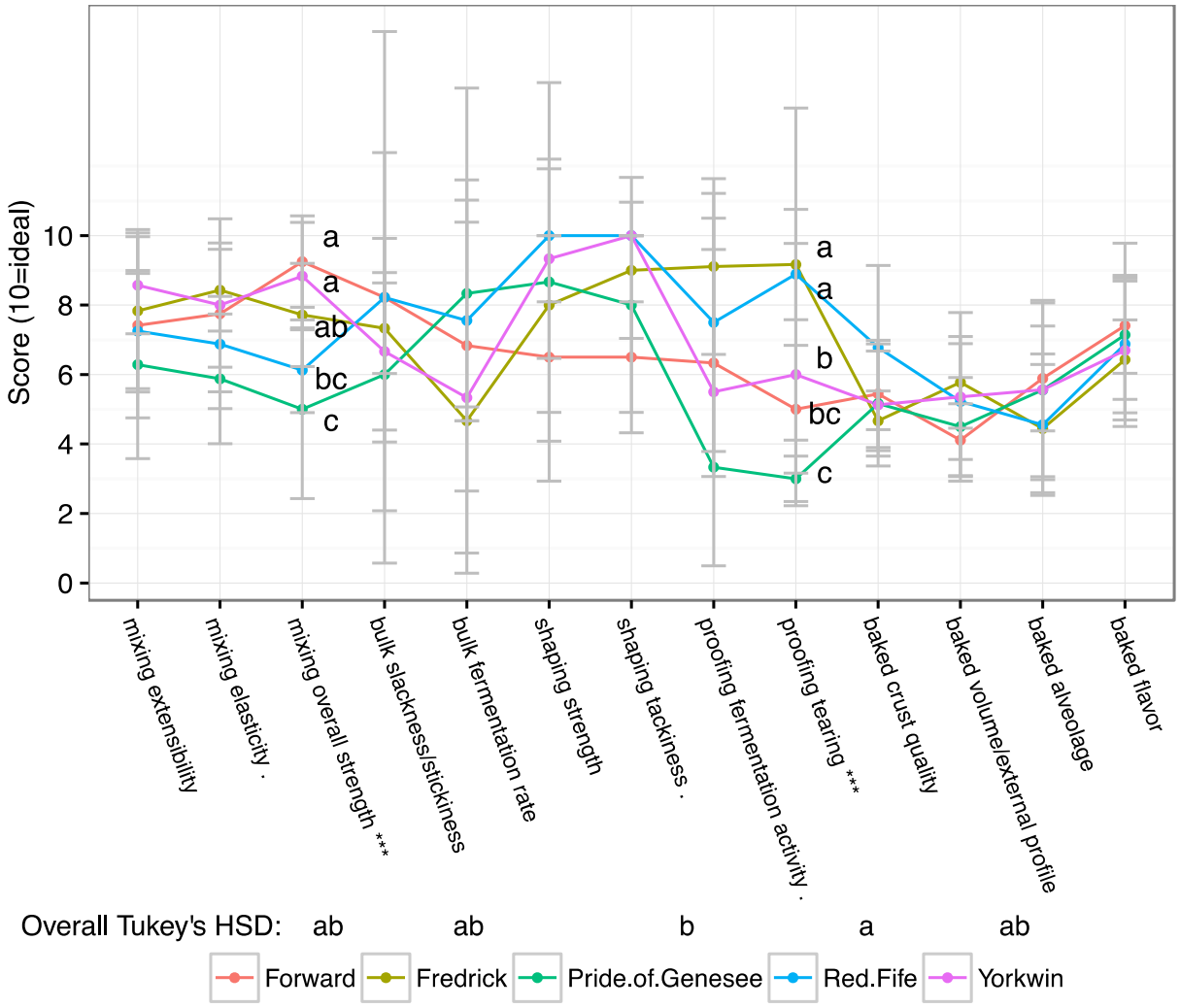

Figure 2.3. Varietal performance as rated by bakers in the yeast bread baking evaluation. Significant differences are indicated by '.' (p<0.10) and *** (p<0.001). Error bars show 95% confidence intervals.

In the production of matzah crackers, 'Forward' was rated as better than 'Pride of Genesee' (p=0.0024) (Figure 2.4). 'Pride of Genesee' had insufficient extensibility compared with all other varieties (p=0.0201). 'Yorkwin' and 'Susquehanna' needed more hydration than the other two varieties, which could reduce production costs by requiring less flour in the final product.

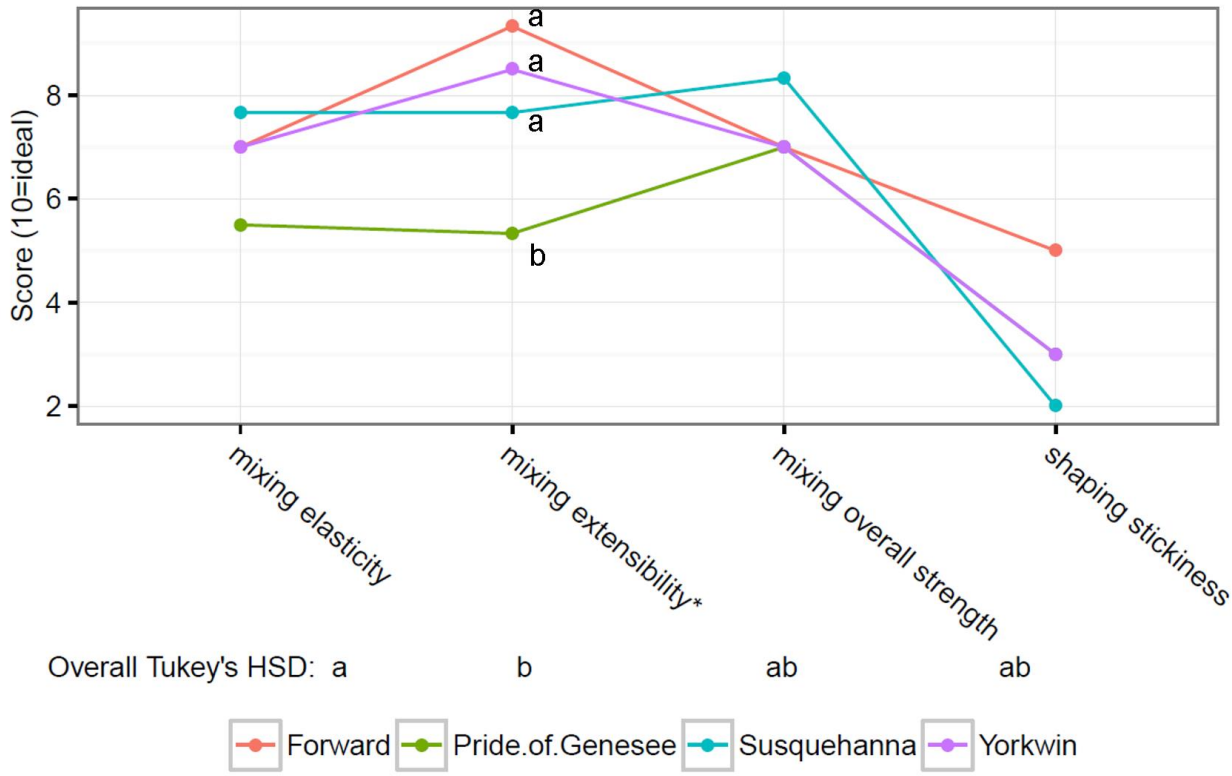

Figure 2.4. Varietal performance as rated by bakers in the matzot baking evaluation. Significant difference is indicated by * (p<0.05). Letters show Tukey's significant difference at p<0.05 for overall variety score in the matzah baking evaluation.

As a shortbread, 'Yorkwin' received a higher ranking than the other varieties for overall shortbread baking quality (p=0.0060) (Figure 2.5). 'Pride of Genesee' tended to have excessive stickiness during mixing, when compared to the top-rated variety, 'Yorkwin' (p<0.001). On the other hand, 'Pride of Genesee' could potentially lower production costs by absorbing less butter. Bakers tended to prefer the flavor of 'Forward' more than 'Susquehanna,' although the difference did not meet the threshold of significance (p=0.0615).

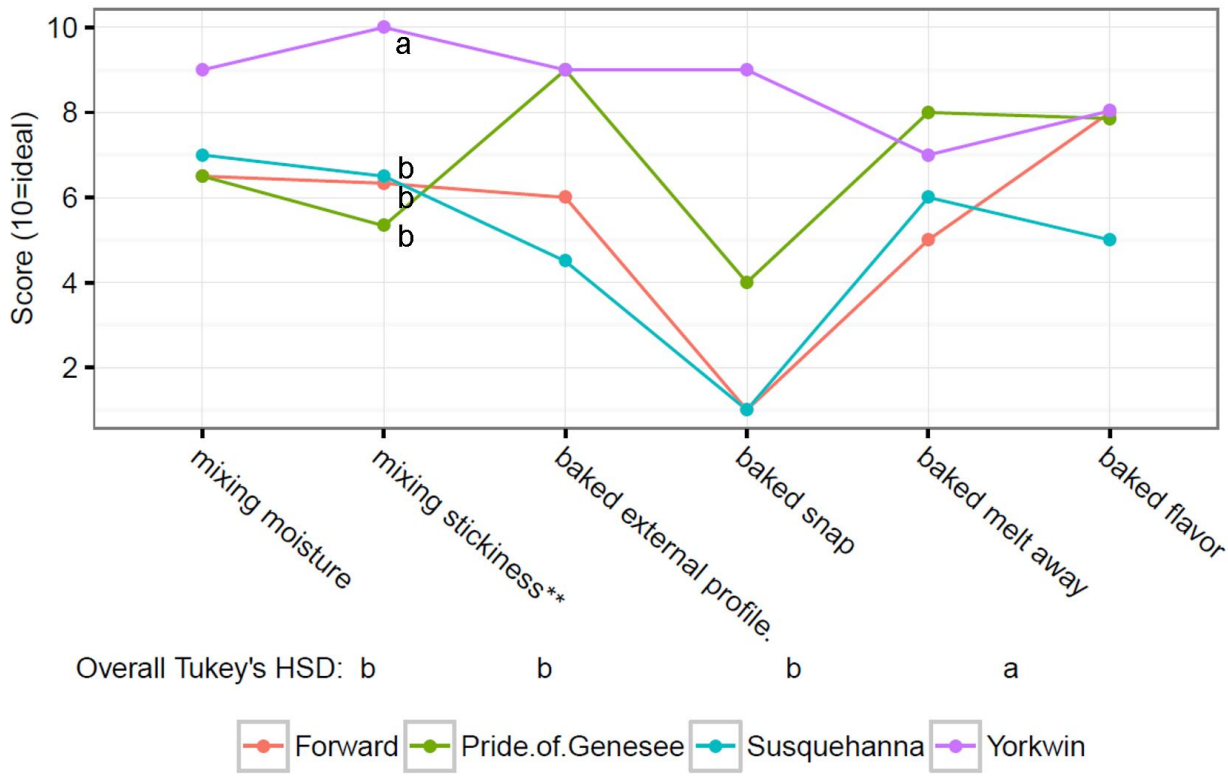

Figure 2.5. Varietal performance as rated by bakers in the shortbread baking evaluation. Significant differences are indicated by '.' (p<0.1) and ** (p<0.01). Letters show Tukey's significant difference at p<0.05 for overall variety score in the shortbread baking evaluation.

2.3.1.3 Pasta making evaluation

The pasta makers rated 'Lucille' and 'Red Vernal' as better than 'North Dakota Common' for pasta making. 'Lucille' and 'Red Vernal' received overall scores of seven out of ten, while 'North Dakota Common' scored four out of ten. 'Lucille' had the best technical performance, as it was strong and easy to roll out and cut with the machine. 'Red Vernal' produced the best texture and had the most preferred flavor by the pasta chefs. 'North Dakota Common' produced a very tacky dough, which demanded additional flour and more time in the pasta roller to obtain the right texture.

2.3.2 Sensory evaluations

2.3.2.1 Sourdough bread and cooked grain evaluation

There were significant differences among varietal sourdough for surface texture, texture of crumb, size of air bubbles, graininess, dryness, and ability to dissolve (Table 2.2). Although panelists assigned 'Red Fife' the highest and 'Warthog' the lowest flavor intensity, the difference was only significant if order of tasting was removed from the model (p=0.0278). 'Fulcaster' had lower odds of being described with bitter flavors, particularly when compared to 'Glenn' (p<0.0001) (Figure 2.6b). Variety also influenced the odds of nutty flavors in a sample (p=0.0498). Rather than variety, replicate impacted the aroma and sour flavor of samples. Overall aromatics of bread samples (p=0.0085), wheat aroma of crumb (p=0.0410), and odds of sour flavor (p=0.0218) were higher in the second replicate than the first.

When tasted as cooked grain, the trained panelists recorded differences among varieties for flavor intensity (Table 2.2). 'Warthog' had higher flavor intensity than 'Appalachian White' (p<0.001), 'Glenn' (p<0.001), 'Red Fife' (p=0.0040), and 'Fulcaster' (p=0.0271). When describing cooked grain samples, variety was significantly associated with the likelihood of dairy flavors (p=0.0291), with 'Fredrick' having the highest odds being described with dairy flavors (Figure 2.6a). While there were no significant differences in cooked grain dryness among varieties, panelists rated the first sample they tasted as moister (p=0.0434).

2.3.2.2 Matzah cracker and cooked soft wheat grain evaluation

The trained panel found differences in woody (p=0.0297) flavor intensity among varietal matzot, with 'Susquehanna' receiving the woodiest flavor (Figure 2.6d). 'Susquehanna' also had

lower odds of earthy flavors than 'Yorkwin' (p=0.0123) and 'Pride of Genesee' (p=0.0233). Replicate, rather than variety, influenced the fresh flavor intensity (p=0.0320) and odds of bitter flavor (p=0.0013), with higher values in the first replicate. There were no significant differences among varieties in visual texture, shape, roughness, graininess, firmness, and cohesion (Table 2.2). Order significantly influenced texture responses, with samples tasted first receiving heavier texture ratings (p=0.0137).

The trained panel found significant differences in dryness and texture of cooked grain from soft wheat varieties (Table 2.2). 'Susquehanna' was moister and less chewy than 'Pride of Genesee' and 'Yorkwin' (p=0.0410 and 0.0374, respectively). There were no significant differences in flavor characterization of cooked grain among the varieties (Figure 2.6c). Order was associated with fresh flavor (p=0.0184), with highest odds when tasted first, and lowest when tasted last. Replicate influenced the odds that a sample would be described as warming sweet (p=0.0386) and fresh (p=0.0010), with higher likelihood when tasted during the first replicate.

Participants in the public cooked grain tasting concurred with the findings from the trained panel, selecting 'Susquehanna' as the moistest variety (p<0.0001), and 'Pride of Genesee' and 'Yorkwin' as the chewiest varieties (p=0.0030). Among varieties ranked for personal preference by tasters, 'Pride of Genesee' was the most preferred, while 'Yorkwin' was the least preferred variety (p=0.0015). Moreover, tasters indicated that they would be more likely to purchase 'Pride of Genesee' than 'Yorkwin' (p=0.0146). There were also significant differences in the selection of the most flavorful variety (p=0.0015), with 'Yorkwin' having the lowest odds. The order in which cooked grain samples were tasted impacted preference. Samples tasted first were most preferred, while those tasted last were least preferred (p=0.0118).

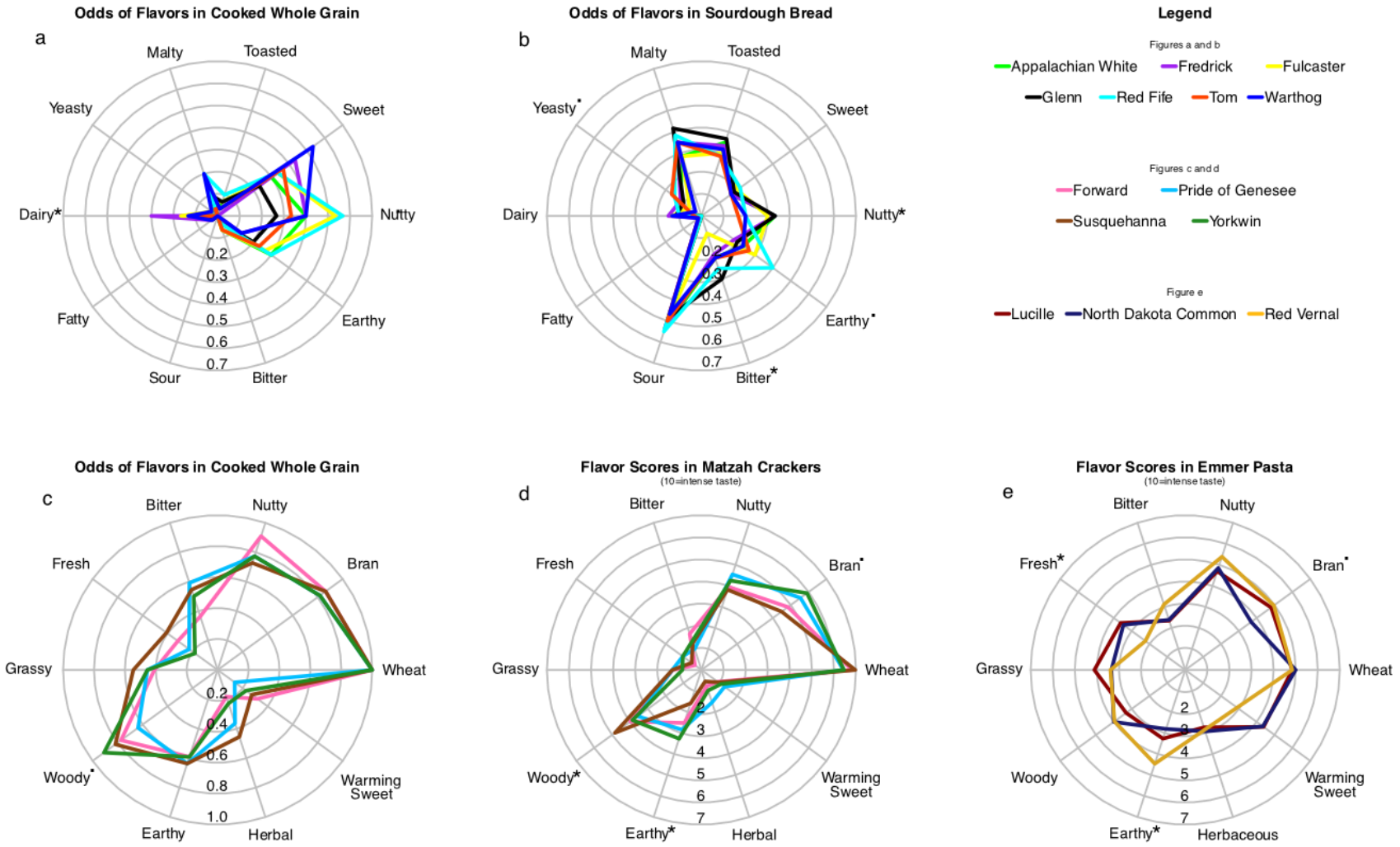

Figure 2.6. Flavor profiles of varietal products. Varietal effect on the likelihood that certain flavors would be used to describe (a) cooked wheat grain (b) sourdough breads, and (c) cooked soft wheat grain; and the intensity of certain flavors in (d) matzah crackers and (e) emmer pasta Significance is indicated by '.' at $P<0.10$ and * at $P<0.05$).

2.3.2.3 Pasta and cooked emmer grain evaluation

There were significant differences among emmer varieties for pasta roughness, graininess, firmness, and cohesion (Table 2.2). Shininess, surface stickiness, and starchiness of texture were not significantly different among varieties. Although there was no significant difference in preference for variety, 'Lucille' tended to have higher odds of being preferred than the other two varieties (p=0.0894). 'Red Vernal' was described as having earthier flavor (p=0.0101), and less fresh flavor (p=0.0434) than the other two varieties (Figure 2.6e). When panelists chose the least and most prominent flavors to describe each variety [data not shown], 'Red Vernal' had higher odds of nutty being described as the most prominent flavor (p=0.0034), and herbaceous as the least prominent flavor (p=0.0242).

When comparing emmer varieties tasted as cooked grain, the panel rated 'Lucille' as more delicate and less chewy than 'North Dakota Common' (Table 2.2). There were no significant differences among varieties for cooked grain flavor intensity (p=0.4406) or dryness (p=0.4076). 'Lucille' was also most likely to have nutty described as the most prominent flavor (p=0.0197) [data not shown]. 'North Dakota Common' was more likely to be preferred than 'Lucille,' although the difference was not significant (p=0.0915).

In the untrained public tasting of cooked grain, 'Lucille' was more likely to be rated with the highest flavor intensity than the other varieties (p=0.0004). Although tasters were twice as likely to seek out 'Lucille' for purchase than 'North Dakota Common,' the difference was not significant (p=0.2150).

2.4 Discussion

2.4.1 Baking evaluations

Varieties differed in baking quality for sourdough bread, yeast bread, matzah crackers, and shortbread cookies. However, the ranking of a variety differed among products. ‘Forward’ was the top scoring variety for making matzah crackers, yet it fell in the lowest ranked category for shortbread cookies. Although ‘Fredrick’ performed poorly in sourdough baking, it was not the lowest performer for yeast breads. Consequently, artisan bakers will not find one variety that performs best for all types of baked goods.

Many heritage wheat varieties that are classified as soft may be semi-hard. Bakers in the northeastern United States have wondered whether soft heritage varieties could, therefore, be appropriate for bread baking. Among the four soft heritage wheat varieties included in our evaluations, many did contain relatively high concentrations of protein (Table 2.1). However, the soft heritage wheat varieties included in this study represented a low to moderate spectrum of baking quality. In the sourdough trial, the soft heritage wheat ‘Fulcaster’ received intermediate scores for baking. It ranked better for overall baking, bread height, and weight than the soft modern wheat ‘Fredrick’ yet had lower bread height and wider circumference than the hard modern winter wheat ‘Warthog.’ When baked into yeast bread, three soft heritage varieties (‘Forward,’ ‘Pride of Genesee,’ and ‘Yorkwin’) received intermediate or low scores. Two of these soft heritage varieties did not significantly differ in overall baking scores from the high-quality baking check. However, all three varieties excessively tore when compared to both the high quality (‘Red Fife’) and low quality (‘Fredrick’) baking checks.

2.4.2 Sensory evaluations

Our results show that wheat and emmer varieties can differ in sensory characteristics, especially in terms of texture and mouthfeel attributes. Flavor differences among varieties were also detected, but tended to be subtler. However, sensory characteristics and preference for a variety often changed depending on what product was being evaluated. In all three sensory evaluations, the variety with the highest preference or taste intensity as a cooked grain received the lowest rating as a processed product. 'Warthog' was rated with the most intense flavor as a cooked grain, but received the lowest rating for flavor intensity when tasted as a sourdough bread. Similarly, the trained emmer taste panel gave 'North Dakota Common' the highest preference as a cooked grain and the lowest preference as a pasta. The least preferred cooked soft wheat grain was 'Yorkwin,' although this variety was most preferred when tasted as a varietal matzah cracker [data not shown]. A significant interaction between variety and product statistically demonstrates this point. For an emmer variety, the likelihood for preference, bran, nutty, fresh and earthy flavors depended on whether the variety was tasted as a cooked grain or pasta ($p<0.05$). Similar to Section 2.4.1, selecting the best variety depends on what product will be made from the selected variety.

Preference and overall flavor were correlated. There was a significant and positive correlation ($p<0.0001$, r=0.557) between odds of the variety being most flavorful and preference rating for cooked grain of soft wheat. There was also a significant and positive correlation ($p=0.01473$, r=0.2341) between the odds of the most intense and most enjoyable flavor. While preference is influenced by sensory factors beyond flavor, such as texture (Heiniö et al. 2016), the association between flavor and preference was also found in tomato (Baldwin et al. 1998) and carrot (Simon et al. 1980).

The order in which samples were tasted did influence many sensory responses, particularly the assessment of preference. The sample that was tasted first tended to be evaluated differently than other samples for preference, fresh flavor, and some texture components. This finding concurs with the documented "first sample effect" in sensory science (Stone et al. 2012), and emphasizes the importance of an experimental design that balances the placement of varieties in the first and last orders.

2.4.3 Inference from results

The complexity and diversity of wheat processing complicate the evaluation of genotypes for baking and sensory quality. Interpretation of our results may be limited, since all material was derived from one site (Freeville, NY). Moreover, the flour extraction rates (85% to 100% extraction rates) and baking methods used in this study will not always match the practices of regional millers and bakers. It becomes expensive and time consuming to add additional treatments to baking and sensory evaluations, such as: including varieties grown under multiple field conditions; using flour with varying extraction rates; and changing fermentation cultures, time, and temperature. Although the presented experimental design did not allow the assessment of genotype by environment, genotype by milling technique, and genotype by baking method interactions, inference from previous studies can illuminate the potential impact of these interactions on our results. Little is known about genotype by environment interactions on sensory characteristics in wheat, but results from other species indicate that there may be an effect. Significant genotype by environment interactions were detected for sweetness, bitterness, and roasted flavors in peanut (*Arachis hypogaea* L.) (Pattee et al. 1997) and protein, sucrose, citric acid, and malic acid in common bean (*Phaseolus vulgaris* L.) (Florez et al. 2009). Previous

studies also showed that genotype by baking technique influenced quality. In Katina et al. (2006), longer sourdough fermentation enhanced roasted and pungent acid flavors, while use of *S. cerevisiae* reduced roasted flavors by metabolizing the amino acids associated with those flavors. The authors also demonstrated that longer fermentation increased loaf volume. Genotype by milling technique, however, may exert the largest influence on quality parameters. In Kihlberg et al. (2004), milling technique influenced bread quality more than the environment in which the wheat was grown. Katina et al. (2006) documented more bitterness and aftertaste in bread made from higher bran flour. In their study, ash content influenced sourdough bread flavor more than temperature, length of fermentation, and type of sourdough culture.

To assess genotype by environment and genotype by milling technique interactions in the present study, the results can be compared to another variety evaluation completed by Mallory et al. (2014, 2015). The study tested similar varieties and sourdough baking techniques, but used different growing environments (Alburgh, VT 2010-2012) and milling techniques (85 to 95% extraction) than the present paper (Freeville, NY 2012-2014 and 100% extraction, respectively). In both evaluations, bakers felt that all varieties made satisfactory loaves of bread, apart from 'Fredrick.' The top-rated spring and winter wheat varieties in both evaluations included 'Glenn' and 'Warthog.' However, in Mallory et al. (2014), 'Tom' displayed excessive dough extensibility and low volume, while the bakers in the current study gave 'Tom' the best score for dough extensibility and second highest score for loaf volume. In another contrast, 'Appalachian White' received the second highest baking score in Mallory et al. (2015), while bakers in the present study rated it second lowest. The differences in variety rankings between the evaluations confirm that genotype by environment and/or genotype by milling interactions influence sourdough baking quality for organically-grown wheat.

2.4.4 Recommendations for high-throughput evaluations

Descriptive sensory analysis is costly and time-intensive. Moreover, the number of tested varieties is limited, since panelists can only handle a small number of samples before reaching sensory fatigue (Stone et al. 2012). Plant breeding, which handles large numbers of genotypes, requires more high-throughput sensory analysis methods. Our results suggest that unreplicated designs could improve throughput. For 58 continuous responses included in the sensory analysis, only four had a significant interaction between variety and replicate (wheat aroma of crust, matzot nutty flavor intensity, pasta grassy flavor intensity, and pasta fresh flavor intensity). These results indicate that unreplicated or partially replicated designs may generate accurate data for most sensory descriptors, thereby allowing the evaluation of more varieties at lower cost.

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CHAPTER 3

A GROUNDED GUIDE TO GLUTEN: HOW MODERN GENOTYPES AND PROCESSING IMPACT WHEAT SENSITIVITY[3]

Abstract

The role of wheat, and particularly of gluten protein, in our diet has recently been scrutinized. This article provides a summary of the main pathologies related to wheat in the human body, including celiac disease, wheat allergy, nonceliac wheat sensitivity, fructose malabsorption, and irritable bowel syndrome. Differences in reactivity are discussed for ancient, heritage, and modern wheats. Due to large variability among species and genotypes, it might be feasible to select wheat varieties with lower amounts and fewer types of reactive prolamins and fructans. Einkorn is promising for producing fewer immunotoxic effects in a number of celiac research studies. Additionally, the impact of wheat processing methods on wheat sensitivity is reviewed. Research indicates that germination and fermentation technologies can effectively alter certain immunoreactive components. For individuals with wheat sensitivity, less-reactive wheat products can slow down disease development and improve quality of life. While research has not proven causation in the increase in wheat sensitivity over the last decades, modern wheat processing may have increased exposure to immunoreactive compounds. More research is necessary to understand the influence of modern wheat cultivars on epidemiological change.

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[3] Lisa Kissing Kucek, Lynn D. Veenstra, Plaimein Amnuaycheewa, and Mark E. Sorrells. A Grounded Guide to Gluten: How Modern Genotypes and Processing Impact Wheat Sensitivity. *Comprehensive Reviews in Food Science and Food Safety*, 14(3), pp.285–302. DOI: http://doi.wiley.com/10.1111/1541-4337.12129 with kind permission from John Wiley and Sons

3.1 Introduction

Wheat (*Triticum* spp.) has been consumed by humans for over 8500 years, and currently supplies about 20% of global dietary protein (Braun et al. 2010). Recently, the role that wheat, and particularly gluten proteins, should play in our diet has been scrutinized. The public discourse, however, often vacillates between extreme viewpoints on the basic question, "Is gluten good or bad for human health?" The facts are often muddled and incomplete on both sides. Gluten-free diet promoters have described modern wheat as a "perfect, chronic poison" (Davis 2011), while commodity groups have countered that "wheat gluten isn't bad" (National Association of Wheat Growers 2013).

Divided viewpoints also exist when interpreting epidemiological trends. Although studies have suggested that celiac disease has increased two- to four-fold over the last 50 years (Lohi et al. 2007; Rubio-Tapia et al. 2009), causes have not been fully determined. Several authors have questioned whether the last 60 years of breeding produced wheat varieties with more reactivity (Davis 2011; Junker et al. 2012), while others consider modern wheat processing to be implicated in epidemiological changes (Di Cagno et al. 2010). Without understanding why wheat sensitivity has increased in the population, policy makers cannot effectively address the problem. To help inform consumers, researchers, and policy makers, this article provides a comprehensive summary of: (1) the compounds in wheat that can cause wheat sensitivity; (2) the pathologies associated with wheat components in the human body; (3) the differences in reactivity among ancient, heritage, and modern wheats; and (4) the impact of processing methods on wheat components and wheat sensitivity.

3.2 Components in wheat that can cause sensitivity

A grain of wheat is mostly composed of carbohydrates, proteins, lipids, and minerals (Figure 3.1). While these components can provide basic dietary sustenance for most people, consuming wheat causes negative responses in a small subset of the population. Not all components of the wheat kernel, however, are equally causative of sensitivity to wheat. The compounds implicated in wheat sensitivity, which are labeled in gray in Figure 3.1, tend to have structures that are difficult for digestion to break apart. This section introduces wheat proteins and fructans, which are most commonly implicated in various types of wheat sensitivity.

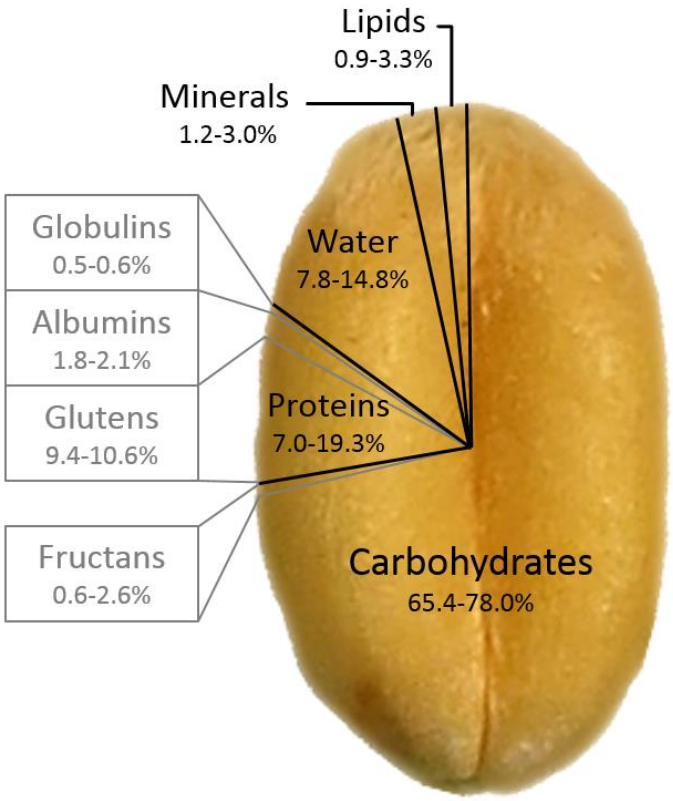


Figure 3.1 Wheat kernel composition. Components of a wheat kernel with variability reported in the literature (Davis and others 1980, 1981; Wadhawan and Bushuk 1989; Wieser and others 1998; Posner 2000; Gafurova and others 2002; Huynh and others 2008; Veenstra 2014). Major components are labeled in black and specific compounds implicated in gluten sensitivity are labeled in gray. Protein fractions reported in the literature were converted to proportion of total grain by multiplying by an average protein content of 12.6%.

Wheat proteins can be classified into three main types called gluten, globulin, and albumin. While glutens mainly supply nitrogen to growing seedlings, globulin and albumin proteins serve other specific functions, such as for enzymes, enzyme inhibitors, and structural elongation. The term gluten defines a very diverse and complex group of two water-insoluble wheat proteins: gliadin and glutenin. Gliadins are prolamin proteins which are rich in proline and glutamine. The hydrophobic proline is relatively bulky, and thus provides viscosity to dough, allowing it to flow and rise. With a classification system based on repetitive amino acid sequence patterns, gliadins can be grouped into α-, β-, γ -, and ω- types. Glutenins are polymeric proteins that provide the elasticity and strength to dough, allowing bread to hold its shape. Glutenins can be classified based on electrophoretic mobility at acidic pH into high molecular weight (HMW) and low molecular weight (LMW) types. Similar storage proteins in barley (*Hordeum vulgare* L.) and rye (*Secale cereale* L.) are termed hordein and secalin, respectively.

During gastrointestinal digestion, each type of wheat protein breaks down into a wide array of peptides of varying lengths. The rich proline residues in glutens create tight and compact structures that can be difficult to digest (Arentz-Hansen et al. 2002). Certain types of these digestion-resistant gluten peptides are found to mediate adverse immune reactions in predisposed individuals.

Amylase-trypsin inhibitors (ATIs), which are albumin proteins, are also implicated in wheat sensitivity. As plant defense proteins, ATIs can block animal enzymes from digesting starch and glycogen in the grain. ATIs have diverse conformational structures that are specific to the enzymes of different animal species, leading some ATIs to affect insect pests without acting strongly against human enzymes (Franco et al. 2000). ATI fractions 0.19 and 0.38, which are classified based on fractionation in chloroform and electrophoretic mobility, were found to be

active against α-amylase in human saliva and pancreas, respectively (Choudhury et al. 1996). ATIs are also found in wheat, rye, triticale, and barley.

In addition to seed proteins, wheat also contains clinically relevant carbohydrates known as fructans. Fructans are fructose polymers with, or without, one glucose conjoined by β-glycosidic linkages (Haska et al. 2008). Fructans can be classified based on their β-glycosidic bond pattern (linear or branched) and the degree of polymerization (short or long). Linear fructans include inulin and levan/phlein which contain β(2–1) and β(2–6) bonds, respectively. Branched fructans are graminan-type and contain a mixture of β(2–1) and β(2–6) bonds. In wheat, these polymers serve the purpose of increasing tolerance to cold and drought (Calderon and Pontis 1985; Hendry 1993).

Fructans are considered dietary fiber as humans are unable to hydrolyze the β-glycosidic bonds. Fructans pass through the upper gastrointestinal tract without undergoing digestion and arrive in the large intestine, where *Bifidobacteria* and other probiotics can utilize and cleave the β-linkages (Playne and Crittenden 1996). Fructans are generally beneficial for most individuals by promoting the growth of healthy gut probiotics, improving stool frequency, and adding fecal bulk (Den Hond et al. 2000; Roberfroid 2005; Kleessen et al. 2007). Evidence indicates that fructans may reduce fasting insulin levels and thus regulate satiety (Jackson et al. 1999; Maziarz 2013) as well as increase absorption of minerals and trace elements (Scholz-Ahrens and Schrezenmeir 2007). However, consumption of high levels of fructans (>15 g/d) may increase bloating, flatulence, and abdominal discomfort (Grabitske and Slavin 2009). While the United States population consumes an average of 3.91 g fructan/d, which is well below the 15 g/d threshold, other global populations consume up to 20 g/d (Moshfegh et al. 1999; Shepherd and Gibson 2006).

Although wheat is the major source of fructans in American diets, fructans are also found in 15% of all flowering plants, including artichoke, banana, broccoli, garlic, leek bulb, melon, onions, white peach, and rye (Nelson and Smith 1986; Roberfroid 2005; Muir et al. 2007; Fedewa and Rao 2014). Recently, fructans have been grouped into a large family of dietary carbohydrates called fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs), which can be fermented by bacteria in the large intestinal tract. In addition to fructans, FODMAPs includes sorbitol (stone fruits), raffinose (legumes, lentils, cabbage, Brussels sprouts), and lactose (dairy; Shepherd et al. 2008).

3.3 Disease pathologies associated with wheat

This chapter and Table 3.1 review the various sensitivities and intolerances that are found to relate to wheat components, including: wheat allergy, celiac disease, nonceliac wheat sensitivity (NCWS), fructose malabsorption, and irritable bowel syndrome (IBS).

3.3.1 Celiac disease

Celiac disease is defined as a chronic, immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals with human leukocyte antigens (HLAs) DQ2 and/or DQ8 (Ludvigsson et al. 2013). During digestion, some wheat proteins that are resistant to proteolytic degradation create relatively large peptides. In individuals with celiac disease, some gluten peptides behave like stress-inducing agents that modulate intestinal epithelia and immune cells (Tuckova et al. 2002; Londei et al. 2005; Thomas et al. 2006; Cinova et al. 2007), while a few gluten peptides mediate increased intestinal epithelial permeability and increase peptide contact with reactive immune cells (Fasano et al. 2000; Clemente et al. 2003;

Lammers et al. 2008; Tripathi et al. 2009). The digestion-resistant gluten peptides are translocated or absorbed to lamina propria (Terpend et al. 1998; Schumann et al. 2008) where the peptides bind to HLA DQ2 or DQ8 on antigen presenting cells. Due to presence of glutamine and proline in the amino acid sequence, a number of gluten peptides directly bind DQ2 or DQ8 in the binding groove while other peptides require prior modification to enhance binding. The HLA DQ2 and DQ8 receptors preferentially bind peptides with negatively charged amino acids and bulky amino acids at certain anchor residues (Tjon et al. 2010). Moreover, tissue-bound transglutaminase selectively deamidates glutamine to create glutamic acid, which allows certain gluten peptides to fit in the binding pockets of HLA DQ2 and DQ8 (van de Wal et al. 1998; Arentz- Hansen et al. 2000; Vader et al. 2002a; Kim et al. 2004; Stepniak et al. 2005). Once bound to HLA, antigen-presenting cells deliver gluten peptides, called T-cell epitopes, to T-cells. The gluten-restricted T-cells proliferate and differentiate into effector Th1 cells that mediate intestinal inflammation through secretion of proinflammatory cytokine interferon-gamma (IFN-γ). The T-cells reactive to the tissue transglutaminase could also lead to destruction of the epithelia through generation of autoreactive antibody (Salmi et al. 2006; Lindfors and Kaukinen 2012).

Table 3.1. Wheat-related sensitivities, prevalence, and wheat components responsible for the disease pathologies.

Disorder	Prevalence in various populations	Commonly potent reactive components in wheat	Less commonly reactive components in wheat	References
Celiac disease	0.5%–2%	α- and ω-gliadins, CM3 and 0.19 ATIs	γ-gliadins, HMW and LMW-glutenins	Rewers 2005; Tye-Din and others 2010b
Wheat allergy	0.2%–0.5%	–	–	Zuidmeer and others 2008; Vu and others 2014
Baker's asthma	–	ATIs, LTPs, and serpins, α- and ω-gliadins	γ-gliadins, peroxidase, LMW and HMW glutenins	Sanchez-Monge and others 1997; Sandiford and others 1997
Atopic dermatitis	–	LTPs, CM3 ATIs, gliadins and glutenins	–	Kusaba-Nakayama and others 2000; Battais and others 2005b
Urticaria	–	ω-5 gliadin	ω-1,2 gliadin, LMW glutenins, ATIs	Battais and others 2005b
Anaphylaxis	–	ω-5 gliadin, LMW glutenin	ATIs, α-,γ-, and ω-gliadins	Battais and others 2005a; 2005b; Morita and others 2009
Fructose malabsorption	11%–38%[a]	Fructans	–	Truswell and others; Born and others 1995; Ladas and others 2000; Barrett and others 2009
Nonceliac wheat sensitivity	0.55%[a]	ATIs, Fructans (more research is needed to confirm the impact of fructans)	–	Biesiekierski and others 2013; Digiacomo and others 2013; Junker and others 2012
Irritable bowel syndrome	11.5%–14.1%	Fructans with low degrees of polymerization	Fructans with high degrees of polymerization	Roberfroid 1993; Brighenti and others 1995; Rumessen and Gudmand-Høyer 1998; Hungin and others 2005

[a] A large-scale epidemiological study has not been conducted.
LTPs, lipid transfer proteins; ATIs, amylase-trypsin inhibitors.

Glutens are the major causative antigens in celiac disease. Gluten peptides recognized by T cells in the context of DQ2 and DQ8 have been identified in gliadins, glutenins, hordeins, and secalins. Tye-Din et al. (2010b) reported that α- and ω-gliadins appear to harbor most T-cell-recognized epitopes, while fewer T-cell epitopes are found in γ-gliadins and glutenins. A digestion-resistant α-gliadin peptide, LQLQPFPQPQLPYP QPQLPYPQPQLPYPQPQPF, referred to as 33-mer, is one of the highly immunogenic peptides that is often used as a marker for celiac immunoreactivity (Arentz-Hansen et al. 2000). In addition to glutens, some wheat ATIs are also considered causative agents that mediate intestinal inflammation by binding to toll-like receptor 4 (TLR4; Junker et al. 2012).

Celiac disease is diagnosed through villous atrophy in the small intestine, which is associated with symptoms of poor nutrient absorption, diarrhea, pain, and weight loss. Celiac disease can also be manifested though specific skin symptoms, called dermatitis herpetiformis, and neurological symptoms, called gluten ataxia (Fabbri et al. 2012; Jackson et al. 2012). Individuals with celiac disease are more likely to develop other autoimmune disorders during their lifetimes, such as type 1 diabetes and thyroid disease (Ventura et al. 1999). Although the vast majority of cases are likely undiagnosed, celiac disease is considered the most common genetically related autoimmune disease in the world, affecting 0.5% to 2% of global populations (Rewers 2005). It has been estimated that the safe threshold of gluten consumption for individuals with celiac disease ranges from 10 to 100 mg/d (Hischenhuber et al. 2006).

3.3.2 Wheat allergy

Individuals with food allergies have elevated amounts of immunoglobulin E (IgE) antibodies specific to certain food allergens. Upon exposure, allergens bind to IgEs on mast cells

or basophils and trigger the release of histamine and other chemicals that mediate the immediate allergic reactions. Nearly all food allergens are proteins that tend to resist degradation from heat, proteases, or acid hydrolysis. Peanut, milk, egg, tree nuts, wheat, crustaceans, fish, and soybeans are the most common allergenic foods (Taylor and Hefle 2001). Symptoms of wheat allergy encompass baker's asthma and rhinitis, which results from inhaled flour; atopic dermatitis, which relates to skin exposure; urticaria, which forms hives after contact with wheat; and anaphylaxis, which is the most life-threatening type of wheat allergy and affects many body systems (Battais et al. 2008).

An estimated 0.4% of the world's population is allergic to wheat proteins (Zuidmeer et al. 2008; Vu et al. 2014). Various types of wheat allergies differentially affect subsets of the population. The majority of wheat allergy cases are found in children, which are dominated by atopic dermatitis and digestive symptoms (Hischenhuber et al. 2006). On average, sufferers outgrow their wheat allergy by age six (Keet et al. 2009). Wheat-dependent exercise-induced anaphylaxis (WDEIA) results when wheat is consumed before vigorous physical activity and most commonly manifests in teenagers. Baker's asthma and rhinitis are predominantly occupational hazards for bakers and millers, who are exposed to large amounts of airborne flour particles (Walusiak et al. 2004).

Wheat allergens are found in a number of glutens, albumins, and globulins (see Table 3.1 and reviews by Battais et al. 2008; Tatham and Shewry 2008). Omega-5 gliadins are the major allergens in WDEIA and urticaria (Battais et al. 2005a, 2005b; Morita et al. 2009). ATI CM3, α-, and γ-gliadins were linked to atopic dermatitis (Kusaba-Nakayama et al. 2000; Tanabe 2004). While a number of albumins and globulins, including ATIs, lipid transfer proteins, and serpins were found to be the strongest triggers of baker's asthma, α- and ω-gliadins as well as LMW

glutenins were also causative (Weiss et al. 1993; Sandiford et al. 1997). It has been reported that, in general, wheat allergy sufferers can tolerate larger amounts of wheat than celiac patients. More than one gram of wheat was necessary to induce symptoms in most adults with wheat allergy, although a minority of children experienced reactions after less than ten milligrams of exposure (Hischenhuber et al. 2006).

3.3.3 NCWS, fructose malabsorption, and IBS

Apart from celiac disease and wheat allergy, various ill-defined adverse reactions to wheat are grouped as NCWS (Sapone et al. 2012). However, the role(s) of gluten and other wheat components in mediating NCWS remains unclear. Some individuals with NCWS suffer an innate immune reaction that is mechanistically similar to, but less severe than, celiac disease (see reviews by Verdu et al. 2009; Catassi et al., 2013). In comparison to the general population, NCWS patients appear to have a higher incidence of the HLA DQ2 or DQ8 genetic disposition (Wahnschaffe et al. 2001, 2007), and high levels of gluten-specific antibodies (Carroccio et al. 2012; Volta et al. 2012). Individuals with NCWS, however, lack the villous atrophy characteristic of celiac disease. Certain ATIs appear to implicate NCWS as well. Junker et al. (2012) reported that ATI fractions CM3 and 0.19 activated a TLR4-dependent pathway leading to the release of proinflammatory cytokines from monocytes, macrophages, and dendritic cells derived from both celiac and nonceliac patients. The authors hypothesized that individuals with poorly regulated TLR4 could experience inflammation induced by wheat ATIs.

Carroccio et al. (2012) reported that 70 of 276 NCWS patients exhibited disease pathology similar to celiac disease. The remaining majority of patients (206 of 276), however, demonstrated allergy-like hypersensitivity to wheat in addition to a broad array of other foods.

However, a double-blind placebo-controlled challenge on 920 total NCWS patients revealed that 644 patients (70%) did not react to wheat in the diet. Another double-blind placebo-controlled cross-over study compared 22 NCWS individuals consuming a gluten-containing diet and a nondairy whey protein-containing diet. There was no significant difference in pain, bloating, bowel movements, tiredness, gas, or nausea between the two diet types. Patients only experienced reduced gastrointestinal discomfort when placed on a low-FODMAP diet (Biesiekierski et al. 2011, 2013). As Carroccio et al. (2012) did not control for FODMAPs in study diets, it is possible that FODMAPs could have contributed to symptoms of the 70% of NCWS patients who did not respond to wheat alone. The prevalence of NCWS is not known, although one study from 2010 estimated that 0.55% of individuals in the United States follow a gluten-free diet and do not have celiac disease (Digiacomo et al. 2013).

Some individuals with NCWS may suffer from fructose malabsorption rather than gluten sensitivity (see review by Gibson et al. 2007 and Fedewa and Rao 2014). In such cases, consumption of wheat fructans can provoke symptoms *via* fructose malabsorption (Shepherd and Gibson 2006; Ong et al. 2010). Individuals with fructose malabsorption are unable to absorb free fructose present in the digestive tract. The unabsorbed fructose undergoes bacterial fermentation and induces abdominal symptoms, such as pain, bloating, and altered bowel habit. Fructose malabsorption can be easily diagnosed through standard testing of hydrogen and methane in the breath following fructose consumption (Fedewa and Rao 2014). The diagnosis of fructan intolerance is less straightforward than fructose malabsorption; however, a protocol for a diagnosis of fructan intolerance was under development (Fedewa and Rao 2014). Although many studies have examined fructose malabsorption in individuals, the lack of standardization in testing and doses have resulted in no firm estimates of the prevalence of fructose malabsorption

in the population (Latulippe and Skoog 2011). Between 11% and 38% of healthy individuals have experienced fructose malabsorption when consuming fructose levels that are typical of daily consumption rates (Truswell et al. 1988; Born et al. 1995; Ladas et al. 2000; Barrett et al. 2009). The prevalence of malabsorption rises with higher consumption rates of fructose (Gibson et al. 2007).

Individuals with IBS may also react to wheat in the diet. Fructan ingestion likely causes discomfort for all IBS individuals because only 5% to 15% of ingested fructans are absorbed in the small intestine (Fedewa and Rao 2014). The low absorption rate results in large quantities of fructans entering, and undergoing fermentation in the large intestine of IBS-affected individuals. This fermentation can further aggravate symptoms in all IBS individuals, regardless of whether or not individuals are fructose intolerant. Patients with diarrhea-predominant IBS exhibited significantly higher small bowel permeability, reduced expression of tight-junction proteins regulating intestinal permeability, and increased frequency of bowel movements on a wheat-containing diet (Vazquez-Roque et al. 2013). The consumption of low-FODMAP diets is associated with declines in symptom severity for individuals with IBS (de Roest et al. 2013; Halmos et al. 2014). Nevertheless, low levels of fructan consumption may help manage IBS by stimulating *Bifidobacterium* growth in the gut (Roberfroid et al. 2010). Further research is needed to determine the daily fructan intake level that best minimizes symptoms in IBS-affected individuals. IBS is a very prevalent disease that affects an estimated 14.1% of the United States population (Hungin et al. 2005) and 11.5% of Europeans (Hungin et al. 2003).

Misdiagnosis is common among NCWS, fructose malabsorption, and IBS. For example, it is estimated that up to one-third of patients with suspected IBS, particularly IBS with diarrhea-predominant symptoms, actually suffer from fructose malabsorption (Choi et al. 2008). IBS,

NCWS, and fructose malabsorption share a broad array of symptoms (Muir et al. 2007; Verdu et al. 2009). To further challenge correct diagnosis, the mechanisms of disease pathology remain unknown for NCWS and IBS. Regardless of uncertain diagnosis, consumption of fructans by individuals with fructose malabsorption, NCWS, or IBS is not recommended due to potential aggravation of symptoms (Roberfroid et al. 2010).

Given that all fructan types are predominantly composed of fructose, small amounts of any ingested fructan will be digested to fructose and likely aggravate symptoms in fructose-intolerant individuals (Roberfroid 2005; Jenkins et al. 2011). Shepherd and Gibson (2006) indicate that the chain length of fructans, rather than structure type, is an important factor in the amount of discomfort as a result of ingestion. Fructans with a low degree of polymerization induce IBS-like symptoms, have a greater osmotic effect, and are more rapidly fermented than fructans with higher degrees of polymerization (Roberfroid 1993; Brighenti et al. 1995; Rumessen and Gudmand-Høyer 1998).

3.4 Variation in reactivity among species and varieties of wheat

"Wheat" is a term loosely used to include a diverse array of cultivated species and genotypes in the *Triticum* genus (Figure 3.2). As coding regions for wheat storage proteins are highly polymorphic (Payne 1987; Metakovsky et al. 1991; Salentijn et al. 2013), each genotype produces unique types and quantities of glutens, ATIs, and fructans (Nakamura et al. 2005; Veenstra 2014). Consequently, wheat varieties can be assigned a "*reactivity profile*," which indicates the potency and amount of reactive epitopes created after digesting that specific wheat variety. However, the protein and fructan expression of one genotype can change depending on the environment where it was grown. Moreover, reactivity profiles are not universal, as patients

differentially react to glutens, ATIs, and fructans. This section reviews the types of cultivated wheat, and evaluates their relative reactivity for celiac disease, wheat allergy, NCWS, fructose malabsorption, and IBS.

3.4.1 Types of wheat

The world's most widely grown crop species is common wheat (*T. aestivum* L.), which is otherwise known as hexaploid wheat, or bread wheat. Tens of thousands of varieties of common wheat are grown around the world. Modern wheat generally refers to varieties that were developed after the use of dwarfing genes in the 1950s, while heritage wheat varieties were developed before that time period. Landraces, which can be a mixture of genotypes, are categorized as heritage varieties in this review.

Common wheat contains three genomes (A, B, and D) that were derived from different ancestors. Ancient wheat refers to the hulled relatives of common wheat, which are separate species that contain different combinations of the three wheat genomes. The oldest cultivated ancient wheat is einkorn (*T. monococcum* L. ssp. *monococcum*), which is a diploid with only the A genome. Tetraploid species that share the A and B genomes with common wheat are emmer (*T. turgidum* L. ssp. *dicoccum* Schrank ex Schu¨bl.), durum [*T. turgidum* L. ssp. *durum* (Desf.) Husn.], rivet (*T. turgidum* L. ssp. *turgidum*), and Khorasan wheat [*Triticum turgidum* L. ssp. *turanicum* (Jakubz.) A. Löve & D. Löve], for which one variety is marketed under the Kamut® trademark. Although durum wheat is not an ancient grain, but a free-threshing grain primarily used for pasta, it will also be included in this discussion. Spelt [*T. aestivum ssp. spelta* (L.) Thell.] is a hulled species that shares A, B, and D genomes with common wheat.

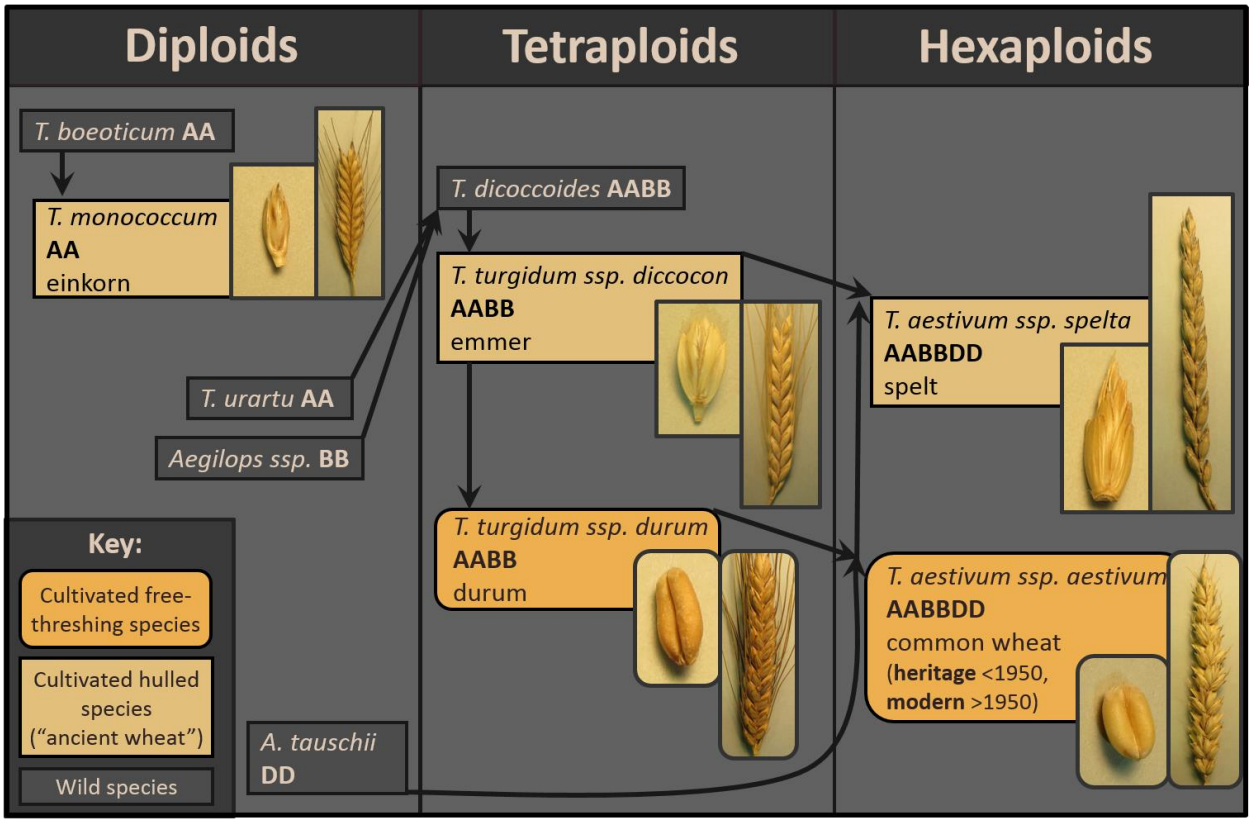
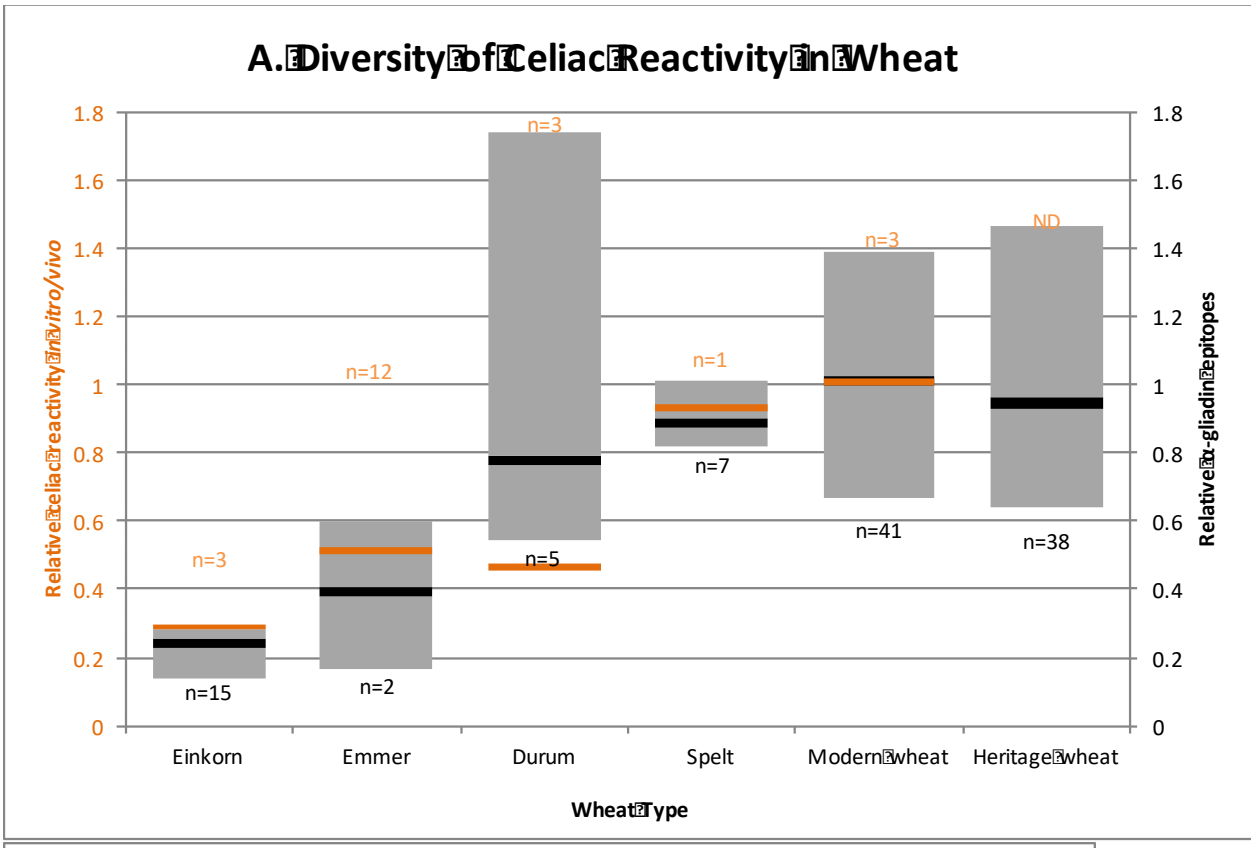


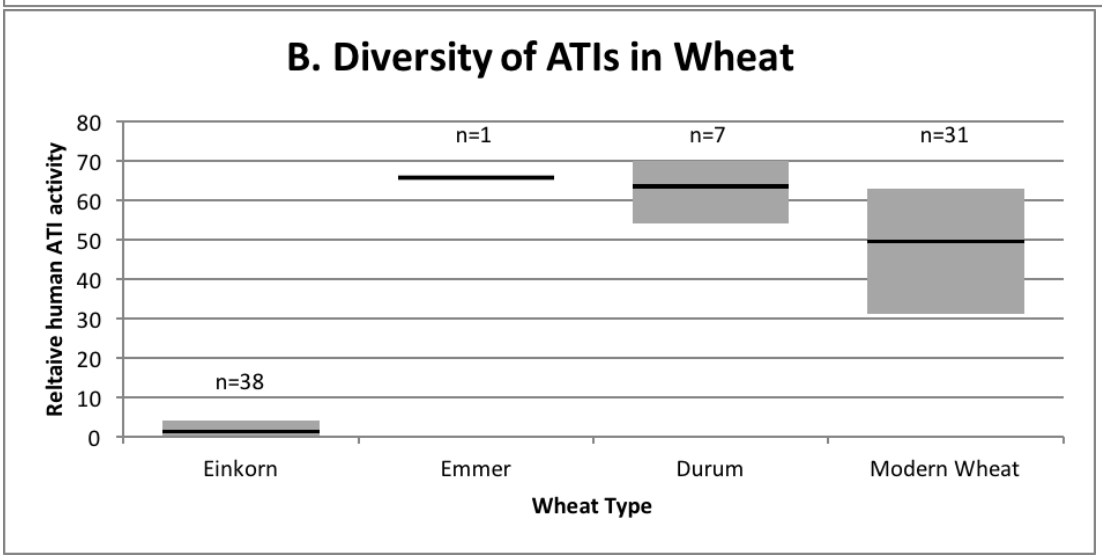
Figure 3.2. The genealogy of cultivated members of the *Triticum* family. Various cultivated ancient wheat species, durum wheat, and common wheat are presented (adapted from Dawson and others 2013).

3.4.2 Celiac disease

3.4.2.1 Celiac disease and ancient wheat species

Genome composition can partially explain the variation in celiac immunoreactivity among species of wheat. Several highly immunogenic α-gliadins are encoded by the D genome of wheat (Molberg et al. 2005; Spaenij-Dekking et al. 2005; van Herpen et al. 2006). Consequently, species that lack the D genome of wheat, such as einkorn, emmer, and durum, appear to exhibit average lower reactivity than common wheat (Figure 3.3A). The B genome of wheat encodes the fewest α-gliadin epitopes implicated in celiac disease (van Herpen et al. 2006). However, diploid species with genomes similar to the B genome of wheat are not cultivated or normally consumed by humans.


A. Diversity of Celiac Reactivity in Wheat
Relative celiac reactivity in vitro/vivo
Relative α-gliadin epitopes
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
n=3
n=15
n=12
n=2
n=3
n=5
n=1
n=7
n=3
n=41
ND
n=38
Einkorn
Emmer
Durum
Spelt
Modern wheat
Heritage wheat
Wheat Type


B. Diversity of ATIs in Wheat
Reltaive human ATI activity
80
70
60
50
40
30
20
10
0
n=38
n=1
n=7
n=31
Einkorn
Emmer
Durum
Modern Wheat
Wheat Type

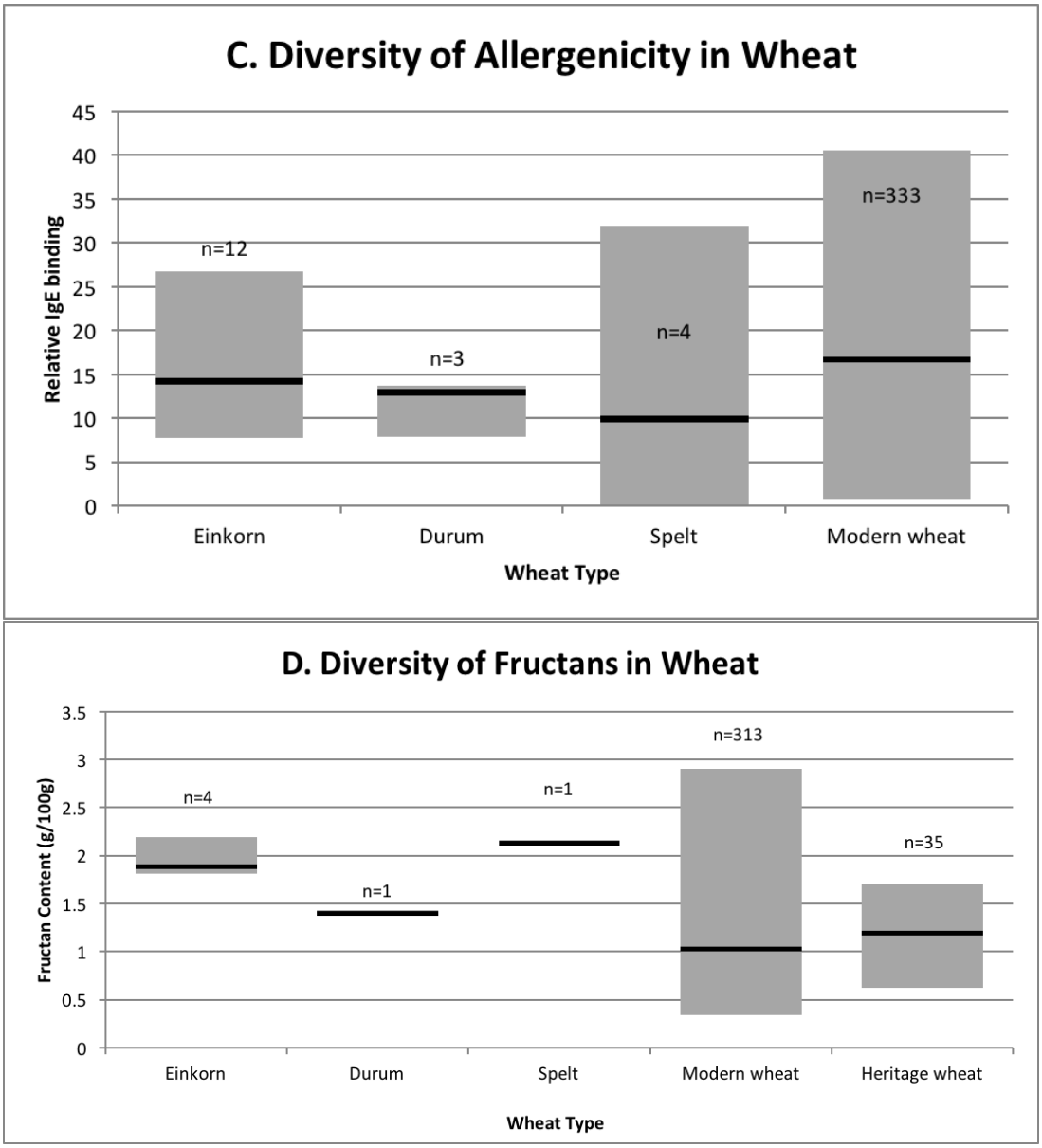


Figure 3.3. Variation in wheat sensitivity among and within wheat species. Values reported in the literature within and among wheat types for (A) celiac reactivity (Molberg and others 2005; Pizzuti and others 2006; Vincentini and others 2007, 2009; van den Broeck and others 2010a, 2010b), (B) human α-amylase inhibitor activity (Bedetti and others 1974; Vittozzi and Silano 1976; Sanchez-Monge and others 1996; Wang and others 2007; Zoccatelli and others 2012), (C) allergenicity (Weiss and others 1993; Sanchez-Monge and others 1996; Klockenbring and others 2001; Nakamura and others 2005; Larre and others 2011; Vu and others 2014), and (D) fructan content (De Gara and others 2003; Huynh and others 2008; Brandolini and others 2011; Hammed 2014; Veenstra 2014). Gray boxes show values of intraspecific variation (maximum to minimum values). Black lines represent means for each wheat type. Labels "*n* = " refer to the number of unique varieties evaluated. Values in A, B, and C were normalized to a relative scale by converting reported average values for modern wheat in each study to a shared value. Modern wheat includes varieties of common wheat that were developed after 1950, while heritage wheat includes varieties and landraces that were developed before 1950.

Since spelt has a D genome, its cytotoxicity was found to be similar to common wheat. Six spelt landraces produced levels of celiac α-9 T-cell epitopes that were similar to 80 common wheat genotypes (van den Broeck et al. 2010b). When comparing spelt and common wheat, Vincentini et al. (2007) measured similar inhibition of cell growth, activation of apoptosis, release of nitric oxide, release of tissue transglutaminase, and alteration of transepithelial electrical resistance on Caco-2/Tc7 and K562 (S) cell agglutination.

Einkorn, which has only the A genome of wheat, expressed the least celiac disease epitopes among cultivated species. Vincentini et al. (2007) reported no cytotoxicity in one einkorn genotype, measured as inhibition of cell growth, activation of apoptosis, release of nitric oxide, release of tissue transglutaminase, and alteration of transepithelial electrical resistance in human colon cancer Caco-2/TC7 and human myelogenous leukemia K562(S) cells. After exposure to gliadin extracted from einkorn, intestinal biopsies of eight individuals with celiac disease showed no reduction in intestinal villi height or production of IFN-γ (Pizzuti et al. 2006). In a rare *in vivo* study, 12 celiac patients experienced no difference in gastrointestinal complaints after 28 days consuming either 2.5 grams of rice or the einkorn cultivar "Monlis" (Zanini et al. 2009). Nevertheless, einkorn still expressed T-cell immunogenic α- and γ-gliadin epitopes (Molberg et al. 2005; van Herpen et al. 2006). Fifteen different einkorn genotypes produced substantially different amounts of these celiac disease epitopes (Molberg et al. 2005). The amino acid sequences of toxic peptides were also found in one einkorn genotype (Vaccino et al. 2009).

Emmer and durum, which have the A and B genomes of wheat, generally appear to be less immunoreactive than common wheat, but more immunoreactive than einkorn. Gliadin derived from two durum varieties were less cytotoxic than those from a common wheat, when exposed *in vitro* to biopsies of children with celiac disease. Five times the concentration of

durum was necessary to match the intestinal villi damage caused by the common wheat (Auricchio et al. 1982). Although the average reactivity of emmer and durum is lower than common wheat, there is a wide range of celiac response depending on genotype. While three emmer landraces induced negligible T-cell proliferation and release of IFN-γ, other landraces induced levels that were not significantly different than common wheat (Vincentini et al. 2009). Similarly, van den Broeck et al. (2010a) identified two emmer and three durum accessions with low amounts of the commonly reactive α-9 epitope (PFPQPQLPY). However, other durum accessions expressed amounts similar to those of common wheat.

Individuals with celiac disease differentially react to the gluten profiles of ancient wheat (Vader et al. 2002b). T-cell activity from four children with celiac disease differed widely after exposure to nine landraces of emmer (Vincentini et al. 2009). Despite lower reactivity overall, einkorn, emmer, and durum still produced reactions in 25% to 38% of tested patients' T-cells (Molberg et al., 2005). Such variability underscores the fact that no varieties or species of wheat have been determined to be safe for individuals with celiac disease.

Unfortunately, the D genome, which is associated with celiac epitope expression, is also responsible for expressing most of the HMW proteins that are essential for bread-making quality. Consequently, ancient and durum wheat are not equipped with the gluten profile for bread baking. Moreover, emmer lines lacked HMW 7+8, LMW-2, and γ -45/ω-35 proteins which are important for pasta quality (Vincentini et al. 2009).

3.4.2.2 A comparison of modern and heritage varieties for celiac disease

Broad diversity in celiac immunoreactivity also exists among varieties of common wheat. Varieties that express more Gli-2 genes from the A or B genomes, rather than the D genome, will

produce fewer α-gliadin celiac T-cell epitopes (Salentijn et al. 2009). Other varieties have mutations in α-gliadin coding sequences that alter expression of celiac disease T-cell epitopes.

Data compiled from a limited number of studies indicate that heritage genotypes, on average, express lower levels of celiac immunoreactive compounds (Figure 3.3A). Van den Broeck et al. (2010b) compared European heritage and modern varieties for the production of α-9 epitopes implicated in celiac disease. Twelve of 44 heritage collections produced low levels of the epitope, compared to only one of 36 modern varieties. In another study, two modern genotypes had lower frequency of α-gliadin expression from the A genome (15%), when compared to five landraces (29%; Salentijn et al. 2009). Among 61 durum accessions, the genotypes expressing the lowest amounts of three α-gliadin epitopes (DQ2.5-Glia-α1 (PFPQPELPY), DQ2.5-Glia-α2 (PQPELPYPQ), and DQ2.5- Glia-α3 (FRPEQPYPQ)) were a mix of landraces, old varieties, and modern breeding lines (Salentijn et al. 2013). However, modern durum varieties tended to fall in the highest categories of epitope expression. Modern breeding lines constituted 91% of varieties in the most immunodominant category, while old varieties and landraces only represented 9% (Salentijn et al. 2013). Genetic linkages between loci for α-gliadins and HMW glutenins may explain why some modern varieties contain more celiac T-cell epitopes. Many modern varieties have been bred for increased HMW glutenin content, which improves bread baking quality when using common wheat and pasta quality when using durum.

Not all heritage genotypes, however, had low T-cell immunoreactivity. Although average intensity of α-9 epitopes was higher in modern varieties, the most immunodominant variety identified by van den Broeck et al. (2010b) was a heritage wheat. As Vincentini et al. (2009) concluded, old varieties and landraces exist with potent celiac epitopes, indicating that humans

have long been exposed to immunoreactive genotypes of wheat. Conversely, studies have identified modern varieties with low expression of α-gliadin epitopes (van den Broeck et al. 2010b; Salentijn et al. 2013) and IgA reactivity (Constantin et al. 2009). Certain varieties of modern wheat have also shown less immunoreactivity than ancient wheat species. In an evaluation of 16 ancient and modern wheats, one line of modern club wheat [*T. aestivum* L. ssp. *compactum* (Host) MacKey] induced the second lowest *in vitro T-cell* response and IFN-γ release (Spaenij-Dekking et al. 2005).

Efforts have been made to create modern wheat genotypes with lower celiac immunoreactivity. Varieties devoid of any immunoreactive glutens would not be functional, as a portion of celiac patients react with HMW glutenins, which are essential for baking quality (Molberg et al. 2003; Dewar et al. 2006; van den Broeck et al. 2009). Due to linkage with some immunoreactive gliadins and HMW glutenins (van den Broeck et al. 2009), traditional breeding methods have not been able to develop celiac-safe bread wheat. Wheat lines which lacked portions of the short arms of chromosomes 1 and 6 expressed fewer celiac T-cell epitopes, although most had reduced baking quality (Molberg et al. 2005; van den Broeck et al. 2009). One deletion line, lacking part of the short arm of chromosome 6D (6DS-2), had reduced celiac T-cell epitopes and improved bread quality, but demonstrated poor kernel size and milling yield (van den Broeck et al. 2011). Lafiandra et al. (1987) developed a mutant line with good baking quality by limiting α-, γ-, and ω-gliadins encoded by the Gli-A2, Gli-D1, Glu-D3 loci. When tested with an *in vitro* organ culture, the mutant line did not cause damage to villi (Frisoni et al. 1995), but it did induce IFN-γ and cytokine IL-2 production (Carroccio et al. 2011).

Transgenic approaches have been successful at reducing celiac T-cell epitopes while maintaining bread quality. Gil-Humanes et al. (2010) used ribonucleic acid (RNA) interference

to down-regulate α-, γ -, and ω-gliadins. The transgenic lines produced up to 91% fewer α-gliadins, 81% fewer ω-gliadins, and no γ-gliadins. When tested against T-cells derived from celiac patients, several lines were able to substantially reduce T-cell responses. As an added benefit, the transgenic lines compensated for gliadin reductions by increasing HMW-glutenins, which resulted in medium to high bread quality. As these lines still induced low levels of T-cell responses, celiac patients would need to limit consumption of these wheat varieties in their diets.

3.4.3 ATIs

Different species and genotypes of wheat produce varying types and amounts of ATIs implicated in celiac disease, wheat allergy, and NCWS (Figure 3.3B). ATIs are encoded by the B and D genomes of common wheat, suggesting that diploid and tetraploid species lacking one or both of these genomes might produce fewer ATIs (Figure 3.3B). Specifically, Wang et al. (2006) mapped the problematic 0.19 fraction to the D genome of wheat. As it lacks both B and D genomes, einkorn contained no coding regions and produced no proteins for ATIs (Wang et al. 2006; Larre et al. 2011; Zoccatelli et al. 2012) and no human α-amylase inhibition was detected in various einkorn genotypes (Bedetti et al. 1974; Vittozzi and Silano 1976; Sanchez-Monge et al. 1996).

Varieties of durum and emmer inhibited total α-amylase activity in human saliva at levels equal to (Vittozzi and Silano 1976) or higher than common wheat (Bedetti et al. 1974; Sanchez-Monge et al. 1996). While ATIs of durum and emmer differ from those of common wheat, they did contain the CM3 ATI that was implicated in celiac disease, wheat allergy, and NCWS (Capocchi et al. 2013). Although significant varietal differences were found among three durum genotypes by Prandi et al. (2013), environment had a stronger influence on CM3 ATI content

than genotype. Locations that yielded more protein content consistently produced lower amounts of CM3 ATI (Prandi et al. 2013).

Types and quantities of ATIs also vary among genotypes within a species. Two-fold intraspecific differences were recorded in α- amylase inhibition among seven durum lines and 113 common wheat lines (Bedetti et al. 1974; Baker et al. 1991; Sanchez- Monge et al. 1996). Although Junker et al. (2012) indicated that ATIs may have been increased through modern wheat breeding programs, no studies that directly compared heritage and modern wheat genotypes for inhibitory activity against human enzymes were found. ATI activity for only one variety that was released before 1950 was reported in the literature. The heritage variety, "Clarkan," induced the fifth highest ATI activity out of 104 common wheat varieties studied (Baker et al. 1991). Hypoallergenic rice has been developed by downregulating ATIs (Tada et al. 1996), but no such varieties have been developed in wheat.

3.4.4 Wheat allergy

As mentioned above, ATIs and glutens implicated in allergic reactions also vary by wheat type (Figure 3.3C). Intraspecific variation may be most influential in determining allergenicity, rather than differences between species. The ω-gliadins are primarily encoded by the B genome of wheat (Altenbach and Kothari 2007; Denery-Papini et al. 2007). However, all cultivated species of wheat, including einkorn, express ω-5 gliadins implicated in WDEIA (Seilmeier et al. 2001). For baker's asthma, Sanchez-Monge et al. (1996) found no significant difference in IgE binding capacity between einkorn, durum, and common wheat. In a general screening of 324 wheat varieties, one variety of einkorn, one rivet, and eight common wheats were the least allergenic (Nakamura et al. 2005).

Among ten einkorn lines, there was a two-fold difference in ATI- IgE binding between the least and most allergenic genotypes (Sanchez-Monge et al. 1996). Allergenicity also varies by patient. Larre et al. (2011) exposed sera from individuals with contact urticaria and dermatitis to albumin and globulin proteins from einkorn and common wheat. While the majority of sera demonstrated lower IgE activity with einkorn, a minority of patients' sera (five out of 18) exhibited similar IgE activity from each species, and serum from one patient bound with more intensity to the einkorn wheat protein extract. Einkorn and emmer contained higher proportions of ω-5 than common wheat. Emmer wheat exhibited a distinct ω-5 amino acid sequence from that of common wheat (Seilmeier et al. 2001), but the impact of this sequence on allergic reactions has not been determined.

Durum wheat showed slightly lower average IgE binding reactivity for ATIs and other albumin/globulin proteins than common wheat (Lupi et al. 2014). However, some durum genotypes exhibited allergenicity equal to common wheat. Among ten varieties of durum, one genotype exhibited 57% less IgE binding reactivity for ATI proteins than the most allergenic genotype (Lupi et al. 2014). When tested against sera of patients with food allergy, Khorasan wheat produced similar IgE binding profiles and skin-prick test reactions as durum wheat (Simonato et al. 2002).

Some, but not all, varieties of spelt have lower allergenicity than common wheat. Spelt produced lower proportions of ω-5 gliadins implicated in WDEIA than common wheat (Seilmeier et al. 2001). In an *in vivo* study of 64 patients with baker's asthma and other types of wheat allergy, only 30% of patients reacted to a variety of spelt (Armentia et al. 2012). In both cases, reactions to spelt corresponded to patients who had a more severe manifestation of wheat allergy. Vu et al. (2014) isolated sera antibodies obtained from 73 individuals with wheat allergy,

and then exposed the sera to two varieties of spelt and one common wheat. Only 57% to 88% of sera that reacted strongly to common wheat also reacted to the spelt varieties (Vu et al. 2014). One hypoallergenic spelt variety, "GWF," has a mutation that alters albumin/globulin proteins (Vu 2014). Other spelt genotypes, however, are not hypoallergenic. Sotkovsky et al. (2008) found one variety of spelt to elicit more IgE reactivity than five of six tested common wheat varieties.

Among genotypes of common wheat, allele variants (for example, *Gli-B1c*) exist that greatly reduce ω-5 expression and immunoreactivity. Twenty-nine different cultivars expressed highly variable amounts of ω-5 gliadins, with one variety producing ten times the amount of ω-5 gliadin of the lowest cultivar (Wieser et al. 1994). Immunoreactivity of these wheat cultivars to sera from nine individuals with WDEIA and urticaria generally corresponded to the amount of ω-5 gliadins present (Denery-Papini et al. 2007). Nakamura et al. (2005) screened 321 wheat varieties for broad allergenicity, and recorded six times more IgE binding in the most reactive genotype when compared to the least. For baker's asthma, the least allergenic of ten common wheat varieties bound only 44% to 63% of the IgE when compared to the most allergenic lines (Weiss et al. 1993; Sanchez-Monge et al. 1996). After comparing seven different common wheat varieties, Weiss et al. (1993, 1997) found an eight-fold difference in IgE binding to a highly reactive 27 kDa albumin/globulin fraction. No studies were found that directly compared allergic reactions incited by heritage and modern wheats. The least allergenic line was a modern cultivar ("CM32859" CIMMYT) in the large screening conducted by Nakamura et al. (2005).

Varieties of wheat have been developed with lower allergenicity. Low ω-5 gliadin expression occurred in wheat lines containing chromosome arm 1RS from rye (Wieser et al. 1994) and corresponded to very low allergenicity among subjects with WDEIA and urticaria

(Denery-Papini et al. 2007). Waga and Skoczowski (2014) developed varieties that were 30% less immunoreactive across a panel of allergic individuals. Through RNA interference technology, lines were developed that partially or completely suppressed the expression of ω-gliadins (Altenbach and Allen 2011; Altenbach et al. 2014). Wheat genotypes developed to express fewer allergens may have suitable baking quality, because downregulating ω-gliadin expression improved bread quality (Waga and Skoczowski 2014). Downregulation of ω-gliadins sometimes corresponded to reduced amounts of other allergens, such as ATIs, lipid transfer proteins, and serpins (Altenbach et al. 2014). However, wheat lines compensated for the removal of ω-gliadins by upregulating α-gliadins (Altenbach et al. 2014; Waga and Skoczowski 2014). So, while these varieties may lessen the suffering of individuals with exercise-induced anaphylaxis, they could be worse for individuals with celiac disease.

To complicate the development of hypoallergenic varieties, individual patients differ in the intensity and specificity of their allergic reactions to common wheat genotypes. Lupi et al. (2014) found a significant interaction for genotype and patient serum, indicating that cultivars with lower reactivity for one individual were not necessarily less reactive for another individual. Moreover, hypoallergenic varieties for one type of allergy may not be hypoallergenic for another type. Constantin et al. (2009) found only one variety out of 13 that bound with less intensity to the serum antibodies obtained from both individuals with baker's asthma and food allergy.

3.4.5 NCWS, fructose malabsorption, and IBS

The content of fructans, which can impact individuals with fructose malabsorption, IBS, and some cases of NCWS, varies among species and genotypes (Figure 3.3D). Like gluten proteins, fructans are present in detectable amounts in all species and varieties of wheat. To date,

the general ranking of wheat species by fructan content, from highest to lowest, is spelt, einkorn, durum, and common wheat (De Gara et al. 2003; Huynh et al. 2008; Brandolini et al. 2011; Hammed 2014; Veenstra 2014). No studies regarding the fructan content of emmer could be located. As studies have evaluated very few genotypes of einkorn, spelt, and durum, the range of fructan content found in each species is not well known.

In an evaluation of 62 common wheat varieties, fructan content ranged from 0.7% to 2.9% dry weight (Huynh et al. 2008). Preliminary measurements of total fructan content in 286 common winter wheat varieties grown in one environment ranged from 0.3% to 1.5% (Veenstra 2014). The 35 heritage wheat varieties studied had an average fructan content of 1.2% dry weight, compared to an average of 1.0% for 313 modern wheats (Huynh et al. 2008; Veenstra 2014). As drought and temperature influence wheat stem fructan content and remobilization, both environment and genotype influence the fructan composition of wheat grain (Bancal and Triboi 1993; Ehdaie et al. 2006). Further research is needed to understand how the type of fructan structures differs among varieties of common wheat. Two popular Swedish winter wheat varieties contained similar types of fructan structures (Haska et al. 2008).

3.4.6 Summary of immunoreactivity among ancient, heritage, and modern wheats

Although the popular press (for example, Davis 2011) has indicated that consuming ancient or heritage wheat prevents sensitivity, the scientific literature does not support this claim. No wheat species or varieties are currently approved for diagnosed celiac and allergic individuals to consume. Nevertheless, some varieties of ancient, heritage, and modern wheat produce fewer amounts and types of reactive prolamins and fructans. Einkorn is particularly promising for producing fewer immunotoxic effects in celiac research studies. Modern breeding efforts have

also produced varieties that are hypoallergenic or less dangerous for celiac individuals. As many celiac and allergic individuals do not always adhere to wheat-free diets (see the review by Hall et al. 2009), these less-immunoreactive wheat products may improve their quality of life. Furthermore, these varieties may be good targets for slowing development of disease in populations genetically predisposed to celiac disease and other wheat sensitivities.

The limited data available on common wheat indicate only a slight increase in average expression of components causing celiac disease in modern compared to heritage varieties (Figure 3.3A). Preliminary data show that modern varieties did not contain higher fructan content than heritage varieties (Figure 3.3D). These data suggest that the introduction of modern wheat varieties may not fully explain the rise in wheat sensitivity over the last 50 years. To understand how modern wheat breeding has impacted wheat sensitivity, a broader array of modern and heritage genotypes must be screened. In particular, published data have not evaluated the differences in ATI activity and allergenicity between heritage and modern varieties.

As a challenge to interpreting data from the literature, many studies have not controlled for variability in growth environments. Nakamura et al. (2005) concluded that allergenicity of a single wheat variety could vary according to where the variety was grown. Omega-5 gliadin content increases with fertilization and temperature during maturity (Wieser and Seilmeier 1998; Altenbach and Kothari 2007; Hurkman et al. 2013). A similar less-pronounced trend was observed for α-gliadins (Hurkman et al. 2013). Many studies included in this review evaluated gliadin content in kernels from plants that were grown under different nitrogen and weather conditions. Consequently, apparent immunoreactive gliadin content may not reflect an inherent genetic difference between varieties, but rather the environmental conditions of the field in

which they were grown. The effects of fertilization also suggest that higher nitrogen inputs characteristic of modern production systems could be directly increasing the amounts of reactive ω- and α-gliadins in wheat products.

As most wheat breeding programs do not screen for celiac, allergy, or fructan reactivity in their lines, scientists and the public have little information on reactivity of modern wheat genotypes used in agriculture. To generate meaningful information for wheat breeders and consumers, a first step would be to standardize screening procedures. No standard screening protocol exists for identifying "reactivity" of a wheat variety. Due to the cost and ethical barriers of *in vivo* testing, the full impact of wheat genotypes on a large number of patients is rarely studied. Instead, research has inferred reactivity *via* genomic information, prolamin sequences, or *in vitro* models. The cumbersome nature of evaluations prevents all potentially reactive prolamins from being evaluated. Similarly, *in vitro* models are often relevant to only a portion of patients. Standardized screening methods could incorporate the most reactive T-cell epitopes in celiac disease, and/or apply varieties to sera from an array of allergenic individuals. Screening of all varieties for fructan content would be beneficial for individuals wishing to control intake levels of fructan. However, the lack of severe health impacts may not justify the cost and time required for widespread fructan screening of varieties.

Even if potential reactivity were determined for all wheat varieties, the variety identity is rarely tracked from farm to mill to bakery to storefront. Moreover, flour is commonly mixed from a variety of wheat genotypes in the milling or baking process. In contrast to potatoes, which are sold under their variety name, such as "Russet" or "Yukon Gold," a wheat variety is rarely labeled in retail products, and most wheat products are sold as combinations of many wheats. Consequently, consumers are not able to distinguish what variety they are purchasing. An

informed consumer would require a processing chain that tracks the reactivity of wheat in marketed products.

3.5 The impacts of food processing on wheat sensitivity

Processing techniques can also impact the nature of reactive components in wheat products. In particular, certain modern processing practices used over the last century may have increased consumer exposure to components implicated in wheat sensitivity. Modern processing can differ from traditional methods by (1) using ungerminated grain, (2) replacing long and diverse fermentation with fast-acting baker's yeast (*Saccharomyces cerevisiae*), (3) using nonacidic dough, (4) adding extracted wheat proteins and inulin to food products, and (5) focusing on refined white flour. The following section reviews the effects of these modern processing practices on wheat reactivity.

3.5.1 Malting and germination enzymes

Wheat, rye, and barley kernels contain their own enzymes that can break down difficult to digest proteins. When the seed imbibes water, proteases in the seed break ATIs into peptides and amino acids to be used in seedling growth. Consequently, ATIs rapidly degrade after germination (Buonocore et al. 1977). During germination, endoproteases also cleave the gliadin and glutenin storage proteins into available amino acids for seedling growth (Hartmann et al. 2006). Germination strongly induces cysteine proteases, which are responsible for breaking down gliadin (Loponen et al. 2007). Five or six days after germination begins, ω-gliadins are the first to be degraded (Bigiarini et al. 1995). Schwalb et al. (2012) documented nearly complete degradation of the immunodominant ω-5 gliadin after seven days of durum germination.

Prolonged incubation of wheat and durum fully degraded gliadins (Bigiarini et al. 1995; Stenman et al. 2009; Schwalb et al. 2012). Germinated and fermented rye and wheat sourdough effectively degraded 99.5% and 95% of prolamins, respectively (Loponen et al. 2007, 2009). Specifically, the toxic 12-mer of α-gliadin QLQPFPQPQLPY was hydrolyzed in gliadin treated with germination enzymes (Stenman et al. 2009, 2010). In addition to reducing the 33-mer by 83% (Stenman et al. 2009), germination and low pH treatment degraded ≥99% of the alpha-gliadin T-cell epitope PQPQLPYPQPQLPY in wheat, emmer, and einkorn (Schwalb et al. 2012). In the study by Luoto et al. (2012), the addition of *Aspergillus niger* prolyl endoprotease was necessary to bring germinated wheat products below the threshold for gluten-free labeling of 20 ppm.

Fewer immunoreactive peptides in germinated products translated into lower celiac disease epitope expression. However, geminated wheat products are generally not safe for individuals with celiac disease. Although *in vitro* Caco-2 cells exposed to endoprotease-treated gluten did not show aggravated barrier function, membrane ruffles, and tight junctions (Stenman et al. 2009, 2010), the degraded gluten still induced T-cell proliferation at levels significantly higher than nongluten controls (Stenman et al. 2009).

The most vigorous enzyme activity and protein breakdown takes place on the eighth day of germination (Bigiarini et al. 1995; Stenman et al. 2010), at which point the seed has nearly transformed into a seedling. Industrial use of such extensively sprouted grain would be a challenge. Moreover, germinating the grain greatly reduces the shelf-life of products made from it. As a positive marketing aspect, germinated grain can improve flavor. Loponen et al. (2009) reported that rye sourdough made from germinated grain had enhanced flavor compared to bread made from nongerminated grain.

To improve applicability for large-scale production, the enzyme supernatants from a small stock of germinated seed have been used to effectively reduce prolamins in large quantities of flour (Stenman et al. 2009, 2010; Schwalb et al. 2012). Enzymes from germinated barley may create the most efficient supernatants, as they degraded the largest amount of gliadins (Stenman et al. 2010). Optimal germination temperature to cleave gliadins varied by species, with 25 °C functioning best in wheat and one emmer variety and 15 °C facilitating more degradation in einkorn, rye, barley, and another emmer variety (Schwalb et al. 2012). Endogenous enzymes from sprouted grain have also been tested as an oral supplement taken during mealtimes (Tye-Din et al. 2010a; Siegel et al. 2012; La ̓hdeaho et al. 2014).

Given that fructans are carbohydrates, rather than proteins, these compounds face a different fate than gluten and ATI proteins in the processes of malting and germination. The exact role and fate of fructans in wheat germination is unclear. Wheat kernels may have hydrolases similar to onion seed, which degrade fructans during the germination process (Pollock and Cairns 1991; Pollock and Lloyd 1994). In barley, fructans are relatively unaffected by the malting process, but >90% of fructans initially present in wort are fermented by yeast (Krahl et al. 2009).

3.5.2 Fermentation and microbial enzymes

Microorganisms involved in fermentation can also contribute to hydrolysis of reactive proteins. A combination of microbial prolyl endopeptidases (PEPs) can be used during wheat processing or during ingestion to break down prolamins (see reviews by Arendt et al. 2007; Gobbetti et al. 2007; M'hir et al. 2012). *Aspergillus niger* PEPs showed promise as an oral supplement for celiac patients to consume with wheat products (Mitea et al. 2008). The

traditional sourdough baking practice also employs a diversity of microbial proteases. Single strains of lactic acid bacteria degraded some storage proteins, releasing more amino nitrogen than uninoculated dough after 24 hours (Wieser et al. 2008). Although single strains were able to degrade 23% to 45% of γ-gliadins, only 11% of ω-5 gliadins were affected. Duar et al. (2014) reported that each set of PEPs derived from *Lactobacillus ruminis*, *L. johnsonii*, *L. amylovorus*, *and L. salivarius* isolated from the small intestine of pigs fed with gluten-containing diets demonstrated distinct capacity to degrade, yet not completely remove, three gliadin peptides harboring T-cell epitopes (the 33-mer, QPQQPFPQPQQPFPWQP, and QLQPFPQPQLPYPQPQ). A combination of enzymes from a diversity of microbes is necessary to effectively break down peptides. The αG-33mer fragment, for example, did not show complete degradation with *Flavobacterium meningosepticum* or *L. sanfranciscensis* alone (Gallo et al. 2005; Matysiak-Budnik et al. 2005), but was successfully degraded with a diverse mixture of *L. alimentarius*, *L. brevis*, *L. sanfranciscensis*, and *L. hilgardii* (De Angelis et al. 2010). Fermenting durum pasta dough with the diverse microbial mixture decreased gluten concentration by 83% (Di Cagno et al. 2005). Microbes also degraded 97% of gluten in bread dough after 48 hours and 70% of the 33-mer after only six hours (Rizzello et al. 2007; Greco et al. 2011).

In testing the dough developed by Rizzello et al. (2007), T-cell proliferation and IFN-γ production was equivalent to nongluten controls. In a double-blind trial using bread made from 30% highly fermented wheat flour, celiac patients did not experience increased intestinal permeability (Di Cagno et al. 2004). There is intriguing evidence that sourdough fermentation alone can reduce celiac disease immunoreactivity, whether or not a grain product contains gluten. Amaranth, corn, and rice products that had been fermented with sourdough bacteria

generated less inflammation (p= 0.045) in celiac patient biopsies (Calasso et al. 2012).

Fermented wheat products, however, have not been determined as safe for individuals with celiac disease. Although extensive fermentation degraded 97% of gluten, two celiac subjects consuming the fermented products still experienced villous atrophy at levels higher than nongluten controls (Greco et al. 2011). Full gluten hydrolysis with sourdough bacteria and fungal proteases was necessary to eliminate elevated intestinal permeability, cytokine expression, and gliadin antibody levels as shown by Di Cagno et al. (2010) and Greco et al. (2011). When 98% of prolamins were degraded using extensive germination and fermentation, the remaining 27 mg/kg of secalin in the rye bread still induced duodenitis, cytokinine secretion, small bowel inflammation, and weight loss in celiac mouse models (Freitag et al. 2014). Due to remnant amounts of reactive peptides, the authors have encouraged gluten-free baking products that incorporate only small amounts of wheat dough that was highly fermented and/or made from germinated grain. In such products, hydrolyzed wheat dough was mixed with flour from nongluten species, such as millet and buckwheat flours (Di Cagno et al. 2004, 2005).

Fermentation often enhances the flavor and shelf-life of baked products. Di Cagno et al. (2006) found that certain sourdough cultures increased bread volume and crumb firmness, eliminating the need for baking texture additives. Furthermore, the sensory qualities of foods made with hydrolyzed-gluten wheat flour are often superior to products made from nonwheat flours (Rizzello et al. 2007). Unfortunately, there is often a tradeoff between degradation of reactive gluten and retention of gluten for basic baking properties. Large amounts of time and heat may be needed for microbial enzymes to break down problematic peptides. To fully degrade the 33-mer α-gliadin peptide in wheat required 24 hours at 30 °C (Gallo et al. 2005), while durum required 72 hours of fermentation at 37 °C to meet gluten-free labeling standards (De

Angelis et al., 2010). HMW glutenins, which are important for baking and pasta integrity, are degraded prior to and more extensively than reactive prolamins during sourdough fermentation (Ganzle et al. 2008; Wieser et al. 2008). Extensively fermented dough has a high ratio of gliadins to glutenins, which is very undesirable for bakers. The disulfide bonds holding together the gluten macropolymer (GMP), an integral component of baking quality, begin to degrade long before glutens. Only 5 hours of fermentation with *Lactobacilli* or acidic chemicals degraded GMP by up to 46% (Wieser et al. 2008). Pentosans, an important component for baking rye bread, were also hydrolyzed in germinated sourdough (Loponen et al. 2009). Consequently, the long and hot sourdough fermentation to hydrolyze prolamins compromises functional baking properties of the dough. Pasta made with highly fermented durum also had lower stickiness and firmness than unfermented pasta (Di Cagno et al. 2005; De Angelis et al. 2010).

While microbial cultures in the sourdough fermentation process also impact fructan content, the mechanisms differ from protein degradation. Only a small portion of lactic acid bacteria, 16 of 712 screened strains, were able to degrade various fructans of forage grasses (Muller and Lier 1994). Some strains of *Lactobacilli* from sourdough cultures, such as *L. plantarum*, *L. brevis*, and some *L. sanfranciscensis* strains, actually synthesized their own fructan structures (Dal Bello et al. 2001; Di Cagno et al. 2006; Bounaix et al. 2009), and subsequently stimulated bifidobacterial growth (Dal Bello et al. 2001; Korakli et al. 2002). Such strains did not, however, synthesize fructans over the relatively short timeframe used for sourdough baking (Di Cagno et al. 2006).

Yeast, on the other hand, produces inulinase and invertase enzymes which work together to effectively hydrolyze fructans (Nilsson et al. 1987). Fermentation with *S. cerevisiae* for 1.7 hours reduced fructan content of whole wheat and white flour by 33% and 48%, respectively

(Knez et al. 2014). Once baked, the leavened bread contained about half the fructan content of unleavened bread. Although the role of nonyeast microbes in degrading fructans is not fully understood, a diverse sourdough rye culture was most effective at degrading fructans (1.9% remaining), when compared to yeast-fermented bread (3.4%), and air-leavened bread (4.7%; Andersson et al. 2009). Similarly, fermented sourdough contained about half the fructosan, a polysaccharide of fructose, content when compared to unfermented dough (Escriva and Martinez-Anaya 2000). Lactic acid bacteria are likely most influential in the fructan degradation process by creating acidic conditions for yeast enzyme activity.

3.5.3 Acidity

Authors have argued that the most important contribution of sourdough fermentation is not the microbial protease activity, but lowering of the pH to levels optimal for wheat endoprotease activity (Hartmann et al. 2006; Ganzle et al. 2008; Loponen et al. 2009). Cysteine proteases operate in a pH range of 3 to 6, with optimal gliadin hydrolysis at 4.25 (Bottari et al. 1996). A pH of 4.0 allowed more of the 33-mer degradation in wheat, emmer, einkorn, and rye, although degradation in barley was more efficient at pH 6.5. Similarly, the optimal pH for yeast enzymatic activity in degrading wheat fructose was 4.5 to 5 (Nilsson et al. 1987). Escriva and Martinez-Anaya (2000) demonstrated that the fructosan degradation in two sourdough cultures was related to the culture's acidification ability.

Acidic conditions alone can help degrade prolamins in wheat and rye (Kanerva 2011). However, chemical acidification has proven less effective than microbial or endoprotease degradation. In celiac patients, chemically acidified bread triggered more intestinal permeability than bread fermented with diverse microbial cultures (Di Cagno et al. 2004). In addition, it has

been demonstrated that the acidic environment promoted nonenzymatic deamidation of gluten peptides leading to more immunogenicity (Arentz-Hansen et al. 2000).

3.5.4 Industrial food products

Since the last half of the 20th century, the food industry has increased its use of wheat proteins (Day et al. 2006). Gluten can be separated from wheat (as in "vital wheat gluten"), or modified for specific uses (referred to as "isolated wheat proteins"). Vital wheat gluten not only improves the structural integrity of industrial bakery products, but it costs less per ton of protein than soy, whey, or casein. In Europe and elsewhere, low-protein flours are often fortified with vital wheat gluten to improve baking characteristics (Day et al. 2006). For the United States market, vital wheat gluten is often added to bind multigrain breads (Atchison et al. 2010). Wheat proteins also act as a binder and protein booster in processed meat, reconstituted seafood, and vegetarian meat substitutes (Day et al. 2006). Commonly used as thickeners, emulsifiers, and gelling agents, wheat compounds were found in 86% of packet soups, 65% of canned soups, 63% of candies, 61% of ice cream, 46% of marinades, 26% of vinegars and dressings, 23% of jams, and 21% of baby food, according to a survey by Atchison et al. (2010). Such extensive food industry uses of gluten contribute to its nearly ubiquitous nature in the marketplace. The authors estimated that wheat is found in 29.5% of supermarket food products.

Neither vital wheat gluten nor isolated wheat proteins contain most endogenous wheat enzymes that assist in the degradation of persistent prolamins. Isolated wheat proteins might also produce *de novo* allergens. Leduc et al. (2003) documented the case of a patient who did not have an allergy to wheat/gluten, but experienced anaphylaxis after consuming a wheat isolate used by the meat industry. Isolated wheat proteins in hair and skin care products could also

provoke contact urticaria in a small subset of patients who are not allergic to gluten (Lauriere et al. 2006). Isolated wheat proteins can be deamidated by chemical acid or enzyme treatment to increase emulsifying applications (Wu et al. 1976). While the impact of industrial deamidation on celiac reactivity remains uncertain, gastrointestinal deamidation from the tissue transglutamase increases the binding of peptides to HLA DQ2/8 and aggravates celiac immune responses (Arentz-Hansen et al. 2000). Deamidated prolamins can also evade detection from commercial gluten screening methods like enzyme-linked immunosorbent assay (Kanerva 2011). Consequently, there is a possibility that products labeled as "gluten-free" contain deamidated gluten above the labeling threshold. The increased prevalence of isolated, and particularly deamidated, gluten in food and other products poses an obvious threat to individuals with wheat sensitivity and increases exposure of the general population to reactive glutens.

The food industry has also increased its use of compounds implicated in fructose malabsorption, IBS, and NCWS. Fructose consumption has risen in the last 30 years, largely due to a 60.8% increase in high-fructose corn syrup sweetener availability since 1978 (Gibson et al. 2007; Marriott et al. 2009). Consumers can also encounter inulin-type fructans in the marketplace. Inulin is added to food products for the purpose of fiber supplementation or fat replacement in low-fat products (Kleessen et al. 2007). Such inulin-type fructans are not derived from wheat, but rather extracted from chicory root and Jerusalem artichoke (Kolida and Gibson 2007). Cereals, muffins, cake mixes, instant oatmeal, granola bars, cookies, and bread are often supplemented with inulin-type fructans (Gibson et al. 2000; Grabitske and Slavin 2008). Although inulin can benefit most consumers when eaten in moderate amounts, inulin may aggravate symptoms of fructose malabsorption, IBS, and NCWS. Of particular interest to individuals with wheat sensitivity, inulin is often used to improve structure, color, taste, and fiber

content in gluten-free breads (Capriles and Areas 2014). Such food products highlight the need for patients with NCWS to understand the true causative agents of their symptoms. For individuals with fructose malabsorption, IBS, and certain cases of NCWS, gluten-free products with added inulin may be a poor dietary choice.

3.5.5 Flour processing

Modern flour processing can also impact wheat sensitivity. Fungal enzymes are commonly added to wheat flour to improve baking properties. Various fungal enzymatic additives, including α-amylase derived from *Aspergillus oryzae*, xylanase, glucoamylase, cellulase, and β-xylosidase, have been associated with allergies, such as baker's asthma and contact dermatitis (Quirce et al. 1992; Morren et al. 1993; Baur et al. 1998; Sander et al. 1998; Quirce et al. 2002). These additives provide an additional exposure risk to bakers (Tatham and Shewry 2008). Although limited research has been conducted, wheat flour treated with γ-irriadiation and microwave radiation were found to elicit more responses from allergic individuals (Leszczynska et al. 2003a, 2003b).

The amount of reactive glutens may change with the level of flour refinement. Most endopeptidase activity was found in the bran rather than the endosperm (Hartmann et al. 2006; Schwalb et al. 2012). This distribution is not surprising, as cysteine proteases are synthesized in the aleurone layer of barley (Hammerton and Ho 1986). Because the bran is removed in the process of making white flour, subsequent products would have fewer enzymes available for prolamin degradation. The total amount of bran also varies by species and variety of wheat, and can impact the amount of endoproteases present.

The content of reactive wheat components is different in various layers of the wheat kernel. ATIs surround starch molecules in the endosperm, protecting them from digestion by insects and mammals. Many of the celiac-reactive α-gliadins are located in the subaleurone layer of the wheat kernel, which can be partially removed by roller-milling. However, the γ-gliadins and the HMW glutenins, which are reactive in a lower number of celiac patients, are concentrated in the endosperm, and will therefore appear in high concentrations in white flour. Omega-gliadins, which are found throughout the grain, will likely not change with the level of flour refinement (Tosi et al. 2011). Wheat bran elicited about twice the IgE activity for baker's asthma than white flour (Armentia et al. 2012). The level of flour refinement on celiac immonoreactivity responses has not been directly assessed.

Fructans are not evenly distributed throughout the wheat grain. In terms of wheat milling fractions, bran, and shorts contain more fructan than the flour (Knudsen 1997; Haska et al. 2008). The inclusion of bran in whole wheat flour likely increases the total fructan content of whole wheat flour relative to white wheat flour. Whole wheat flour also contains fructans with a higher degree of polymerization than white flour. The lower degree of polymerization in white flour makes fructans more available for fermentation in the gut, which can aggravate symptoms in individuals with IBS. On the other hand, as fructans with lower degrees of polymerization were more easily degraded by yeast (Nilsson et al. 1987; Praznik et al. 2002), fructans in white bread were broken down more extensively than those in whole wheat bread (Knez et al. 2014).

3.5.6 Summary of the impacts of food processing on wheat sensitivity

Patients with celiac disease, wheat allergy, and some forms of NCWS should avoid products with added gluten and isolated wheat proteins. Individuals with fructose malabsorption, IBS, and NCWS should limit consumption of inulin and high fructose corn syrup. In seeking

wheat products with less immunoreactivity, consumers would most benefit from products made with germinated grain, and to a lesser extent fermented products. Free amino acid content (a measure of protein breakdown) in germinated wheat sourdough was ten times the concentration of nongerminated sourdough (Loponen et al. 2007, 2009). Similarly, only six hours of fermentation were necessary to break down almost all prolamins in germinated sourdough, but prolamins were still present after 24 hours of fermentation if the grain had not undergone germination (Loponen et al. 2007). Fermented and germinated wheat, however, has not been determined as safe for individuals with celiac disease.

No epidemiological studies have evaluated the impact of wheat processing on the prevalence in wheat sensitivity over the last 50 years. Nevertheless, increases in disease diagnoses correlate with food industry uses of compounds that can trigger sensitivity, such as gluten, inulin, and high fructose corn syrup. Furthermore, modern baking practices used over the last century have focused on short, nonacidic fermentation techniques. Further research is needed to determine how modern wheat processing has influenced epidemiology.

3.6 Conclusions

No wheat species or varieties are currently safe for individuals with celiac disease, wheat allergies, or fructose malabsorption. Individuals or populations who are not symptomatic, but seek to lower the amount of reactive wheat components in their diets, have many options: (1) supporting research efforts to identify, develop, and label less-reactive wheat genotypes; (2) finding varieties of wheat and ancient grains that are known to have lower reactivity for the condition in question; (3) eating products made with the processes of germination and/or diverse microbial fermentation; and (4) avoiding vital wheat gluten, isolated wheat protein, and, in

certain cases, inulin. As a first step to making meaningful diet change, patients need to understand what compounds are causing their symptoms. When correctly matched to disease pathology, less-reactive wheat products can improve the quality of life for individuals with diagnosed wheat sensitivity. Moreover, such products can slow disease development in populations that are genetically predisposed to celiac disease and wheat allergy. Although the cause of increased prevalence of wheat sensitivity over the last several decades remains unknown, modern wheat processing techniques may have increased consumer exposure to immunoreactive compounds.

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CHAPTER 4

GENOTYPE BY ENVIRONMENT INTERACTIONS AND LOCAL ADAPTATION IN ORGANIC WHEAT

Abstract

Optimizing wheat genotypes for organic production will improve lagging yields worldwide. An understanding of genotype by environment interactions can identify top-performing varieties for organic systems. Genotype by environment interactions also inform breeding programs about where to locate selection sites to maximize genetic gain. Over six years at ten organically managed sites in the northeastern and northcentral United States, we assessed genotype by environment interactions for yield, test weight, protein, and falling number. Through Fr tests and parametric bootstrapping in AMMI models, along with GGE biplots, we identified mega-environments with distinct variety performance throughout the region. Results indicate that organic wheat breeding and variety testing programs should decentralize selection into multiple locations. Moreover, breeding for stability should be prioritized for the northeastern and northcentral United States, due to large contribution of genotype by year by location interactions.

4.1 Introduction

Worldwide meta-analyses show that wheat (along with barley and potato) has one of the lowest organic-to-conventional yield ratios in comparison with other crops (Ponisio et al. 2014; Seufert, Ramankutty, and Foley 2012). Identifying and breeding genotypes that optimally perform in organic environments can reduce this yield gap. Prior to selecting top performing

lines, an understanding of genotype by environment interactions (GxE) is necessary. Worldwide, different environments often have distinct winning genotypes for wheat yield and quality (Cooper et al. 1997; Heslot et al. 2014). While studies have documented significant genotype by environment interactions between organic and conventional management systems (Kirk, Fox, and Entz 2012; Hoagland 2009; Reid et al. 2011; Murphy et al. 2007), little is known about the magnitude and structure of GxE among organically managed environments. An analysis of GxE for organically managed environments can identify which genotypes can boost organic production throughout a region.

Designing an effective genetic improvement and variety selection program also requires an understanding of GxE. In particular, GxE can illuminate which and how many testing locations maximize genetic gain for the breeder's region of interest. GxE consists of variance attributed to predictable location effects (σ^2_{GxL}), as well as unpredictable year effects (σ^2_{GxY}) and year by location interactions (σ^2_{GxLxY}) (Equation 4.1). If a large proportion of GxE variance is composed of location effects, opportunities exist to breed for local adaptation (Paolo Annicchiarico 2002). In such situations, mega-environments can be defined as locations within the target breeding region that have unique top performing genotypes. If GxY and/or GxLxY effects dominate over GxL, breeding programs would benefit from genotype stability over years, rather than selecting for local adaptation (Paolo Annicchiarico 2002).

Equation 4.1 $\sigma^2_{GxE} = \sigma^2_{GxL} + \sigma^2_{GxY} + \sigma^2_{GxYxL}$ (Walsh and Lynch 2015b)

While few plant breeding studies have evaluated local adaptation (Annicchiarico 2007; Annicchiarico et al. 2005; Atlin & Frey 1990; Ceccarelli et al. 1998), seventy-one percent of published studies in evolutionary biology document higher performance for locally-adapted

plants (Leimu and Fischer 2008). Locally-adapted plants demonstrate an average of 45% higher fitness than introduced genotypes (Hereford 2009). An organism's superior fecundity and fitness for one environment tend to be suboptimal in other environments ($r = -0.14$, $p = 0.01$, summarized from 74 studies). The failure of adaptations to confer wide-spread advantage may be the consequence of antagonistic pleiotropy, through which best performing alleles in one environment perform poorly in others (Anderson et al. 2011). When large GxL effects indicate an effect of local adaptation, breeding programs can maximize beneficial alleles by making selections and testing varieties in distinct locations.

We analyzed the performance of diverse wheat lines over ten organically managed locations in the northeastern and northcentral United States. First, this chapter presents the magnitude and structure of GxE interactions for wheat yield and the quality characteristics of test weight, protein, and falling number. Second, we identify top performing genotypes for organic systems based on GxE interactions. Third, we assess whether organic wheat breeding for the region of interest should focus on local adaptation and/or stability.

4.2 Methods

4.2.1 Data collection

Datasets included 35 organically managed site-years of data on winter and spring wheat genotypes (Table 4.1). Genotypes included modern and heritage varieties and landraces of all wheat classes: hard and soft, white and red. All lines were replicated three to four times at each site-year and plot sizes varied from 3.8 to 15.9 m^2, depending on location. Data collected on each variety included yield in addition to the quality aspects of test weight, protein, and falling number. As balanced data is essential to determining interaction terms, only genotypes that were

replicated in all site-years were included in each dataset. Winter wheat plots that were entirely lost to winter kill were given yield values of zero. Plots that experienced bird damage, flooding, or erosion were removed from analysis. For each site, mixed models incorporating AR1xAR1 (Gilmour et al. 1997) and fixed effects significant at $p<0.05$ (Gilmour 1997) using ASReml-R v.3.0 (Butler 2009) extracted best linear unbiased predictor (BLUP) values for genotypes, which accounted for spatial correlations between columns and between rows of trial plots.

Table 4.1. Site-years, traits, and genotypes included in analyses.

Dataset Name	Site-Year Names and Codes	Site-Years	Traits Evaluated	Balanced Genotypes
VTME spring wheat[4]	Alburgh, VT 2010-2013 (AL10-AL13); Presque Isle, ME 2013 (PI13); Orono, ME 2010-2012 (OT10-OT12); Sidney, ME 2010-2013 (SD10-SD13); Willsboro, NY 2010-2013 (WB10-WB13)	16	Yield, Test Weight, Protein, Falling Number	7
VTME winter wheat[5]	Alburgh, VT 2010-2013 (AL10-AL13); Athens, ME 2010 (AT10); Houlton, ME 2011-2012 (HT11-HT12); Orono, ME 2010-2013 (OT10-OT13); Willsboro, NY 2010-2013 (WB10-WB13)	15	Yield, Test Weight, Protein, Falling Number	11
PANY spring wheat	Freeville, NY 2012-2015 (FV12-FV15); Carrington, ND 2013-2015 (ND13-ND15); Rock Springs, PA 2012-2014 (PA12-PA14); Willsboro, NY (WB12-WB15)	14	Yield, Test Weight	22
PANY winter wheat	Freeville, NY 2012-2015 (FV12-FV15); Rock Springs, PA 2012-2015 (PA12-PA15); Seneca Castle, NY 2013 (PD13); Willsboro, NY 2012-2015 (WB12-WB15)	13	Yield, Test Weight	36
ALL spring wheat	Combination of VTME and PANY spring wheat: AL10-AL13, FV12-FV15, ND13-ND15, PA12-PA14, PI13, OT10-OT12, SD10-SD13, WB10-WB15	28	Yield, Test Weight	5
ALL winter wheat	Combination of VTME and PANY winter wheat: AL10-AL13, AT10, FV12-FV15, HT11-HT12, PA12-PA15, PD13, OT10-OT13, WB10-WB15	26	Yield, Test Weight	8

[4] Data from Mallory et al. (2014)
[5] Data from Mallory et al. (2015)

4.2.2 AMMI and GGE models

We compared three common methods to assess GxE: AMMI with an Fr test, AMMI with a parametric bootstrap test, and a GGE model. The AMMI model (additive main effects, multiplicative interactions) (Gabriel 1978) allowed an assessment of the magnitude and structure of the GxE interaction. Through singular value decomposition (Equation 4.2) the AMMI model distinguishes the signal from the noise of the GxE interaction, generating composite values that best describe the environmental interaction (η_{kj}) and genetic interaction (Υ_{ki}). The number of significant k multiplicative terms to include in the model was determined by using three separate methods at a significance level of $p<0.05$. First, the method of Forkman and Piepho (2014) identified significant multiplicative terms for the AMMI model *via* a full parametric bootstrap test, implemented through the 'Bilinear' package in R (Santantonio 2016). Second, an Fr test (Piepho 1995) and cross-validation determined the number of significant terms through the AMMISOFT program (Gauch and Moran 2016). Third, an environment-centered model absorbed genotypic main effects into the GE_{ij} term (Equation 4.3) through the 'Bilinear' package in R (Santantonio 2016). Raw values and spatially corrected BLUPs were separately run through the parametric bootstrap analysis and GGE models. As AMMISOFT requires randomized complete block designs, the Fr test was only processed with raw data.

Equation 4.2 $GE_{ij} = \sum_{k=1}^{m} \lambda_k \Upsilon_{ki} \eta_{kj} + \delta_{ij}$ (Walsh and Lynch 2015a)

Equation 4.3 $\mu_{ilk} - (\mu + E_j) = \sum_{k=1}^{m} \lambda_k \Upsilon_{ki} \eta_{kj} + \delta_{ij}$ (Walsh and Lynch 2015a)

4.2.3 Identifying winning genotypes and mega-environments

Winning genotype tables were constructed using the AMMIk and GGE matrices for each environment and plotted following Gauch (2013) and Gauch and Zobel (1997). Plots were constructed using 'Bilinear' in R (Santantonio 2016) and the AMMISOFT program (Gauch and Moran 2016). If the first two terms of the singular value decomposition were significant for the GGE model, matrix values were plotted on GGE biplots using 'Bilinear' in R (Santantonio 2016), and winners were determined following Yan et al. (2007). Mega-environments were defined if testing location(s) had unique winning varieties. If mega-environments were not agronomically-interpretable, AMMI models with fewer multiplicative terms were assessed for more logical division of mega-environments (Gauch 2013).

4.2.4 Analysis of breeding priorities for local adaptation or stability

Using the framework outlined by Annicchiarico (2002), we determined whether an organic wheat breeding program should focus on local adaptation and/or stability. A likelihood ratio test using ASREML-R (Gilmour et al. 2009) tested the impact of the following random effects on yield, protein, falling number, and test weight: genotype *i*, location *j*, year *k*, and corresponding interactions, and replicate *l* (Equation 4.4). Year was nested within location, since not all years were observed in all locations. Significant AR1xAR1 and fixed row or column effects were also included in the model. Random model effects were used because the variance of each term was of primary concern for the analysis. Significance was determined at the level of $p<0.05$.

Equation 4.4 $Y_{ilkl} = \mu + G_i + L_j + Y_k(L_j) + GL_{ij} + G_iY_k(L_j) + r_l(Y_kL_j) + \varepsilon_{ijkl}$ (Annicchiarico 2002)

If GxL explained at least 33% of the variance explained by genotype, local adaptation was considered influential for the dataset. If σ^2_{GxL} was less than 33% of σ^2_G, and σ^2_{GxYxL} was greater than 33% of σ^2_G, genotypes were assessed for stability. Mega-environments, as defined in Section 4.2.3, were analyzed separately for stability. First, least square means of genotypes were calculated using Equation 4.4 and extracted using 'pbkrtest' in R (Halekoh and Højsgaard 2014). Only genotypes with least square means that were not significantly different from the top-performing genotype were included in the stability analysis, determined by a Dunnetts Test at $p<0.05$ through the 'multcomp' package in R (Hothorn et al. 2008). If a single genotype constituted the top category, that genotype was determined the winner, and stability was irrelevant. For each genotype, Type 4 stability was calculated as the variance of year, $Y_k(L_l)$ (Equation 4.5), using lme4 (Bates et al. 2015). Variances of genotypes were compared for homogeneity against the most stable genotype at $p<0.05$ (Hartley 1940). The winner was the genotype in the lowest variance (best stability) group that had the best performance for the response.

Equation 4.5 $Y_{lk} = \mu + L_l + Y_k(L_l)$ (Lin and Binns 1994)

4.3 Results and discussion

4.3.1 GxE magnitude and structure

For all datasets and traits, the GxE interaction was significant, and there was crossing over in rankings of the winners (Table 4.2). Moreover, the AMMI models distinguished large amounts of signal in the interaction. For uncorrected raw data of yield, protein, falling number, and test weight, more significant multiplicative terms were found *via* the AMMISOFT method

than *via* the parametric bootstrap method. Four datasets had more significant multiplicative terms in AMMISOFT than through parametric bootstrapping (Table 4.3). In an additional five datasets, analysis *via* the bootstrap model found no terms significant, although AMMISOFT determined at least one multiplicative was significant. When noise was removed through spatial corrections and derivation of BLUPs, the bootstrap method distinguished a significant term for the three yield datasets that were not significant with raw data. The opposite was found for falling number and test weight. For four data sets, BLUPs reduced or eliminated the number of significant multiplicative terms found in raw data using the parametric bootstrap method.

Our results concur with previous studies showing the bootstrap method to be more conservative than the sequential Fr test, which may overfit models and increase Type 1 errors (1Forkman and Piepho 2014). Bootstrap methods also generated mega-environments that were more relevant for breeding programs. Since the Fr test and boostrap methods determined different significant multiplicative terms, the winners and mega-environments also changed among compared models (Table 4.2). The AMMI models generated with the conservative boostrap method tended to produce fewer mega-environments that were more useful from a breeder's persepective (see Section 4.3.2).

The GGE model was only useful for identifying mega-environments with exactly two significant multiplicative terms in the model. Of 32 analyzed dataset-response combinations, only ten had k=2 significant terms in the GGE model (Table 4.3). The GGE model, therefore, demonstrated limited application for identifying winning varieties and mega-environments. The BLUP-based boostrap AMMI models were chosen for analyses of local adaptation and stability, due to their tendency to avoid overfitting and generate practical mega-environments.

Table 4.2. Winning genotypes in each environment for different models. Colors in the "TRIAL" column define unique mega-environments based on winning genotypes. Blue-colored genotypes indicate different winners than identified in the bootstrap method. A key to variety codes is located in Table A.10. Bootstrap and GGE models used BLUP spatially-corrected data, while Fr tests used raw data.

VTME Spring Wheat

TRIAL	Yield			TRIAL	Test Weight		TRIAL	Protein		TRIAL	Falling Number		
	bootstrap	*Fr*	*GGE*		*bootstrap*	*Fr*		*bootstrap*	*Fr*		*bootstrap*	*Fr*	*GGE*
AL10	FALL	FALL	FALL	AL10	GLEN	TOM	AL10	ACBA	ACBA	AL10	MAGO	ACBA	MAGO
AL11	FALL	FALL	FALL	AL11	GLEN	TOM	AL11	RDFE	RDFE	AL11	MAGO	ACSP	ACBA
AL12	FALL	FALL	FALL	AL12	GLEN	GLEN	AL12	GLEN	GLEN	AL12	MAGO	ACBA	MAGO
AL13	FALL	FALL	FALL	AL13	GLEN	RDFE	AL13	ACBA	ACBA	AL13	MAGO	MAGO	MAGO
OT10	FALL	FALL	FALL	OT10	GLEN	GLEN	OT10	ACBA	ACBA	OT10	MAGO	MAGO	ACBA
OT11	FALL	FALL	FALL	OT11	GLEN	GLEN	OT11	GLEN	RDFE				
OT12	FALL	FALL	FALL	OT12	GLEN	GLEN	OT12	GLEN	GLEN				
				PI13	GLEN	GLEN	PI13	GLEN	GLEN				
SD10	FALL	FALL	FALL	SD10	GLEN	GLEN	SD10	GLEN	GLEN				
SD11	FALL	FALL	FALL	SD11	GLEN	GLEN	SD11	GLEN	GLEN				
SD12	FALL	MAGO	MAGO	SD12	GLEN	GLEN	SD12	ACBA	ACBA				
SD13	FALL	FALL	FALL	SD13	GLEN	GLEN	SD13	ACBA	ACBA				
WB10	FALL	FALL	FALL	WB10	GLEN	GLEN	WB10	GLEN	GLEN	WB10	MAGO	ACBA	ACBA
WB11	TOM	FALL	TOM	WB11	GLEN	TOM	WB11	ACBA	ACBA	WB11	MAGO	ACBA	ACBA
WB12	FALL	FALL	FALL	WB12	GLEN	GLEN	WB12	GLEN	GLEN	WB12	MAGO	MAGO	MAGO
WB13	TOM	FALL	TOM				WB13	ACBA	ACBA	WB13	MAGO	ACBA	MAGO

VTME Winter Wheat

TRIAL	Yield			TRIAL	Test Weight		TRIAL	Protein		TRIAL	Falling Number	
	bootstrap	*Fr*	*GGE*		*bootstrap*	*Fr*		*bootstrap*	*Fr*		*bootstrap*	*Fr*
AL10	ACMO	OVER	JERR	AL10	ACMO	ACMO	AL10	RDEM	RDEM	AL10	WART	WART
AL11	ACMO	ACMO	ACMO	AL11	ACMO	ACMO	AL11	RDEM	RDEM	AL11	WART	RDEM
AL12	ACMO	EXPE	ACMO	AL12	ACMO	EXPE	AL12	RDEM	RDEM	AL12	WART	JERR
AL13	ACMO	ACMO	ACMO	AL13	ACMO	EXPE	AL13	RDEM	RDEM	AL13	WART	WART
AT10	ACMO	OVER	ACMO	AT10	EXPE	WART	AT10	MAXI	MAXI			
HT11	ACMO	ACMO	ACMO	HT11	ACMO	ACMO	HT11	RDEM	RDEM			
HT12	ACMO	ACMO	ACMO	HT12	ACMO	ACMO	HT12	RDEM	RDEM			
OT10	ACMO	ACMO	JERR	OT10	EXPE	EXPE	OT10	MAXI	MAXI			
OT11	ACMO	ACMO	ACMO	OT11	EXPE	EXPE	OT11	RDEM	RDEM			
OT12	ACMO	ACMO	ACMO	OT12	ACMO	ACMO	OT12	RDEM	RDEM			
OT13	JERR	OVER	JERR	OT13	ACMO	ACMO	OT13	RDEM	RDEM			
WB10	JERR	OVER	JERR	WB10	EXPE	EXPE	WB10	MAXI	RDEM	WB10	WART	WART
WB11	JERR	JERR	JERR	WB11	ACMO	ACMO	WB11	RDEM	RDEM	WB11	WART	WART
WB12	ACMO	EXPE	ACMO	WB12	EXPE	EXPE	WB12	RDEM	RDEM	WB12	WART	WART
WB13	ACMO	ACMO	ACMO	WB13	EXPE	EXPE	WB13	RDEM	RDEM	WB13	WART	WART

PANY Spring Wheat

TRIAL	Yield		TRIAL	Test Weight	
	bootstrap	*Fr*		*bootstrap*	*Fr*
FV12	STEE	RB07	FV12	GLEN	GLEN
FV13	SABI	SABI	FV13	GLEN	GLEN
FV14	ACBA	RDFE	FV14	GLEN	GLEN
FV15	TOM	SABI	FV15	GLEN	GLEN
ND14	LOUI	LOUI	ND14	GLEN	GLEN
ND15	ADA	TOM	ND15	GLEN	GLEN
ND13	LOUI	TOM	ND13	GLEN	MN61
PA12	SABI	SABI	PA12	GLEN	GLEN
PA13	LOUI	ACBA	PA13	CERE	THAT
PA14	TOM	TOM	PA14	GLEN	GLEN
WB12	MN78	MN78	WB12	GLEN	GLEN
WB13	TOM	TOM	WB13	GLEN	GLEN
WB14	SABI	TOM	WB14	GLEN	GLEN
WB15	LOUI	MIDA	WB15	CERE	ADA

PANY Winter Wheat

TRIAL	Yield		TRIAL	Test Weight	
	bootstrap	*Fr*		*bootstrap*	*Fr*
FV12	ARRW	YKST	FV12	NUEA	NUEA
FV13	YKST	YKST	FV13	NUEA	NUEA
FV14	ACMO	ACMO	FV14	WART	NUEA
FV15	SUSQ	ARRW	FV15	NUEA	NUEA
PA12	NUEA	ARS9	PA12	PRGE	NUEA
PA13	ARRW	NUEA	PA13	NUEA	NUEA
PA14	ACMO	ACMO	PA14	PRGE	PRGE
PA15	ARRW	ARRW	PA15	PRGE	PRGE
PD13	WART	WART	PD13	NUEA	ARS9
WB12	NUEA	ARS9	WB12	ARS9	NUEA
WB13	ACMO	ARRW	WB13	ARS9	ARS9
WB14	WART	WART	WB14	ZORO	ZORO
WB15	PRGE	PRGE	WB15	ACMO	PRGE

ALL Spring Wheat

TRIAL	Yield			TRIAL	Test Weight		
	bootstrap	*Fr*			*bootstrap*	*Fr*	*GGE*
AL10	TOM	TOM		AL10	RB07	TOM	RB07
AL11	TOM	TOM		AL11	TOM	TOM	RB07
AL12	TOM	TOM		AL12	GLEN	GLEN	GLEN
AL13	TOM	TOM		AL13	GLEN	RDFE	GLEN
FV12	TOM	TOM		FV12	GLEN	GLEN	GLEN
FV13	TOM	TOM		FV13	GLEN	GLEN	GLEN
FV14	RDFE	RDFE		FV14	GLEN	GLEN	GLEN
FV15	TOM	TOM		FV15	GLEN	GLEN	GLEN
ND14	TOM	TOM		ND14	GLEN	GLEN	GLEN
ND15	TOM	TOM		ND15	GLEN	GLEN	GLEN
ND13	TOM	TOM		ND13	GLEN	GLEN	GLEN
OT10	TOM	TOM		OT10	GLEN	GLEN	GLEN
OT11	TOM	TOM		OT11	GLEN	GLEN	GLEN
OT12	TOM	TOM		OT12	GLEN	GLEN	GLEN
PA12	TOM	TOM		PA12	GLEN	GLEN	GLEN
PA13	TOM	TOM		PA13	TOM	RB07	GLEN
PA14	TOM	TOM		PA14	GLEN	GLEN	GLEN
				PI13	GLEN	GLEN	GLEN
SD10	TOM	TOM		SD10	GLEN	GLEN	GLEN
SD11	TOM	TOM		SD11	GLEN	GLEN	GLEN
SD12	TOM	TOM		SD12	GLEN	GLEN	GLEN
SD13	TOM	TOM		SD13	GLEN	GLEN	GLEN
WB10	TOM	TOM		WB10	GLEN	GLEN	GLEN
WB11	TOM	TOM		WB11	TOM	TOM	RB07
WB12	TOM	TOM		WB12	GLEN	GLEN	GLEN
WB13	TOM	TOM		WB13	GLEN	GLEN	GLEN
WB14	TOM	TOM		WB14	GLEN	GLEN	GLEN
WB15	TOM	TOM		WB15	TOM	TOM	RB07

ALL Winter Wheat

TRIAL	Yield			TRIAL	Test Weight	
	bootstrap	*Fr*	*GGE*		*bootstrap*	*Fr*
AL10	ACMO	EXPE	ACMO	AL10	ACMO	WART
AL11	ACMO	ACMO	ACMO	AL11	ACMO	HARV
AL12	ACMO	WART	RDEM	AL12	ACMO	EXPE
AL13	ACMO	ACMO	ACMO	AL13	ACMO	EXPE
AT10	ACMO	ACMO	ACMO	AT10	ACMO	ACMO
FV12	ACMO	ACMO	ACMO	FV12	ACMO	EXPE
FV13	ACMO	ACMO	ACMO	FV13	ACMO	EXPE
FV14	ACMO	ACMO	ACMO	FV14	ACMO	ACMO
FV15	ACMO	ACMO	ACMO	FV15	ACMO	ACMO
HT11	ACMO	ACMO	ACMO	HT11	ACMO	EXPE
HT12	ACMO	ACMO	ACMO	HT12	ACMO	HARV
OT10	ACMO	ACMO	ACMO	OT10	ACMO	EXPE
OT11	ACMO	ACMO	ACMO	OT11	ACMO	WART
OT12	ACMO	ACMO	ACMO	OT12	ACMO	EXPE
OT13	ARAP	ACMO	ZORO	OT13	ACMO	HARV
PA12	ACMO	ARAP	ZORO	PA12	ACMO	EXPE
PA13	ACMO	WART	MAXI	PA13	ACMO	WART
PA14	ACMO	ACMO	ACMO	PA14	ACMO	HARV
PA15	ACMO	ACMO	ACMO	PA15	ACMO	EXPE
PD13	ACMO	ACMO	ACMO	PD13	ACMO	EXPE
WB10	ARAP	ZORO	ZORO	WB10	ACMO	HARV
WB11	ARAP	ZORO	ACMO	WB11	ACMO	ACMO
WB12	ACMO	EXPE	ACMO	WB12	ACMO	WART
WB13	ARAP	ACMO	ZORO	WB13	ACMO	EXPE
WB14	ACMO	WART	ACMO	WB14	ACMO	ACMO
WB15	ARAP	ZORO	ARAP	WB15	ACMO	HARV

Table 4.3. Comparison of three models for significant multiplicative terms. Red values show differences in significance of multiplicative terms between Fr tests and parametric bootstrapping. **Bold** values show differences in the number of significant model terms between AMMISOFT and AMMI using Forkman and Piepho. Blue values show differences in the number of significant terms between corrected BLUPs and raw data in the parametric bootstrapping method. Table 4.1 contains details on analyzed datasets.

Dataset	Habit	Data type	Method*	Significant multiplicative terms			
				Yield	Protein	Falling Number	Test Weight
VTME	Spring	corrected	Parametric Bootstrap AMMI	PC1	PC3	NS	NS
VTME	Spring	corrected	Bilinear GGE	PC2	PC3	PC2	PC1
VTME	Spring	uncorrected	Parametric Bootstrap AMMI	NS	**PC2**	PC1	PC1
VTME	Spring	uncorrected	AMMISOFT Fr test	PC2	**PC3**	PC1	**PC3**
VTME	Spring	uncorrected	Bilinear GGE	PC2	PC2	PC1	PC3
VTME	Winter	corrected	Parametric Bootstrap AMMI	PC1	PC1	NS	PC1
VTME	Winter	corrected	Bilinear GGE	PC2	PC3	PC1	PC3
VTME	Winter	uncorrected	Parametric Bootstrap AMMI	NS	PC2	NS	PC1
VTME	Winter	uncorrected	AMMISOFT Fr test	PC2	PC2	PC1	PC1
VTME	Winter	uncorrected	Bilinear GGE	PC1	PC1	PC1	PC2
PANY	Spring	corrected	Parametric Bootstrap AMMI	PC4			**PC2**
PANY	Spring	corrected	Bilinear GGE	PC5			PC4
PANY	Spring	uncorrected	Parametric Bootstrap AMMI	**PC2**			**PC2**
PANY	Spring	uncorrected	AMMISOFT Fr test	**PC4**			PC2
PANY	Spring	uncorrected	Bilinear GGE	PC2			PC6
PANY	Winter	corrected	Parametric Bootstrap AMMI	PC3			PC5
PANY	Winter	corrected	Bilinear GGE	PC4			PC7
PANY	Winter	uncorrected	Parametric Bootstrap AMMI	**PC3**			PC4
PANY	Winter	uncorrected	AMMISOFT Fr test	**PC5**			PC4
PANY	Winter	uncorrected	Bilinear GGE	PC4			PC8
ALL	Spring	corrected	Parametric Bootstrap AMMI	PC1			PC1
ALL	Spring	corrected	Bilinear GGE	PC1			PC2
ALL	Spring	uncorrected	Parametric Bootstrap AMMI	PC1			PC1
ALL	Spring	uncorrected	AMMISOFT Fr test	PC1			PC1
ALL	Spring	uncorrected	Bilinear GGE	PC1			PC2
ALL	Winter	corrected	Parametric Bootstrap AMMI	PC1			NS
ALL	Winter	corrected	Bilinear GGE	PC2			NS
ALL	Winter	uncorrected	Parametric Bootstrap AMMI	NS			NS
ALL	Winter	uncorrected	AMMISOFT Fr test	PC2			PC1
ALL	Winter	uncorrected	Bilinear GGE	PC1			NS

* AMMISOFT requires randomized complete block designs, and cannot analyze spatially-corrected BLUPs.

4.3.2 Mega-environments and winning genotypes

Locations with unique winning genotypes suggest multiple selection and variety testing locations in the region. For both spring and winter wheat, Willsboro, NY tended to be a unique mega-environment for yield and test weight (Figure 4.1). North Dakota, the most distant of the locations, also merits a unique testing site. North Dakota had distinct winning genotypes for yield, with the overall best genotype at all sites, 'Tom,' never winning at that location (Table 4.2). Using the AMMI model with k=3 significant terms, mega-environments were not apparent for the PANY winter wheat dataset, with winners changing among individual site-years, rather than clustering by location (Table 4.2). For such situations, Gauch (2013) recommended grouping mega-environments by simpler models with fewer multiplicative terms, until an agronomically-explicable trend emerges for differences in variety performance. The simpler model of k=1 terms for PANY winter wheat yield and test weight logically separate the dataset's three locations (Rock Springs, PA; Freeville, NY; and Willsboro, NY) as unique mega-environments. Figure 4.2 shows an identification of mega-environments by winning genotypes for yield at k=1 multiplicative terms. Seventy-five percent of the years at Pennsylvania and Freeville, NY grouped into two different mega-environments by winning genotype. In contrast, the Willsboro location spans the entire breadth of environment scores for the GxE interaction, with each year claiming a different winning genotype. Although Willsboro site-years did not share a common winning genotype in the PANY dataset, the location's high GxY variance validate its distinction as a separate mega-environment.

No clear mega-environment designations were identified for protein or falling number. Rather than grouping by location, winter wheat protein grouped by year (Figure 4.3a). Abnormally warm and dry conditions in 2010 selected a different winning genotype than in other

years (Figure 4.3b). The low resolution of site-years for falling number likely inhibits the identification of mega-environments (Annicchiarico 2002).

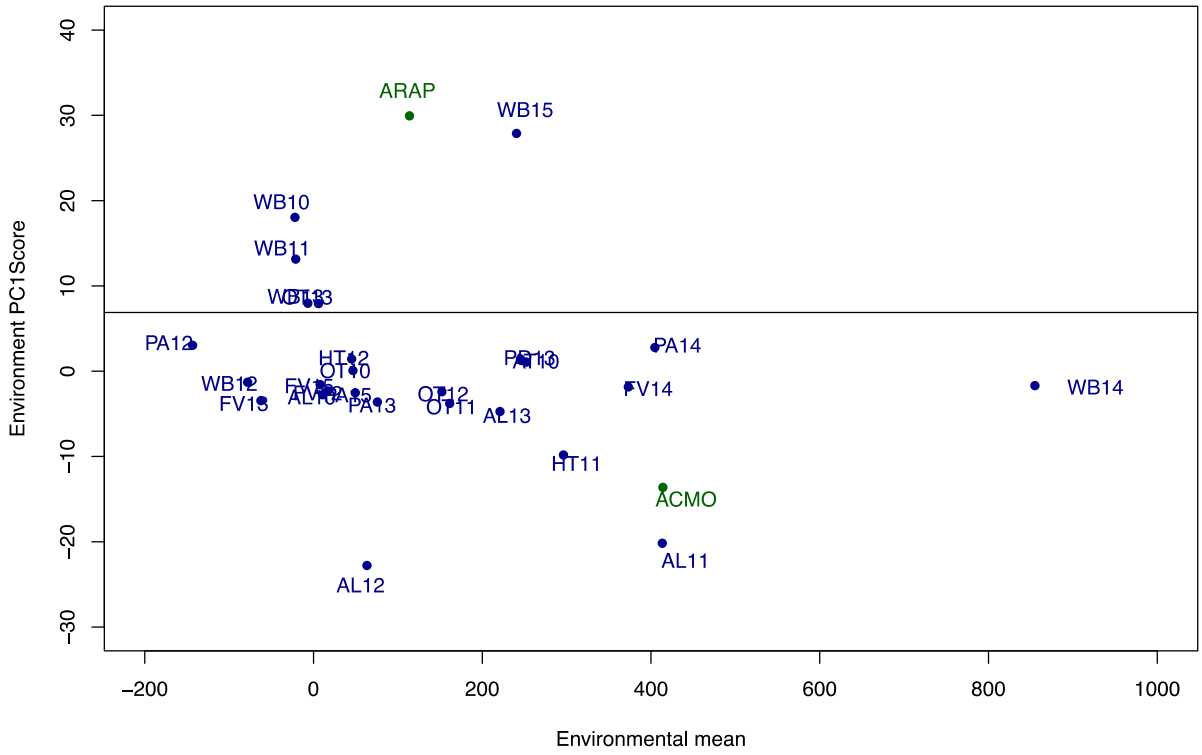

Figure 4.1. Winning genotypes and mega-environments in the ALL winter wheat dataset. Horizontal lines separate mega-environments defined by winning genotypes. Willsboro, NY represents a separate mega-environment for winter wheat yield. In four of six years, Willsboro had a distinct environmental PC score and winning genotype, 'Arapahoe' (ARAP), compared with the other sites, where 'AC Morley' (ACMO) won.

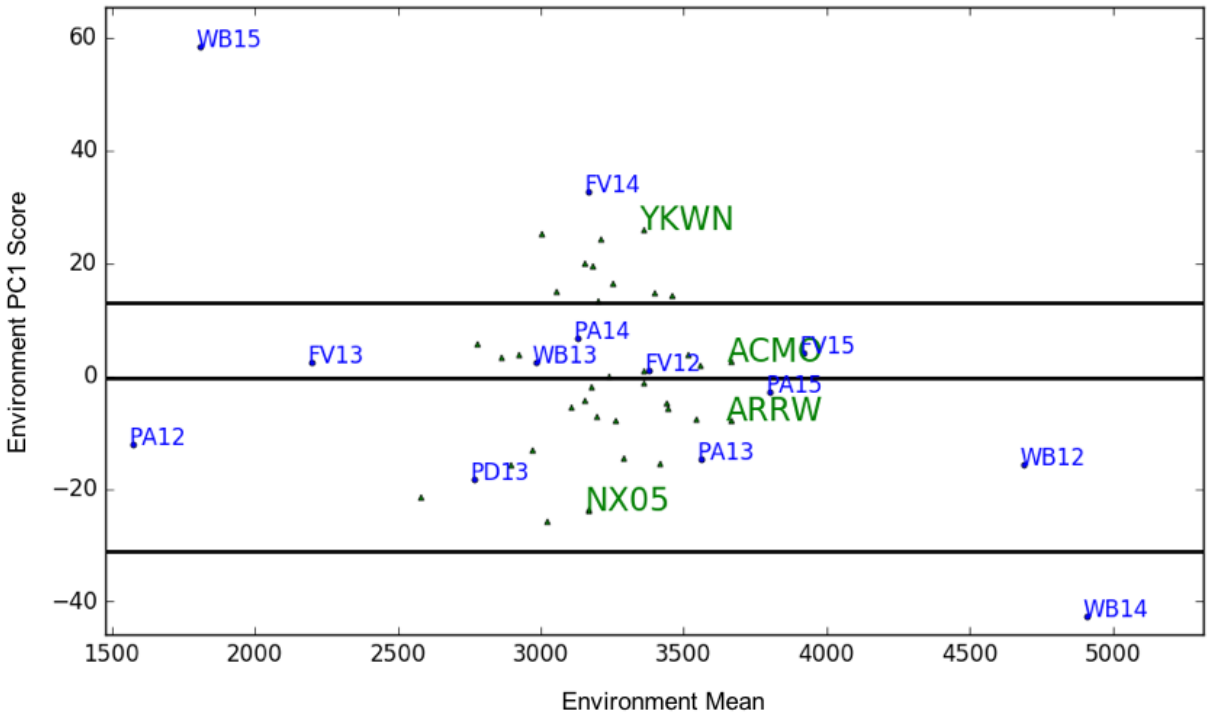

Figure 4.2. Winning genotypes and mega-environments in the PANY winter wheat dataset. Horizontal lines separate mega-environments defined by winning genotypes. The Pennsylvania (PA) and Freeville (FV) locations tend to group into two different mega-environments, and may be good choices for separate selection and variety testing environments. The years at Willsboro (WB) span the breadth of the environmental scores for the GxE interaction term, showing high GxY variation. A key to variety codes is found in Table A.10.

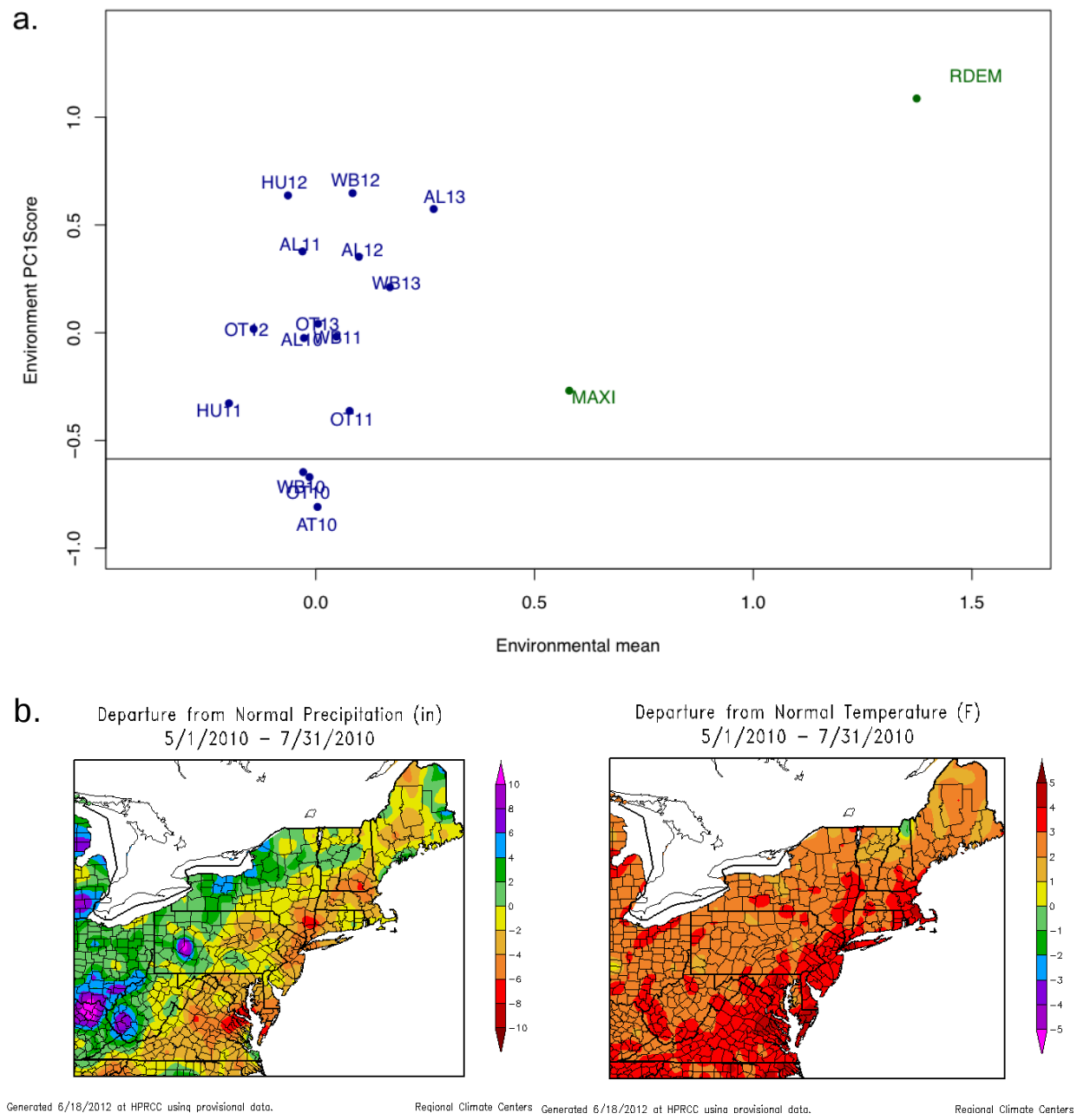

Figure 4.3. Winning winter wheat genotypes and mega-environments for protein. (a) Horizontal lines separate mega-environments defined by winning genotypes. The site-years group by year rather than location. 2010 led to a different winning genotype, 'Maxine' (MAXI), compared to 'Redeemer' (RDEM) that won at all other site-years. (b) Abnormally hot and dry conditions in the 2010 testing locations likely triggered this difference (map modified from Northeast Regional Climate Centers 2016).

4.3.3 Magnitude of GxL interactions

Similar to mega-environments identified through AMMI models for test weight and yield, GxL was significant ($p<0.05$) and borderline significant ($p<0.1$) for yield and test weight in various datasets (Table 4.4). For winter wheat yield in the combined dataset, GxL interaction variance also represented more than 33% of the genotypic variance. The data validate that winter wheat will benefit from local adaptation by separating northern New York as a unique mega-environment. Although GxL variance was less than 33%, evidence suggests that separate selection and testing sites in Pennsylvania, central New York, and North Dakota are also merited (Figure 4.2).

GxY was significant in nearly every case and explained more than 33% of genotypic variance in all but four dataset responses (Table 4.4). The influence of GxY indicates high variability in genotype performance among years within locations, and emphasizes stability as a priority for organic wheat breeding. More selection locations and years could help buffer the large variability in site-year variety rankings for yield, test weight, protein, and falling number. Winning genotypes with and without stability are presented in Table 4.5. The stability analysis changed the winning genotype for one quarter of analyzed datasets. Consequently, stability analyses are meaningful for choosing top-performing genotypes in regional breeding programs.

Table 4.4. Analysis of genotype, location, year, and subsequent interactions on yield, protein, falling number, and test weight. Significance is indicated by '.' (p<0.1), * (p<0.05), and *** (p<0.001).

Dataset	Habit	Trait	% Variance GxL/G	% Variance GxY(L)/G
VTME	Spring	Yield	15.47.	72.91***
VTME	Spring	Protein	<0.001	32.44***
VTME	Spring	Falling #	4.12	18.24***
VTME	Spring	Test Weight	5.76	31.14***
VTME	Winter	Yield	17.63	97.71***
VTME	Winter	Protein	2.10	24.13***
VTME	Winter	Falling #	2.35	25.90***
VTME	Winter	Test Weight	23.73*	35.26***
PANY	Spring	Yield	<0.001	142.0***
PANY	Spring	Test Weight	9.41***	18.16***
PANY	Winter	Yield	<0.001	137.2***
PANY	Winter	Test Weight	0.214	94.40***
ALL	Spring	Yield	16.35.	21.59
ALL	Spring	Test Weight	4.45	88.12***
ALL	Winter	Yield	45.12	199.9***
ALL	Winter	Test Weight	<0.001	179.94.

Table 4.5. Winning genotypes based on stability analysis. If only one variety was in the top category, it was the winning genotype, regardless of stability. Blue text indicates a change in winning variety based on stability. FV indicates Freeville, NY; ND indicates Carrington, North Dakota; PA indicates Rock Springs, Pennsylvania; WB indicates Willsboro, NY; '-ND' means all sites other than ND, '-WB' means all sites other than WB. A key to variety codes is located in Table A.10. Significant difference in genotype Y(L) variances at . $p<0.1$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Dataset	Habit	Trait	Mega-environment	Varieties in top means category	Differences in variety stability	Varieties in top stability category	Winner without stability	Winner with stability
VTME	Spring	Yield		1			FALL	FALL
VTME	Spring	Protein		2	NS	2	ACBA	ACBA
VTME	Spring	Falling #		3	NS	3	ACBA	ABCA
VTME	Spring	Test Weight		2	NS	2	GLEN	GLEN
VTME	Winter	Yield	WB	12	***	2	MILL	ACMO
VTME	Winter	Yield	-WB	5	*	2	ACMO	ACMO
VTME	Winter	Protein		1			RDEM	RDEM
VTME	Winter	Falling #		4	**	1	WART	CAME
VTME	Winter	Test Weight	WB	7	***	1	EXPE	EXPE
VTME	Winter	Test Weight	-WB	7	*	7	RDEM	RDEM
PANY	Spring	Yield	ND	15	***	8	LOUI	LOUI
PANY	Spring	Yield	-ND	8	*	8	TOM	TOM
PANY	Spring	Test Weight		6	**	4	GLEN	TOM
PANY	Winter	Yield	FV	31	*	24	SUSQ	SUSQ
PANY	Winter	Yield	PA	31	**	19	NUEA	NUEA
PANY	Winter	Yield	WB	36	***	8	ARRW	ZORO
PANY	Winter	Test Weight	FV	18	**	1	PRGE	FULC
PANY	Winter	Test Weight	PA	13	***	6	NUEA	PRGE
PANY	Winter	Test Weight	WB	24	**	23	ARS9	ARS9
ALL	Spring	Yield		1			TOM	TOM
ALL	Spring	Test Weight		2	.	2	GLEN	GLEN
ALL	Winter	Yield	WB	8	***	4	ZORO	ZORO
ALL	Winter	Yield	-WB	2	NS	2	ACMO	ACMO
ALL	Winter	Test Weight		6	.	6	WART	WART

4.4 Conclusions

Yield, protein, falling number, and test weight of organically managed spring and winter wheat were influenced by genotype by environment interactions. Winning genotypes differed among site-years for all four traits. In comparing models, AMMISOFT distinguished more GxE signal than a parametric boostrapping method. However, the more conservative results of the bootstrapping method tended to generate more interpretable GxE structure. For yield and test weight, mega-environments were identified that had unique top performers among locations. Organic wheat breeding programs should separate testing and selection sites in northern New York, southern New York, central Pennsylvania, and North Dakota from sites in New England. Local adaptation of varieties to specific mega-environments indicates that decentralized breeding will increase genetic gain for yield and test weight. Due to the large influence of year on genotypic performance, stability should also be a focus of breeding programs for organic wheat in the northeastern and northcentral United States.

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CHAPTER 5

SELECTING WHEAT FOR WEED-COMPETITIVE ABILITY: A SUCCESS IN PARTICIPATORY BREEDING FROM THE NORTHEASTERN UNITED STATES

Abstract

Competition from weeds often reduces wheat yields and discourages farmers from transitioning to organic production. Improved yields can be achieved by breeding wheat for increased competitive ability with weeds. Selecting directly for weed-competitive ability, however, is challenged by difficult field measurements, genotype by environment interactions, and low heritability. To identify more effective secondary selection traits that breeding programs could use for weed-competitive ability, we conducted meta-analysis of the published literature. Worldwide, early vigor and, to a lesser extent, early plant height, proved to be easy measureuments that were consistently correlated with weed-competitive ability. To inform the design of a breeding program for the northeastern United States, we assessed the genotype by environment interactions for weed-competitive ability and its correlated traits of early vigor and height. Results indicated that multiple selection sites would maximize gain in selection for weed-competitive ability and early vigor. We tested the effectiveness of a decentralized participatory plant breeding method to improve weed-competitive ability for organic wheat. Participating farmers were effective at indirectly selecting for weed-competitive ability using early vigor and strong weed pressure in the field. Developed lines preferentially performed at farms where they were selected, and at sites with similar genotypic correlation to the selection site. This study concludes that weed-competitive ability and early vigor can benefit from highly-decentralized breeding.

5.1 Introduction

Wheat yield under organic production suffers when compared to yield under conventional production systems. Global meta-analyses conducted by Seufert et al. (2012) and Ponisio et al. (2014) revealed that organic wheat production yielded an average of 29 to 38% less than conventional systems. Heavy weed pressure is a major cause of lower realized yields in organic systems (Teasdale et al. 2007; Cavigelli et al. 2008). In the U.S. and the U.K., weeds are the major barrier preventing conventional farmers from transitioning to organic production (Bond and Grundy 2001; Walz 2004).

Selecting varieties with superior competitive ability is an important component of weed management in agricultural systems. In organic agriculture, competitive varieties can buffer mechanical and cultural weed management practices, which can occassionally fail to control weeds and reduce yield under certain field conditions (Kolb et al. 2010, Rasmussen 2004). Moreover, competitive crop genotypes can decrease the complexity of in-field weed management (Bastiaans et al. 2008). Organic wheat farmers in the northeastern United States echoed the need for varieties that could suppress weeds, rating it as the most important priority for breeding (see Section 1.2.1).

This chapter aims to (1) identify secondary selection traits in wheat that are correlated with weed-competitive ability, (2) assess the genotype by environment interactions associated with competitive ability in wheat, and (3) evaluate a participatory plant breeding program for improving weed-competitive ability in spring wheat.

5.1.1 Weed-crop interactions

Weed-competitive ability (WCA) is a measure that describes ecological interactions

between weeds and wheat plants. By reducing wheat emergence *via* allelopathy and suppressing growth by competing for light and nutrients (Blackshaw 1994; Huel and Hucl 1996), weeds can reduce tillering in wheat (Huel and Hucl 1996; Seefeldt and Ogg 1999) and ultimately lower grain yield (Blackshaw 1994; Huel and Hucl 1996; Worthington et al. 2013). Weeds will exert maximum interference at times during the season that correspond to peak light, nutrient, and water demands for their growth. Earlier weed interference is likely to affect wheat seed set and kernel number (spikes per m^2 x kernels per spike) more than kernel weight, which is affected by later weed interference during grain fill (Mason et al. 2007b).

Wheat plants can also fight back. Crop interference, which will be referred to as **weed suppression** from this point forward, describes a crop's ability to reduce weed growth and reproduction (Jordan 1993). Weed suppression is generally measured as weed biomass and/or weed seed production in competition with wheat – two measures that are highly correlated (Mason et al. 2007b; Worthington et al. 2013). Due to their relatively large seed size compared with weeds, field crops such as wheat, maize, soybean, oat, and rye have a competitive advantage early in the season (Mohler 1996). Large-sized crop seeds provide ample resources for early growth and competition, while also reducing susceptibility to allelochemicals released by other plants (Liebman and Davis 2000). However, weeds compensate for their small seed size by exhibiting high relative growth rate after germination (Seibert and Pearce 1993). In a classic experiment by Pavlychenko and Harrington (1934), flowering broadleaf weeds had up to 52 times the carbon assimilation surface of wheat. Wheat also tends to be less effective than weeds at competing for nutrients and water later in their growth cycle. For example, 21 days after emergence, the root systems of three weed species exceeded the root depth of four wheat varieties (Pavlychenko and Harrington 1934).

Crop tolerance is the ability of a crop to produce seed yield in the presence of weeds. This measure of WCA is influenced by direct competition with weeds (weed suppression), but also by the avoidance strategies employed by the crop. Crops may have high tolerance if their peak resource demands occur at times when weed demand for light, nutrients, or water use is low. For example, winter wheat can reduce competition from warm season annuals by conducting much of its growth during cold times of the year. Crop tolerance is reported as either (1) the absolute yield of a variety under weedy conditions, or (2) the percent yield loss under weedy conditions as compared with weed-free conditions. In previous studies, absolute yield showed stronger correlations with weed seed or biomass production than with percent yield loss (Worthington et al. 2013, Coleman et al. 2001). Nevertheless, percent yield loss is a factor that influences farm profitability during years with variable weed densities. In years of low weed pressure, a farmer could lose revenue if using a weed-suppressive variety that has low yield potential in weed-free conditions (Jordan 1993, Lemerle et al. 2001). Some studies (Blackshaw 1994, Ogg and Seefeldt 1999) avoid this problem by only testing varieties with comparable potential yield under weed-free conditions.

5.1.2 The ideotype

An ideal wheat plant for weed-competitive ability would express both high weed suppression and crop tolerance. Genotypes with high weed suppression would limit weed growth and reduce the soil weed seedbank. Genotypes with high crop tolerance would ensure good wheat yields regardless of varying weed seedbank conditions between farms and year-to-year variability within one farm.

Lemerle, Gill, et al. (2001) compared the theoretical ideotype for grain yield of a plant in isolation, a plant in a mixed community with weeds (“the competition ideotype”), and a plant in

a dense monoculture. The ideotype of a plant in competition with weeds is nearly identical to the ideotype of an individual plant, except that the competition ideotype puts more emphasis on early growth (Figure 5.1). The competition ideotype, however, is very different than the ideotype of a plant in dense monoculture. Under weedy conditions, the weight of grain per plant is the most important determinant of yield, rather than the weight of grain per hectare, as is the case for a dense monoculture (Lemerle, Gill, et al. 2001). There is often no correlation between wheat yields under dense monoculture (without weeds) and wheat yields when weed competition is present (Lemerle et al. 1996), although exceptions do exist (Challaiah et al. 1986). Mokhtari et al. (2002) hypothesized that high-yielding varieties in weed-free conditions set more seed early in the season, and then struggle to fill so many kernels when weeds heavily compete for resources late in the season. Since most modern lines have been bred for grain yield in dense monoculture, new breeding priorities are required to develop wheat that is highly competitive with weeds.

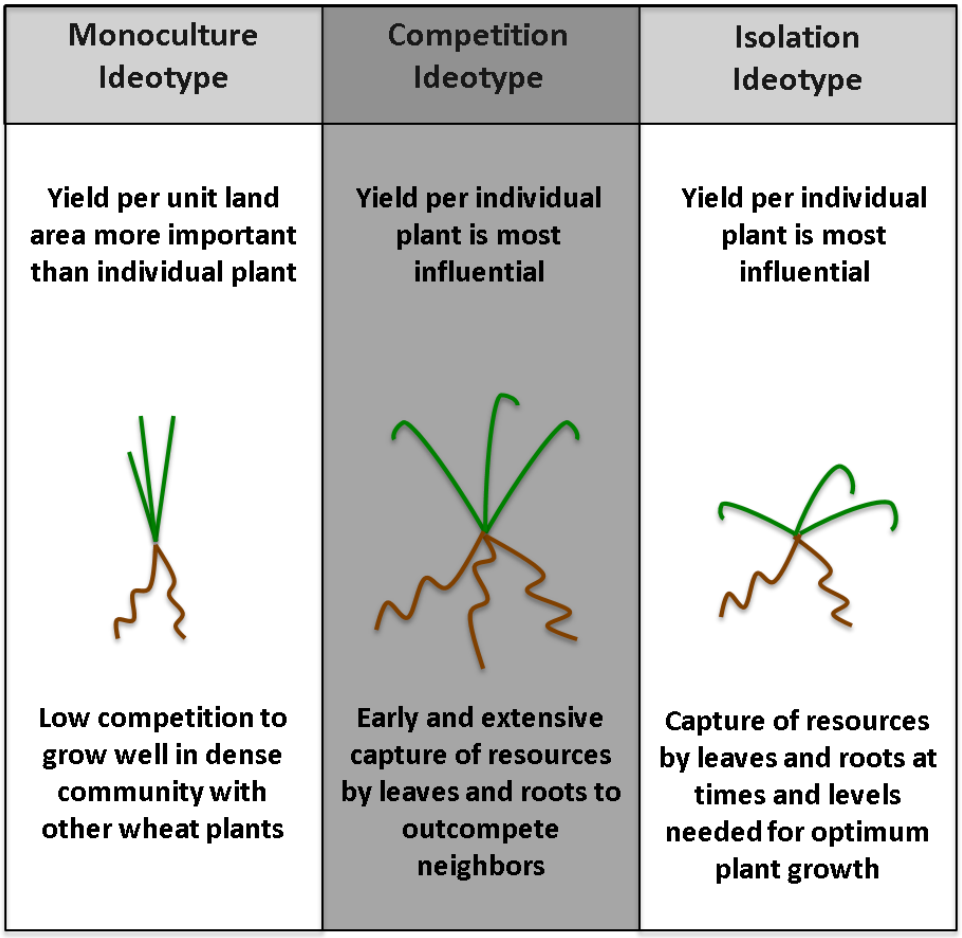

Figure 5.1. Comparison of ideotypes of wheat when grown in monoculture, competition with other species, and in isolation without competition. Figure based on Lemerle, Gill, et al. (2001).

5.1.3 Breeding for weed-competitive genotypes

5.1.3.1 Genetic gains for WCA

The Breeder's Equation (Equation 1.1) (Falconer 1981) outlines how genetic improvement can be maximized for weed-competitive ability. First, breeding populations with more variability in WCA (σ) allow for more gains in selection. Wheat genotypes vary in competition with weeds, indicating the potential to breed for the trait. When compared to highly suppressive lines, poorly suppressive genotypes allowed 79% more *Aegilops cylindrica* Host (jointed goatgrass) seed weight (Ogg and Seefeldt 1999), 29% more *Avena sativa* L. (oat)

biomass (Huel and Hucl 1996), double the *Bromus tectorum* L. (downy brome) biomass (Blackshaw 1994), seven times the *Lolium rigidum* biomass (Lemerle et al. 1996), and up to 5.7 times the resident weed biomass (Wicks et al. 1986; Murphy et al. 2008). Percent yield loss from weed competition varied from 23 to 60% (Huel and Hucl, 1996; Blackshaw, 1994; Mason et al., 2007b; Baylan et al. 1991). Experimental design that shows differences in weed suppression and tolerance is integral to variability in selection (Worthington et al. 2013).

Second, high selection intensity (i) improves weed-competitive ability by advancing only the very best genotypes. Third, high heritability traits generate more gains in selection. Narrow-sense heritability (h^2) describes the amount of observed variation in a trait that can be passed on to future generations (see Equation 5.1) (Falconer 1981). WCA is a complex and quantitative trait with low heritability (Coleman et al. 2001). Moreover, genotype by environment interactions (GxE) reduce heritability (Equation 5.1). Rankings for WCA correlate among some site-years, but not for others (Lemerle, Verbeek, et al. 2001; Worthington et al. 2015a). In a comparison of 11 cultivars grown in four diverse environments in Australia, only one cultivar had high crop tolerance to *L. rigidum* competition across all sites (Cousens and Mokhtari 1998). Certain cultivars either performed very poorly or very well at individual sites, but not consistently among sites.

Equation 5.1 $$h^2 = \frac{\sigma_a^2 \text{ (additive genetic variance)}}{[(\sigma_g^2 \text{ (genetic variance)} + \sigma_e^2 \text{ (environmental variance)} + \sigma_{ge}^2 \text{(interaction variance)}]}$$

5.1.3.2 Indirect selection

Due to low heritability, direct selection for WCA is not efficient for breeding programs. Moreover, the large amounts of seed, land, and labor needed to screen for weed suppression and

crop tolerance makes direct selection very expensive (Worthington and Reberg-Horton 2013). Indirect selection seeks to identify a secondary trait (x) that is strongly correlated (ρ) with the primary trait of competitive ability (y), yet has higher heritability and is easier to evaluate in the field (Equation 5.2) (Acquaah 2012). When two traits are highly correlated in their response to selection (CR_y), these traits are likely associated due to mechanisms such as pleiotropism or linkage disequilibrium.

Equation 5.2 $CR_y = i\rho h_x \sigma_{gy}$

5.1.4 Traits correlated with weed-competitive ability

Linear regression demonstrated that many traits contribute to the WCA of wheat (Challaiah et al. 1986; Lemerle et al. 1996; Bertholdsson 2005; Murphy et al. 2008). Competition for light is influenced by plant height, tillering, leaf angle, canopy structure, seedling ground cover, leaf area index (LAI), early leaf area expansion rate, and alleloopathy. Crops can acquire more nutrients with early root growth, high nutrient uptake rates, and roots located near nutrient supplies (Wicks et al. 1986; Huel and Hucl 1996; Lemerle, Gill, et al. 2001). Competition for water involves root distribution at the location of water storage, maturity timed to seasonal water availability, and water use efficiency (Huel and Hucl 1996; Mason et al. 2007a). Suppression of neighboring weed plants can also take place through the secretion of allelopathic compounds.

Correlations between WCA and some traits change depending on the environment and weed community evaluated (Ogg and Seefeldt 1999; Bertholdsson 2005, Coleman et al., 2001). The most well-known example of GxE for a secondary selection trait is flowering time. Later flowering genotypes are effective at competing with weeds in climates with high nutrient and

water availability late in the season. However, early maturity is important for drier climates in which wheat varieties must compete for limited early rainfall (Mason et al. 2007a). In general, maturity is confounded with weather conditions, and its relationship to competitive ability will vary by climate and year (Challaiah et al. 1986; Lemerle et al. 1996; Bertholdsson 2005; Murphy et al. 2008).

GxE interactions were also found for tillering and growth habit (*i.e.*, leaf angle), which demonstrated crossover interactions between weedy and weed-free plots (Challaiah et al. 1986). Consequently, tillering and growth habit may be poor parameters to use in the many breeding programs that screen genotypes in weed-free conditions. Allelopathic activity will only be a useful selection trait if the regional weed species of concern are susceptible to the chemicals excreted by wheat. In Bertholdsson (2011), allelopathy was significantly and positively correlated with the control of *Sinapsis alba* L. (mustard), but not that of *L. perenne* L.

Each breeding program would benefit from a region-specific model that identifies which WCA traits are most effective in their region (Worthington and Reberg-Horton 2013). No published studies on have evaluated correlated traits with WCA in the northeastern United States. To inform our organic wheat breeding program, we first completed a meta-analysis of the literature to find traits correlated with WCA. Second, we conducted an evaluation of WCA trait correlations of spring wheat in the northeastern United States. Third, through an assessment of GxE interactions for WCA and correlated traits, we also determined how many selection sites would maximize genetic gain in the region. Fourth, we tested the effectiveness of a highly decentralized breeding model to select spring wheat for WCA at various organic farms representing a diversity of environments in the Northeast.

5.2 Methods

5.2.1 Meta-analysis of secondary traits for selection of WCA

A meta-analysis of the literature (Figure 5.2) sought traits that were correlated with WCA, highly heritable, and easy to evaluate. Thirteen studies were included from diverse global environments (Figure 5.2), which met the minimum inclusion criteria of multiple site-years studied, many genotypes with broad phenotypic diversity for WCA screeed, adequate weed pressure applied to obtain variability in competition phenotypes; and weed competition sampled through biomass or visual means. The analysis also included two additional datasets from the northeast United States (see Sections 5.2.2 and 5.2.3 for study details). The quality of studies varied widely (Table 5.1). To help weight more robust results, which included more site-years and number of genotypes studied, each correlation identified in the literature was visually rated using an index of (site-years + number of genotypes studied) × 20.

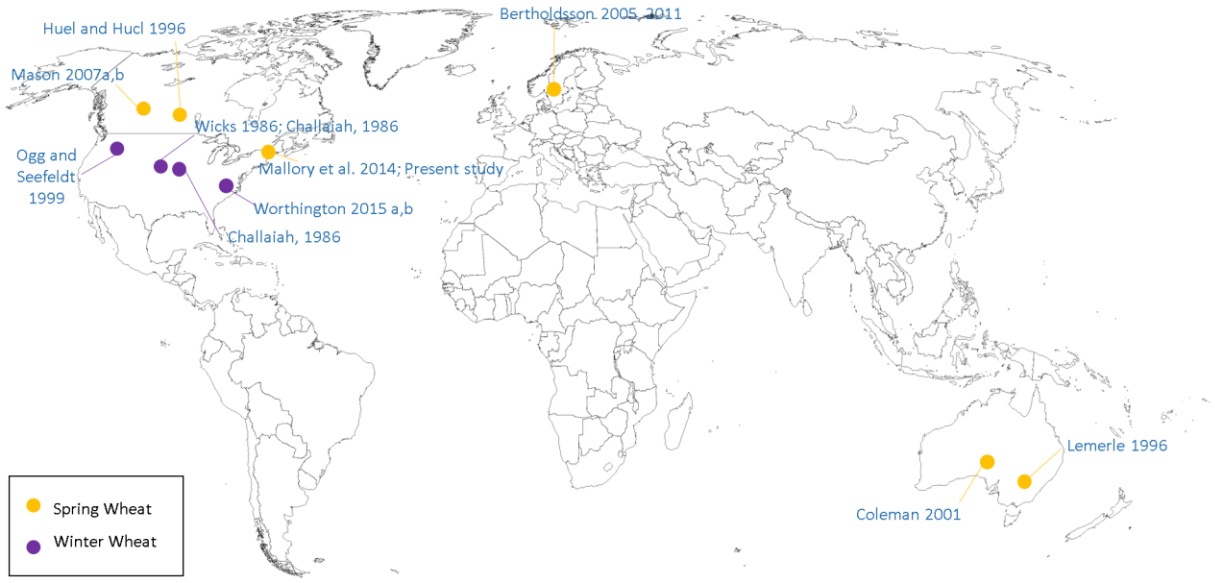

Figure 5.2. Locations of studies included in the meta-analysis. Background map provided by Wikimedia Commons <https://commons.wikimedia.org/wiki/File:World_map_blank_black_lines_4500px_monochrome.png>.

Table 5.1. Studies included in the meta-analysis that measured traits correlated to weed-competitive ability in wheat.

Reference	Wheat habit	Sites studied	Genotypes evaluated	Broad diversity of lineage and trait phenotypes included?	Years studied	Plot replications	Weeds seeded	Model weed species planted	Weed stand counts	Wheat stand counts	Weed-free control	Plot size for biomass sampling (m^2)
Bertholdsson 2011	Winter	1	12	Y	2	3	Y	*Brassica napus, Apera spica-venti*			N	0.5
Bertholdsson 2005	Spring	1	20	Y	2	3	N	resident			N	0.25
Challaiah et al. 1986	Winter	2	10	N (all tall)	2	6	Y	*Bromus tectorum*				1.44
Coleman et al. 2001	Spring	1	161	N (one cross)	2	2	Y	*Lolium rigidum*	N	N	Y	0.5
Huel and Hucl 1996	Spring	2	16	N (two crosses)	3	4	Y	*Avena sativa, Brassica juncea*	Y	Y	Y	1.23
Lemerle et al. 1996	Spring, Durum	1	250*	Y	2	1	Y	*Lolium rigidum*	Y	Y	Y	0.75
Mason, A Navabi, et al. 2007	Spring	2	9	Y	2	3	Y	*Avena sativa*	Y		N	0.13
Mason, A. Navabi, et al. 2007	Spring	2 - 4	27	Y	3	4	N	resident			N	0.13
Murphy et al. 2008	Spring	1	63	Y	3	3	N	resident			N	3.13
Ogg and Seefeldt 1999	Winter	1	7	moderate	2	4	Y	*Aegilops cylindrica*		Y	Y	0.75
Wicks et al. 1986	Winter	1	20*	moderate	3	3 - 10	N	resident		Y	Y	visual estimate
Worthington et al. 2015b	Winter	4	53	Y	2	3	Y	*Lolium perenne*			N	weed heads in 0.5 m^2
Worthington et al. 2015a	Winter	4	9	Y	2	4	Y	*Lolium perenne*	Y		Y	weed heads in 1 m^2
Present study, data from Mallory et al. 2014	Spring	3	33*	Y	1	4	N	resident	N	Y	N	0.3019 to 0.4024
Present study, 2016 data	Spring	1	30	Y	1	3	Y	*Sinapsis alba*	N	N	Y	0.5

*Indicates that varieties changed among site-years.

5.2.2 Correlated traits and genotype by environment interactions for WCA in the Northeast

We analyzed a dataset previously published by Mallory et al (2014) that assessed diverse wheat varieties for weed suppression, crop tolerance, and early vigor in the Northeast United

States. Twenty varieties of spring wheat were grown in four replicates at five site-years: Alburgh, VT 2010 and 2011; Willsboro, NY 2011; Old Town, ME and Sidney, ME in 2010. An additional 13 varieties were included in some, but not all site-years. Vigor was visually rated on a 1 to 5 scale between 2nd leaf stage and early tillering, with 5 being the most vigorous. At the three site-years in 2010, weed and wheat biomass were each determined by collecting biomass from three to four 0.1 m^2 quadrats per plot (total of 0.3 to 0.4 m^2), separating out wheat from weed biomass, drying at 55°C, and weighing. Best linear unbiased predictors (BLUPs) reduced error variance caused by spatial correlation using AR1xAR1 (Gilmour et al. 1997) and fixed effects significant at $p<0.05$ (Gilmour 1997) in ASReml-R v.3.0 (Butler 2009). Weed biomass and the ratio of wheat-to-weed biomass was logarithmically-transformed due to many low values for weed biomass.

For data compiled from the 2010 locations at Alburgh, Old Town, and Sidney, we evaluated whether crop tolerance (measured as grain yield and wheat biomass) and weed suppression (measured as weed biomass and the ratio of wheat to weed biomass) were correlated with height and early vigor. For the responses of weed biomass, the ratio of wheat to weed biomass, and early vigor, the AMMI (additive main effects, multiplicative interactions) model (Gabriel 1978; Equation 4.2) assessed the significance and structure of the GxE interaction. A parametric bootstrap test determined the number of significant k multiplicative terms to include in the AMMI model (Forkman and Piepho 2014), which was implemented through the 'Bilinear' package in R (Santantonio 2016). Site-years were also compared for magnitude and significance of correlations (at $p<0.05$) in variety BLUP ranks (Spearman 1904).

5.2.3 Measuring the effectiveness of a participatory plant breeding model for WCA

5.2.3.1 Field design

Researchers at The University of Vermont and Butterworks Farm chose parental varieties with a broad diversity of weed-competitive ability, based on the results of trials explained in Section 5.2.1. The parent 'AC Walton' tended to have lower vigor; parents 'Kelse,' 'Helios,' and 'Faller' were generally higher in vigor; and other parents' performance varied among environemnts (Figure A.1). Eight bi-parental family populations were created by bulking progeny of each cross. After increasing seed to the F4 generation, the bi-parental populations were planted on representative organic farms of the region. Five participating farmers (Figure 1.2, "Spring wheat farmer collaborator") planted bulked F4 bi-parental families from spring wheat crosses in 2014. Each farm established five to six bi-parental family populations in a randomized complete block design with two replicates (Figure 5.3). Plot sizes varied from 5.6 to 7.3 m^2, depending on the size of regional planting equipment. As mentioned in Section 5.1.3, yield under weed competition is determined on an individual plant basis, rather than total yield per land area. Fortuitously, heritabilities of competitive traits, such as early vigor, are also high for individual plants (Rebetzke and Richards 1999). Consequently, space planting was used to screen for competitive traits on an individual plant basis. Farmers and researchers at three farms (Butterworks Farm, Rusted Rooster Farm, and Grange Corner Farm) selected 20% of plants with the most ground cover between the 3rd and 5th leaf stage. An additional two farms (Essex Farm and Adirondack Organic Grains) subjected all plants to intense competition from resident weed populations, and then selected the best plants at the end of the season.

Concurrent with farmer selection for the best individual plants for WCA, I randomly collected the same number of spikes from each bi-parental family plot (Figure 5.3). These

collections formed F4:F5 spring wheat baseline populations to track gains in selection. Farmer-selected F5 and randomly collected F4:F5 seed from the two replicate plots of each biparental family at each farm was pooled, and replanted during a second year. The same plot layout was repeated during the second year of selection, with the addition of adjacent plots seeded with randomly collected baseline F4:F5 populations for each biparental family (Figure 5.3). Unfortunately, we lost one farm trial in 2015 due to mice herbivory. From the remaining four farms, farmer-selected F6 and randomly collected F4:F6 populations were increased in a winter nursery in California and a greenhouse in Ithaca, NY to obtain enough seed for multi-location trials in 2016.

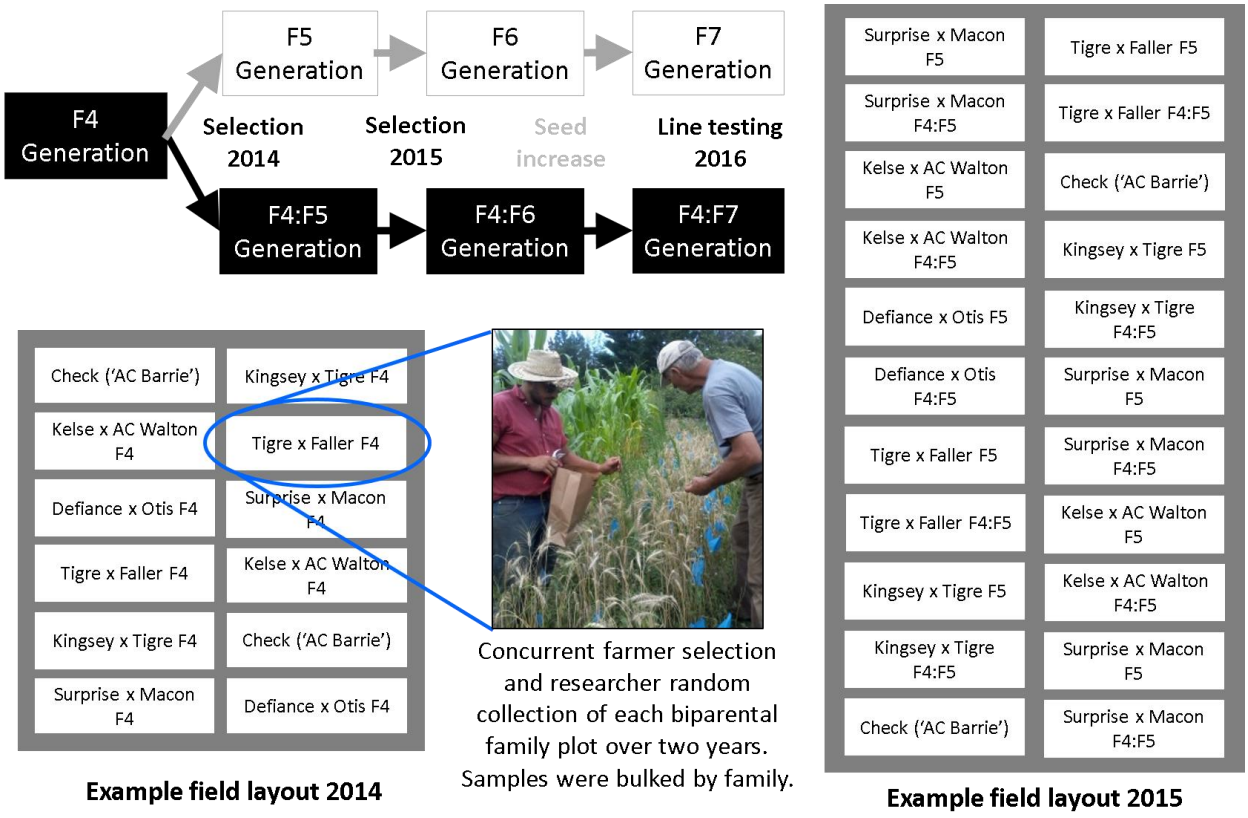

Figure 5.3. Development of populations to evaluate participatory breeding for weed-competitive ability. Farmers selected F4 and F5 biparental families for weed-competitive ability, which were increased for one year to form F7 breeding populations. Random plants were concurrently collected from the F4 generation of each biparental family on each farm. Random collections of the F4:F5 familiy plots, followed by seed increase, formed an F4:F7 baseline population to track gains in selection.

5.2.3.2 Measuring gains in selection

In 2016, farmer-selected F7 populations and randomly-collected F4:F7 populations were planted in split plot pairs to measure gains in selection at five research station locations (Old Town, ME; Borderview, VT; Fusarium Headblight Nursery in Ithaca, NY; Ketola in Ithaca, NY; Helfer in Ithaca, NY) (Figure 1.2, "Advanced line trial site"). These sites were chosen to represent mega-environments defined in 5.3.3.2. Recorded data at Borderview and Old Town included yield at 12% moisture, test weight at 12% moisture, height, lodging (1 to 9), and early vigor between 4th and 5th leaf stage (1 to 9). At Old Town, ME, plots were overseeded with the surrogate weed *Sinapsis alba* cv. 'Idagold' using a Brillion seeder at a rate of 75 live seeds per m^2. Weed and wheat biomass were measured in each plot by sampling two 0.25 m^2 quadrats (0.5 m^2 total sample size), separating out wheat from weed biomass, drying at 55°C, and weighing. At Ketola and Helfer, six-row one meter miniplots were assessed by two to three evaluators for early vigor at 4th leaf stage (1-9). Ground cover was also assessed at Ketola and Helfer using a 16 Megapixel camera and Canopeo App (Patrignani and Ochsner 2015) at one meter height during 3rd, 4th, and 5th leaf stages. Due to errors incurred in the Canopeo processing software during high light conditions (Figure A.2), shade cloth was added to camera equipment for the 4th and 5th leaf stages.

A calibration trial was also conducted to assess the consistency of visual early vigor genotype rankings among evaluators. Six graduate student evaluators were trained on visual rating of early vigor for five minutes, and then asked to rate 20 spring wheat genotypes for early vigor over three replicates. Through the package 'lme4' [version1.1-10] (Bates et al. 2015), the random effects of genotype, replicate, evaluator, and the interaction between genotype and evaluator were tested for variance in early vigor scores.

To evaluate if selection was effective, an F-test compared the fixed effect of population type (F7 and F4:F7) for WCA traits using R [version 3.2.2] (R Core Team 2015), package 'lme4' [version1.1-10] (Bates et al. 2015) (Equation 5.3). The model also included family and farm where selection took place as fixed effects, and block as a random effect.

Equation 5.3 $\mathbf{Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + d_l + \varepsilon_{ijkl}}$

H_0: $\mu_{F7} = \mu_{F4:F7}$; $\alpha \leq 0.05$

y_{ijkl}: trait of interest for type i, family j, farmer k, and replicate l;
μ: overall mean response;
α_i: fixed effect of type i (e.g. F7, F4:F7);
B_j: fixed effect of family j;
γ_k: fixed effect of farmer k;
d_l: random effect of block l;
ε_{ijkl}: experimental error associated with response i,j,k,l

5.2.3.3 Tracking local adaptation

To measure local adaptation of selected populations, the F7 populations were placed on four farms in a replacement experiment in 2016. Trials followed a randomized complete block design with three replicates. Each farmer grew the five to six "local" populations that he had selected in addition to three to four populations selected by each of the other farmers, for a total of 10 to 12 "introduced" comparison populations. Plots were screened for visual early vigor on a 1-9 scale, and visual measures of weed-competitive ability. We were not able to seed a surrogate weed species on participating farms, due to concerns about increasing farm weed seedbanks. However, visual measures of weed-competitive ability have been shown to correlate highly with biomass measurements (r=0.87), while saving considerable time and effort (Worthington et al., 2013). Measures of WCA varied by farm due to different weed competition at each farm. At Essex and Adirondack, the visual ratio of wheat to weed biomass was measured. At Butterworks, intense natural weed competition prevented an accurate visual estimate of wheat to weed

biomass, and heads of wheat were counted as a more reliable measure. At Grange Corner, where there was little to no weed presence, visual ratings of weed competition were not possible. An ANOVA with a random blocking factor tested differences in mean values of weed-competitive ability between local and introduced populations using using R [version 3.2.2] (R Core Team, 2015), package 'lme4' [version1.1-10] (Bates et al. 2015) (Equation 5.4). Data were plotted using 'forestplot' (Gordon 2014).

Equation 5.4 $\mathbf{Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + d_l(f_m) + \varepsilon_{ijklm}}$

H_0: $\mu_{local} = \mu_{introduced}$; $\alpha \leq 0.05$

y_{ijklm}: trait of interest for type i, family j, farm k, rep k, trial m;
μ: overall mean response;
α_i: fixed effect of type i (local or introduced);
β_j: fixed effect of family j;
γ_k: fixed effect of farm where selected k;
$d_l(f_m)$: random effect of rep l, nested in trial m;
ε_{ijklm}: experimental error associated with response i,j,k,l,m

Seeding rates in all 2016 trials were adjusted based on germination rates and thousand kernel weight to obtain 376 viable seeds per square meter. Right-skewed responses were log transformed to obtain normal distributions for ANOVA models (e.g., data for the number of wheat heads at Butterworks and weed to wheat ratio at Old Town).

5.3 Results

5.3.1 Meta-analysis of secondary selection traits for WCA

The studies included in the meta-analysis assessed sixteen potential secondary selection traits for weed-competitive ability: allelopathic activity; early biomass; early and mature height; early and mature LAI; early spectral vegetation indices, including the normalized difference vegetation index (NDVI) and ratio vegetation index (RVI); early and mature photosynthetically

active radiation (PAR); early and mature vigor; length and width of early leaves and the flag leaf; tillering; and growth habit. Most traits showed negative and nonsignificant correlations with weed suppression and/or crop tolerance in some trials (Figure 5.4). Early vigor and early height, however, were positively correlated for both measures of WCA among many studies.

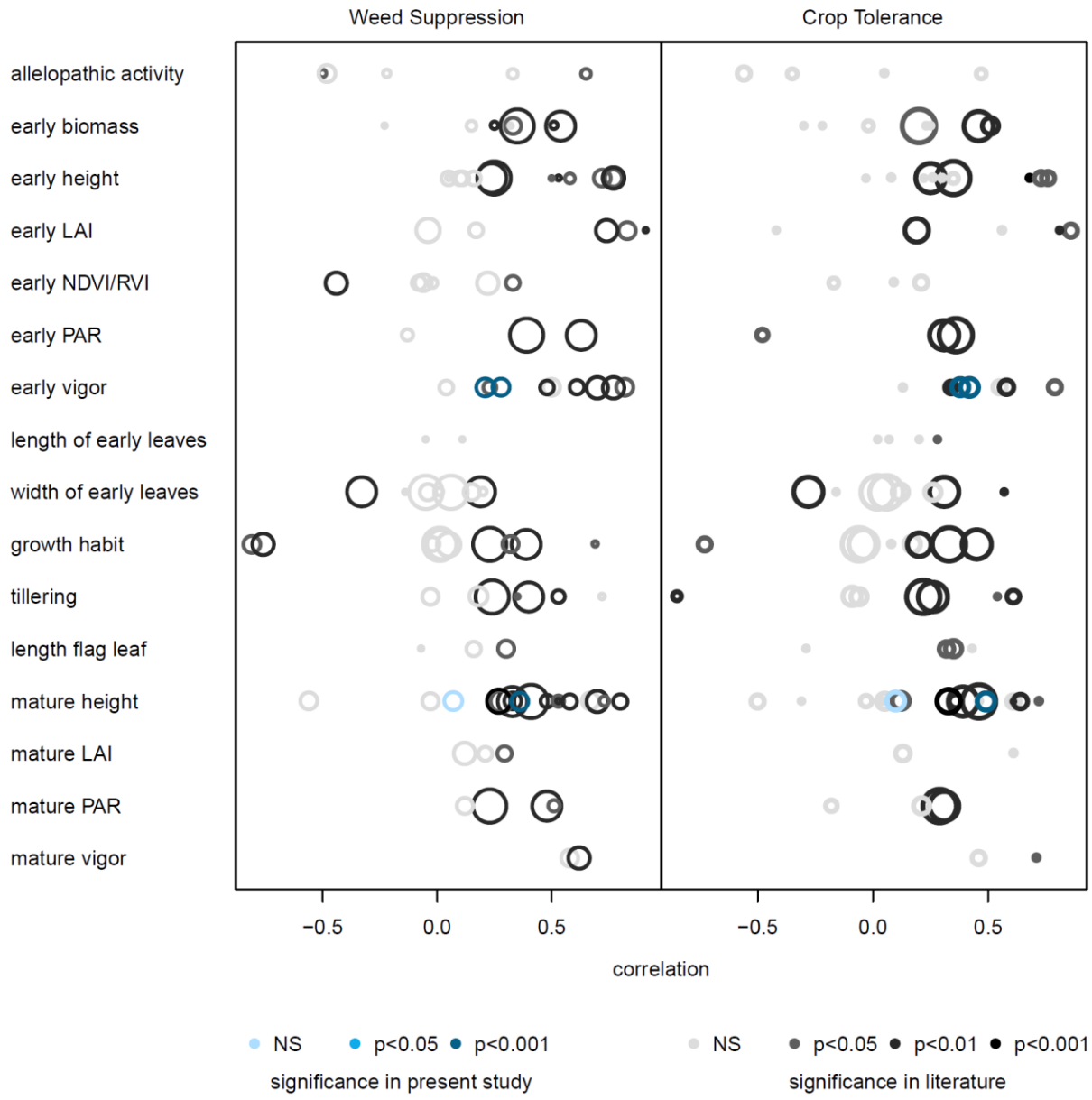

Figure 5.4. Meta-analysis of secondary traits correlated with weed suppression and crop tolerance. Each circle represents one value presented in the literature. Larger circles denote studies that included more site-years and diversity of genotypes, based on the index of (site-years + number of genotypes studied) × 20.

5.3.1.1 Early vigor

The meta-analysis revealed early vigor to be the most promising secondary selection trait for weed-competitive ability. Although encompassing many complex processes, such as emergence, growth rate, and resource use efficiency, early vigor is a simple visual rating of seedling size. Early wheat growth is essential for successful competition with weeds. If a wheat plant fails to establish an effective early cover to shade weeds, it struggles to compete with weeds later in the season (Jordan 1993). Among all reviewed studies, early vigor was positively correlated with WCA. Moreover, early vigor was significantly correlated with weed suppression and crop tolerance in 73 and 75% of trials, respectively. As described in Section 5.1.1, seed size is a strong contributor to early vigor (Rebetzke and Richards 1999, Cousens and Mokhtari 1998). Since seed size is strongly influenced by environmental conditions during grain fill (Jannink et al. 2001), breeders should increase the seed of tested genotypes in the same environment prior to screening for early vigor.

One disadvantage of using early vigor as a secondary selection trait is the effect of GxE interactions, which can reduce heritability (Coleman et al. 2001). While studies have evaluated components of early vigor that have higher heritability, most are not ideal secondary selection traits. Seedling biomass and leaf area are good surrogates for early vigor, but they have moderate heritability at h^2=0.35 and 0.3, respectively, and are more laborious to measure (Rebetzke and Richards 1999). Moreover, seedling biomass is not well-correlated with crop tolerance (Figure 5.4). Although the length and width of early leaves are highly heritable (h^2 = 0.67 and 0.76, respectively) (Rebetzke and Richards 1999), the meta-analysis indicates that these traits are not consistently correlated with WCA (Figure 5.4). Measures of early biomass cover— such as percent ground cover imaging, LAI, and PAR below the canopy— are related to visual early

vigor. However, these metrics of early biomass cover are more difficult to measure than early vigor and do not appear to increase correlations with WCA. Consequently, conducting visual estimates of early vigor remains the best secondary selection trait for WCA.

5.3.1.2 Early height

Early height is another promising secondary selection trait. Taller plants exponentially decrease the amount of PAR available in the canopy (Ford 1980). Therefore, tall genotypes reduce available light for weeds (weed suppression), while simultaneously avoiding shade cast by tall weeds (crop tolerance). Height has the added benefit of being highly heritable (h^2= 0.9 in Coleman et al. 2001). However, height of mature wheat is not always positively correlated with WCA. Twenty-two percent of trials in the meta-analysis found negative correlations between mature height and WCA (Figure 5.4). Ogg and Seefeldt (1999) reported the rate of height gain, particularly early in the season, was more related to weed competition and crop tolerance than the mature height of the variety. Indeed, the meta-analysis shows that early height was consistently positively correlated with WCA, when mature height was not (Figure 5.4). Since tall varieties can reduce harvest index and yield, it is not surprising that 41% of studies reported non-significant correlations between mature height and crop tolerance (Challaiah et al. 1986; Wicks et al. 1986; Seefeldt and Ogg 1999; Coleman et al. 2001). Breeding programs can improve crop tolerance by selecting genotypes with tall height early in the season, but intermediate height at maturity. While many dwarfing alleles (Rht1 and Rht2) reduce gibberellin sensitivity and seedling growth (Seefeldt and Ogg 1999; Murphy et al. 2008; Rebetzke and Richards 1999, Addisu et al. 2009), one semi-dwarf gene (*Rht8c*) maintained the early vigor needed for weed competition (Addisu et al. 2009). For all dwarf and semi-dwarf alleles, the study showed that

early growth could be partially recovered when paired with the photoperiod insensitivity allele, *Ppd-D1a*. Selection for early height can identify such genotypes that meet both weed suppression and crop tolerance.

5.3.2 Correlated traits and genotype by environment interactions for WCA in the Northeast

5.3.2.1 Correlated traits in the northeast United States

For the three site-years studied in the northeastern United States, early vigor was correlated ($p<0.001$) with measures of weed suppression (weed biomass and the ratio of wheat to weed biomass) and crop tolerance (grain yield and wheat biomass). Visual estimation of early vigor showed consistency in varietal ratings among different evaluators, indicating its promise for use as a consistent field measurement tool. After six evaluators visually rated early vigor of 20 spring wheat genotypes over three replicates, 70% of the variance in early vigor was explained by genotype. Although evaluators did have slightly different rating scales, with 11% of the variance in early vigor explained by the evaluator, evaluators did not differ in their ranking of varieties for early vigor. Only 0.09% of variance in early vigor was explained by the interaction between evaluator and replicate. Consequently, visual estimation seems to be a reliable measure of early vigor among genotypes, even if different evaluators complete field measurements.

Visual early vigor measurements were also correlated with canopy cover measurements using Canopeo ($r=0.3238$ at $p<0.0001$). However, canopy cover measurements and data processing took 408% more person-hours than visual estimates of early vigor. Worthington et al. (2013) found similar results, and recommended visual estimations of early vigor instead of ground cover imaging.

Mature height was significantly and moderately correlated to wheat biomass and the

wheat to weed biomass ratio across all sites, likely due to large amounts of straw (Figure 5.5). Weed biomass, however, not correlated with mature height. Similar to the global meta-analysis results, mature height does not appear to be a consistent secondary trait to select for weed suppression in the northeastern United States.

Grain yield was the most highly correlated and consistently significant trait with weed-competitive ability among site-years (Figure 5.5). Our results show that under organic growing conditions with substantial weed pressure (which is typical for spring wheat in the northeastern United States), grain yield may be a more reliable trait to select for weed-competitive ability than early vigor or mature height. As grain yield is already measured in advanced breeding trials, it may also be the most cost-effective measure of WCA screening. For early-stage breeding, however, when lack of seed makes grain yield measurement impractical or unreliable, early vigor remains the best secondary selection triat for WCA.

5.3.2.2 Genotype by environment interactions for WCA

There were significant GxE interactions among site-years for weed biomass, the ratio of wheat to weed biomass, and early vigor. Separate mega-environments, defined as locations with unique winning genotypes, were identified for improving WCA in the northeastern United States.

Weed biomass was highly structured, with 92.52% of the interaction variance explained by one significant principal component in the AMMI model. Although the model produced a unique winning genotype for each site in 2010, the Maine sites (Old Town and Sidney) shared a common four of five top-performing genotypes (Table 5.2) and a moderate correlation in genotype rank (Table 5.3). Three top-genotypes at the Vermont site were unique from those at the Maine sites (Table 5.2). Moreover, the Vermont site had low or negative nonsignificant correlations with the Maine sites.

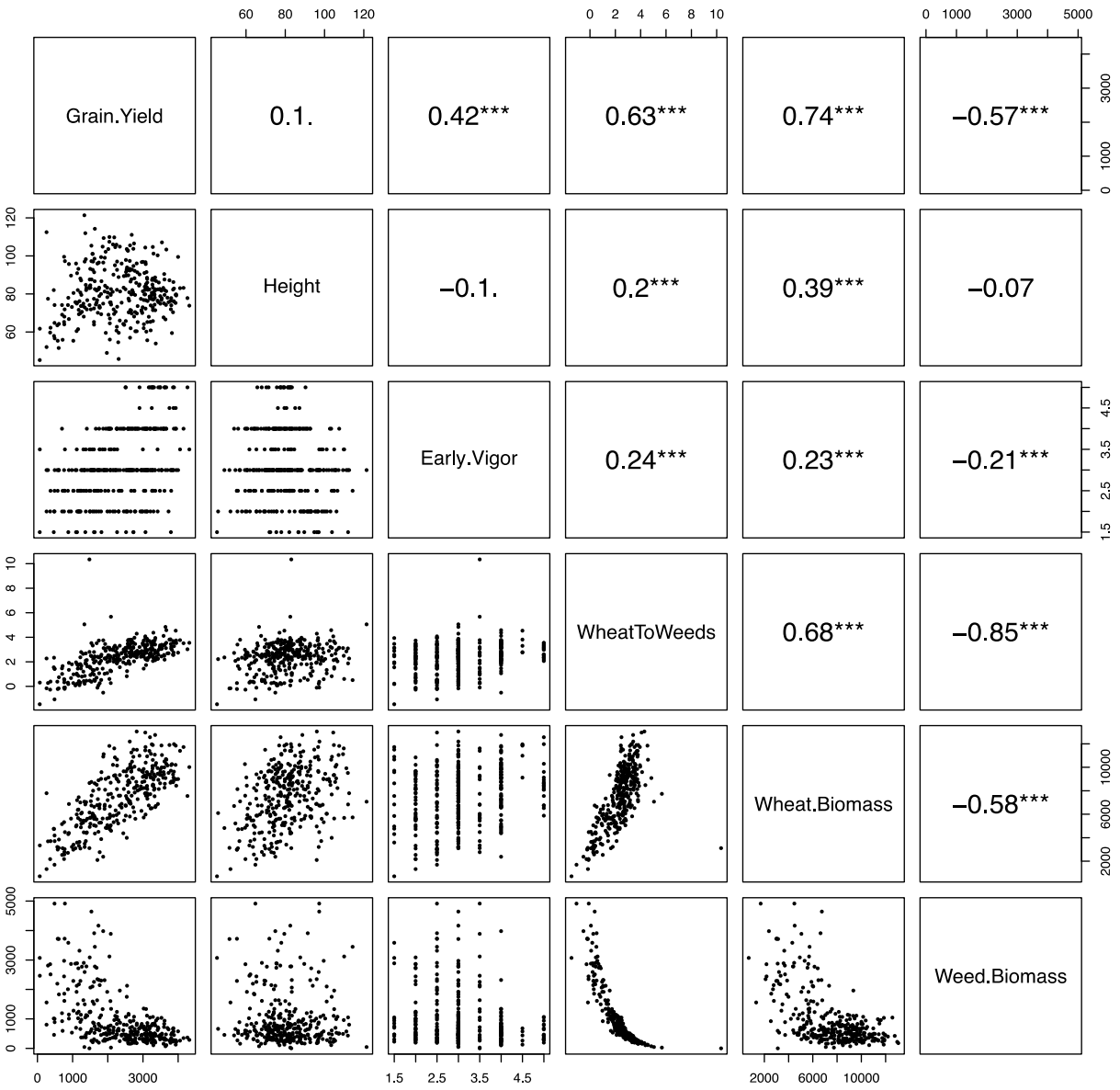

Figure 5.5. Weed-competitive ability was correlated with early vigor and grain yield. Results include three sites in the northeastern United States in 2010. Significance is reported as "." (p<0.1), * (p<0.05), ** (p<0.01), *** (p<0.001)

Early vigor GxE interactions were also highly structured, with 88.60% of variance explained by two significant principal components of the AMMI model. Early vigor showed a similar, but more pronounced, regional grouping of genotypic performance (Table 5.4). The Maine sites (Old Town and Sidney) tended to share winning genotypes, as did sites near one

another in northern Vermont and northern New York (Alburgh and Willsboro). However, rank correlations tended to be stronger within years than within regions (Table 5.3).

For both WCA and vigor in wheat, coastal Maine and northern Vermont/New York represented unique mega-environments. Breeding and variety testing programs should split selection sites at least between these two regions. However, the most competitive genotypes even differed within defined mega-environments. Such patterns of GxE interactions suggest that a decentralized breeding model may select optimal genotypes for WCA.

Table 5.2. Winning genotypes for weed suppression among sites.

Site-year	**rank1**	**rank2**	**rank3**	**rank4**	**rank5**
Sidney.2010	Kingsey	Faller	AC Superb	Tom	Bastican
Old Town.2010	Tom	Kingsey	AC Superb	Sabin	Faller
Alburgh.2010	RB07	Faller	Red Fife	Bastican	FBC Dylan

Table 5.3. Rank correlations for weed suppression in the upper triangle and early vigor in the lower triangle. Significance is indicated by * (p<0.05) and *** p<0.001).

Site-year	Alburgh.2010	Alburgh.2011	Old Town.2010	Sidney.2010	WB.2011
Alburgh.2010		-	-0.21	0.13	-
Alburgh.2011	-0.18		-	-	-
Old Town.2010	0.54*	-0.08		0.37	-
Sidney.2010	0.31	-0.30	0.70***		
WB.2011	0.11	0.34	0.21	-0.03	

Table 5.4. Winning genotypes for early vigor among site-years.

Site-year	**rank1**	**rank2**	**rank3**	**rank4**	**rank5**
Old Town.2010	AC Superb	Tom	Ulen	Howard	Faller
Sidney.2010	AC Superb	Tom	Ulen	Sabin	Ada
Alburgh.2010	Ada	Bastican	Ulen	Oklee	Tom
Alburgh.2011	Bastican	Malbec	Ada	Magog	Oklee
WB.2011	Bastican	Malbec	Magog	Faller	Cabernet

5.3.3 Measuring the effectiveness of a participatory plant breeding model for WCA

5.3.3.1 Gains in selection

Farmers made gains in selection for WCA. The farmer-selected populations reduced weed biomass by an average of 173.2 kg/ha compared to the randomly collected comparison populations. This represents a 11.46% gain in weed-competitive ability after two years of selection (p=0.0271). Farmer-selected populations also produced 115.7% of the wheat biomass to weed biomass ratio (p=0.0274), and 116.3% of grain yield (p=0.0050) relative to the randomly selected controls. Farmer selections scored 105.8% of the early vigor compared with random controls (p=0.0043). For ground cover at 4th leaf stage, measured through Canopeo, farmer selections produced 104.2% of the canopy cover of F4:F7 controls (p=0.0498). Although farmer selections generated 106.7% of the wheat biomass of the randomly collected controls, the difference between the groups was not significant (p=0.1148). It appears that spring farmers were more effective at selecting for their first-ranked trait of weed-competitive ability than for their second-ranked priority trait of straw production (Figure 1.3). There were no significant differences between height in farmer selections and randomly collected populations at both Old Town (p=0.7446) and Alburgh (p=0.4264) trials, indicating that farmers improved weed-competitive ability without increasing height in biparental families. The resulting intermediate height varieties should improve spring wheat farmers' third ranked trait of lodging resistance (Figure 1.3), while still meeting the needs of weed-competitive ability.

The testing site strongly influenced measured gains in selection. Selected lines varied in adaptation to different trial sites. Gains in selection showed an expected dependence (Equation 1.2) on the genetic correlation between the farm where selection took place and the testing environment (r_g) (Table 5.5). Figure 5.6 shows the relationship between gains in selection and

the genetic correlation between selection and testing sites. The trend was strong and significant for early vigor (r=0.7678, p=0.0035) and canopy cover at 3rd, 4th, 5th leaf stages (r=0.73878, p=0.0005). As an example, Adirondack lines demonstrated 11.13% gains in selection for early vigor at the Old Town testing site. These two sites were highly and significantly correlated for genotypic rank (Table 5.5). In contrast, Adirondack lines showed negative and zero gains in selection for early vigor and cover at Helfer and Ketola, which had low genotypic rank correlations with the Adirondack selection site. Due to differences in the selection and testing environments, it is likely that the full potential of Adirondack lines was not revealed at the Helfer and Ketola testing sites. Similarly, Grange Corner lines showed a mean 6.45% increase in ground cover from the baseline F4:F7 populations when tested at Ketola, a site that correlated strongly in genotype rank with Corner Grange Corner. In sharp contrast, the same Grange Corner lines *decreased* 0.89% in mean ground cover from the baseline population when tested at Helfer, a site with low genotype rank correlation with the Grange Corner farm. Gains in selection for the ratio of wheat to weed biomass also tracked with r_g (r=0.8782, p=0.3175), but few observations at one site limited significance testing.

These results indicate that lines should be tested in environments with similar genotypic performance to the site where they were selected. The merits of lines developed through PPB are often evaluated at only one research station (*e.g.*, Rivière et al. 2013; Kirk et al. 2015). Such studies are likely underestimating the true potential of PPB populations, since testing occurs in environments that differ from farms where selection took place. Conversely, selection and testing sites should be used that correlate with regional farms. Among our testing sites, Helfer had low genetic correlation to all farms, indicating that it is unrepresentative of regional organic farms and a poor testing site.

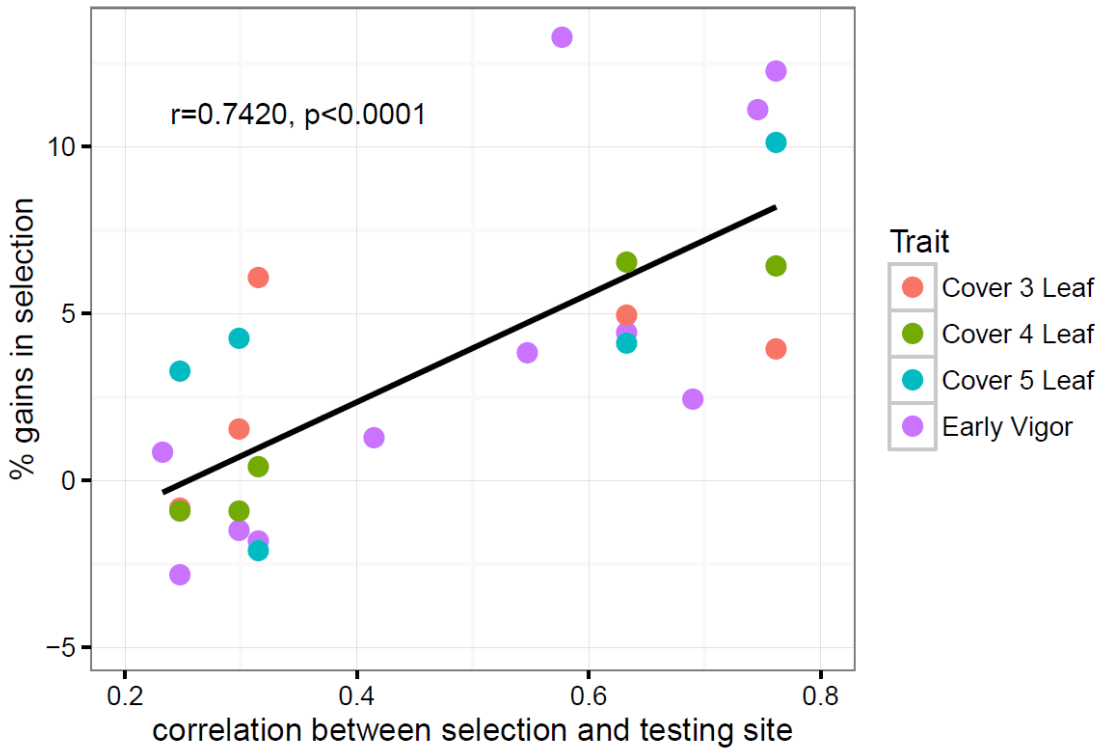

Figure 5.6. Gains in selection depended on the similarity between the selection and testing environments. There was a significant correlation between gains in selection for a breeding population and the rank correlation for early vigor and canopy cover between selection and testing sites. Gains were calculated as (F7 farmer selected populations – F4:F7 randomly collected populations)/F4:F7 randomly collected populations*100.

Table 5.5. Rank correlations between 2016 sites for early vigor (bottom triangle) and ratio of wheat to weeds (upper triangle).

Site	Adirondack	Border-view	Butter-works	Essex	Grange Corner	Helfer	Ketola	Old Town
Adiron-dack		-	0.30	0.43	-	-	-	0.42
Border-view	0.55.		-	-	-	-	-	-
Butter-works	-	-		0.37	-	-	-	0.25
Essex	0.29	0.23	-		-	-	-	0.49.
Grange Corner	0.66*	0.41	-	0.59*		-	-	-
Helfer	0.19	-0.29.	-	0.32	0.24		-	-
Ketola	0.30	0.11	-	0.63*	0.76**	0.38*		-
Old Town	0.7458***	0.2794	-	0.6900*	0.5766*	0.2953.	0.2216	

5.3.3.2 Local adaptation

Farmer-selected lines demonstrated evidence of local adaptation. Local populations were significantly more competitive with weeds than introduced populations (p=0.0302, Table 5.6). Across all trials, populations demonstrated an average of 9.35% higher weed-competitive ability when grown on the farms where they were selected. The magnitude of local adaptation varied by farm (Figure 5.7a). Essex and Butterworks lines showed more local adaptation to their selection site compared with introduced lines, while Adirondack lines did not. As an example of local adaptation, Butterworks received the highest mean WCA ratings when grown at Butterworks, but did not receive the highest mean ratings at any of the other sites. The participatory breeding program developed lines that were specifically adapted to various regional farms for WCA. Similar to Mangione et al. (2006), our results provide further evidence that participatory and decentralized selection can reduce the amount and cost of advanced line testing, since developed lines were already adapted to regional farm environments.

Locally selected lines also demonstrated a mean 5.41% increase in early vigor, although the effect was not significant (p=0.5057, Figure 5.7b). In contrast, height did not show local adaptation (p=0.9747, Figure 5.7c), which is expected for a trait that has low GxE and high heritability.

Table 5.6. Preferential performance of populations at the farm where they were selected for weed-competitive ability. Tests of local adaptation compared the mean of populations selected on the test farm minus the mean of the populations not selected on the test farm for WCA, vigor and height. Significant difference in mean performance for WCA is indicated as * for p<0.05. The 95% confidence intervals for each trait are indicated by ±.

Trait	Mean of local lines	Mean of introduced lines	Significance of local adaptation
Ratio of wheat to weeds	5.23 (±1.34)	3.89 (±0.50)	p=0.0302*
Vigor (1-9)	6.11 (±0.52)	5.80 (±0.39)	NS
Height (cm)	91.87 (±2.27)	91.10 (±3.17)	NS

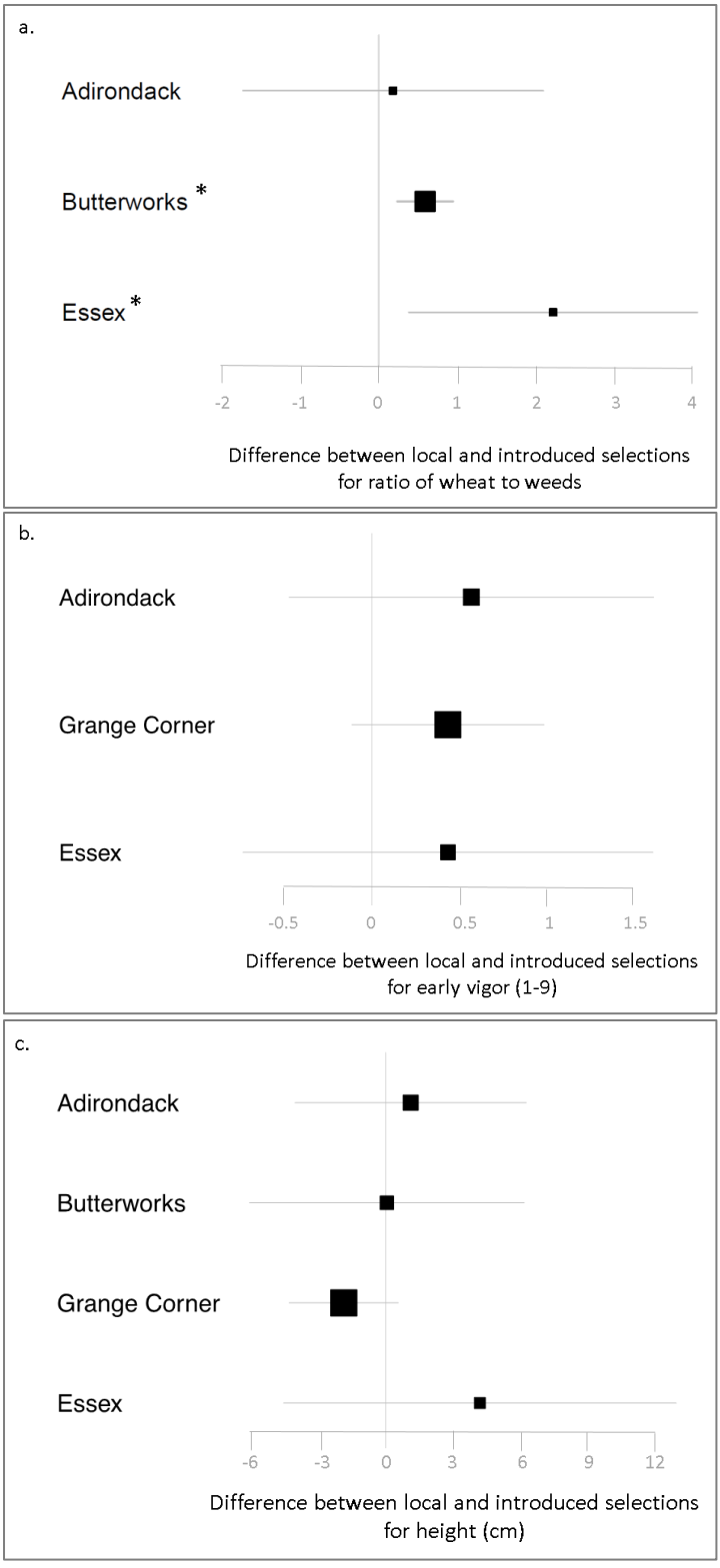

Figure 5.7. Local adaptation for WCA, vigor, and height among farms. Tests of local adaptation at each farm for (a) ratio of weeds to wheat (WCA), (b) vigor, and (c) height. Significant difference in mean performance for each farm is indicated as * for $p<0.05$. Error bars show 95% confidence intervals.

Different winning genotypes among test sites also indicated local adaptation for weed-competitive ability and early vigor. The most competitive breeding lines at the northern New York sites were different from the winning lines at Maine and Vermont (Table 5.7). The distinct ranking of genotypes for weed-competitive ability in northern New York matches well to the mega-environments identified for yield and test weight in Section 4.3.2. Mega-environments for early vigor also follow the mega-environments identified in Section 5.3.3.2, separating Maine as a unique region for genotypic performance. Early vigor tended to create mega-environments based on region: Maine sites (Old Town and Grange Corner) shared winning lines as did sites in central New York (Helfer and Ketola) (Table 5.8).

Unlike the patterns seen for WCA, yield, and test weight, sites in northern New York did not form a mega-environment for early vigor, as Adirondack and Essex Farms had unique winning genotypes. Mega-environments for early vigor also did not correspond to the genotypic rank correlations among sites (r_g). Sites that were highly correlated by genotype rank (*e.g.*, Old Town and Adirondack; Grange Corner and Ketola) did not share winning genotypes (Table 5.5 and Table 5.8). Since winning varieties differed within mega-environments, and did not follow genotypic correlation, optimal gains in selection for early vigor would likely happen at a highly decentralized level.

Table 5.7. Winning genotypes by site for weed suppression. Sites that have the same rank1 genotype constitute a mega-environment. A key to variety codes is located in Table A.11.

Site	rank1	rank2	rank3	rank4	rank5
Adirondack	AKTF	BWSM	EXKH	BWDO	AKKW
Essex	AKTF	BWSM	BWDO	ACBA	AKKW
Butterworks	EXKH	BWSM	AKKW	BWDO	AKTF
Old Town	EXKH	BWSM	AKTF	BWDO	AKKW

Table 5.8. Winning genotypes by site for early vigor. Sites that have the same rank1 genotype constitute a mega-environment. A key to variety codes is located in Table A.11.

Site	rank1	rank2	rank3	rank4	rank5
Adirondack	BWSO	GCSO	AKSM	GCKW	BWSM
Borderview	GCSO	EXKT	EXSO	AKSM	GCKW
Grange Corner	AKKW	BWDO	GCFT	EXKT	GCKW
Essex	AKFT	GCFT	EXKT	EXSO	AKKH
Helfer	BWSO	GCFT	AKFT	BWSM	BWKT
Ketola	BWSO	GCFT	AKFT	BWSM	AKKW
Old Town	AKKW	AKSM	GCKW	GCFT	AKTF

5.4 Conclusions

Weed-competitive ability and its most consistently correlated trait, early vigor, show opportunity to breed for local adaptation. Genotype by environment interactions were significant and structured for both traits, and indicate separate mega-environments for selection and variety testing in the northeastern United States. A decentralized participatory breeding program for weed-competitive ability showed effectiveness for gains in selection. Lines developed by organic farmers showed greatest gains in selection when grown in environments with high genotypic correlation to the selection site. When evaluating breeding lines at sites that are very different from the selection site, the variety's potential performance is likely underestimated. Lines also showed higher mean performance when grown on the farm where selections took place. Such proof of local adaptation for weed-competitive ability is unique in the literature. Because developed lines were adapted to regional organic farms, this research validates that PPB can reduce breeding program costs for advanced line testing.

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APPENDICES

Table A.1. Sourdough* and yeast bread§ formulas.

Component	Ingredient	Percent weight of overall flour Sourdough	Weight (g) Sourdough	Percent weight of overall flour Yeast Bread	Weight (g) Yeast Bread
Overall Formula	Flour	100.00	750.00	100.00	530
	Water	71.90	539.23¥	70.00	371+
	Salt	2.00	15.01	1.89	10
	Culture	5.00	37.52	0.38	2
	Baker's Yeast	-	-	0.38	2
	Total	178.89	1341.76	172.64	915
Levain	Flour	25.00	187.50	5.66	30
	Water	15.50	116.23	6.60	35
	Salt	0.50	3.75	-	-
	Culture	5.00	37.52	0.38	2
	Total	46.00	345.00	12.64	67
Final Dough	Flour	75.00	562.50	94.33	500
	Water	56.40	423.00¥	63.34	336+
	Salt	1.50	11.26	1.89	10
	Levain	46.00	345.00	12.64	67
	Baker's Yeast	-	-	0.38	2
	Total	178.89	1341.76	172.64	915

*Sourdough method: Each varietal flour prefermented overnight with a rye sourdough culture (levain). The final dough was mixed in an Italian fork mixer until optimal consistency: four minutes for 'Fulcaster' and 'Red Fife;' six minutes for 'Appalachian White,' 'Fredrick,' 'Glenn' and 'Tom;' and seven and a half minutes for 'Warthog.' After a 20 to 25 minute autolyse, the bakers added the prefermented levain and additional salt. Final doughs bulk fermented for two hours and received two folds during the fermentation process. Using a wood fired oven with steam at Wide Awake Bakery (Trumansburg, NY), breads baked at to at least 95.6°C internal temperature. Oven temperatures varied between 221.7 and 238.3°C, due to opening and closing of the oven door.

§Yeast bread method: Bakers hand mixed the final dough (2 to 5 minutes) and allowed a 36 to 50 minute autolyse. Following the addition of prefermented levain, bakers folded doughs during a two-hour fermentation. 'Yorkwin' and 'Red Fife' received two folds, while the other three varieties received three folds. Loaves baked at 232°C in a propane oven at Bread Alone Bakery (Boiceville, NY), until internal loaf temperatures reached 97.8°C.

¥Bakers added additional water to the final sourdoughs in the following amounts: 50g for 'Appalachian White,' 46g for 'Fredrick,' 129g for 'Glenn,' 152g for 'Tom,' and 60g for 'Warthog.'

+Bakers added additional water to the final yeast doughs in the following amounts: 14.84g for 'Forward,' 13.25g for 'Fredrick,' 43g for 'Pride of Genesee,' 24.4g for 'Red Fife.'

Table A.2. Matzah* formula.

Ingredient	Percent weight of overall flour	Weight (g)
Flour	100.00	540
Water	33.30	180
Salt	0.56	3
Canola Oil	0.52	28
Total	134.40	751

*Matzah method: After mixing, the dough rested for 15 minutes. The dough was hand rolled to reach 2 to 3 mm thickness, pricked with a fork, brushed with water, and baked 10-12 minutes at 232°C.

Table A.3. Shortbread cookie* formula.

Ingredient	Percent weight of overall flour	Weight (g)
Flour	100.00	240
Unsalted Butter	94.50	227
Sugar	47.10	113
Baking Powder	1.67	4
Salt	0.83	2
Total	244.10	586

*Shortbread method: After sifting dry materials, bakers mixed all ingredients in a Hobart mixer until just incorporated. After bakers rolled the dough to 12.7 mm thickness, the shortbread cooled for four hours in a refrigerator. The cookies baked at 176.7°C for 15 to 20 minutes.

Table A.4. Emmer pasta* formula.

Ingredient	Percent weight of overall flour	Weight (g)
Emmer Flour	63.78	560.00
Antico Molino Caputo 00 Flour	36.22	318.00[§]
Egg Yolks	21.73	190.80[¥]
Whole Eggs	31.51	276.66[¥]
Water	0.98	8.59[¥]
Salt	0.46	4.00
Total	154.68	1358.05

*Pasta method: After mixing ingredients in a Hobart mixer, dough rested overnight in a refrigerator, was rolled to 1 mm thickness in an industrial pasta roller, and cut into 10 cm strips of fettuccine. Pasta was cooked until *al dente*.

[§]Pasta makers added 166g of Antico Molino Caputo 00 flour to 'North Dakota Common' and 26g of 00 flour to 'Red Vernal' doughs

[¥]Pasta makers increased the amount of liquid ingredients for 'North Dakota Common' by 15.30%

Table A.5. Flavor attributes for sourdough bread and cooked grain evaluation.

Flavor	Materials for training	Descriptors of attributes
Nutty	almond, walnut, hazelnut, brazil nut	oily, creamy, astringent
Sweet	vanilla, cultured cream, butterscotch, sweetened condensed milk	caramel, egg-like, honey, flan
Toasted*	roasted nuts	burnt
Malty	malt	malt, sweet, milky
Yeasty	yeast	fermented, pungent
Dairy	milk, cultured cream, buttermilk	sweet, refreshing, haylike, floral
Fatty	butter	oily, clean, waxy
Sour*		sour dairy
Bitter		alkaline, phenolic
Earthy*		dusty, musty, fresh earth

*Not included in the training but included with examples in a flavor wheel provided to all panelists.

Table A.6. Visual and mouthfeel characteristics for sourdough bread and cooked grain evaluation.

Characteristic	Product	Range
Dryness Rate the overall dryness of the sample.	cooked grain	(1) very dry (10) moist
Flavor Rate the intensity of the flavor.	cooked grain	(1) no flavor (10) intense
Aromatics - Sample as a whole Hold sample and smell deeply, moving sample back and forth making sure to smell the crust along with the interior. Rate aromatic characteristics or lack of aroma.	sourdough bread	(1) no aroma (10) intense
Wheat aroma of crust Hold sample and smell only the crust, be sure to smell the top and bottom crusts of sample.	sourdough bread	(1) no aroma (10) intense
Wheat aroma of crumb Hold sample and smell crumb.	sourdough bread	(1) no aroma (10) intense
Surface texture	sourdough bread	(1) even and smooth (10) heavily textured
Texture of crumb Feel a small portion of the sample and consider the visible texture and how it feels.	sourdough bread	(1) delicate (10) most hearty
Flavor What is the general, overall taste of the bread? Rate the intensity of flavor.	sourdough bread	(1) no flavor (10) intense
Dryness Saliva taken from tongue	sourdough bread	(1) very dry (10) moist
Graininess of mass - amount of small particles Describe the mouthfeel of the bread in terms of graininess	sourdough bread	(1) no graininess (10) overwhelming graininess
Cohesion of mass - degree in which chewed sample holds together Take a sample the size of a quarter and count as you chew at a normal rate. Count the seconds of chewing until the mass of the bread breaks apart into a generally even particle range.	sourdough bread	seconds
Ability to dissolve Record the seconds to dissolve	sourdough bread	seconds

Table A.7. Flavor attributes for matzah cracker, cooked soft wheat grain, pasta, and cooked emmer grain evaluations.

Flavor	Materials for training	Consensus descriptors of attributes for soft wheat (matzah and cooked grain)	Consensus descriptors of attributes for emmer (pasta and cooked grain)
Wheat	100% wheat crackers	delicate, floury	flour, soft, light, delicate
Bran	100% bran cereal	buttery, caramel	buttery, caramelized, molasses, toast, brown sugar
Nutty	mixture of chopped almond, cashew, walnut	buttery, oil, sweet-nutty	buttery, sweet, toast, fatty
Bitter	quinic water, phenolic compound, rubbing alcohol, white vinegar (smell only)	medicinal, piercing, back of throat	astringent, sharp, medicinal, abrasive, back of the throat
Fresh	apple, cucumber	acidity, refreshing	crisp, cleansing
Woody	toothpicks	drying, flat, rough on tongue, one-dimensional	dry, rough, coarse, bland
Grassy	fresh grass, fresh grass steeped in hot water (hay included in pasta training)	fresh, crisp, sweet	fresh, sunshine
Earthy	raw potato, raw potato mixed with potting soil (smell only)	musky, bloomy, umami, mushroom	mushroom, clay, musk
Herbal	mixture of dried sage, basil, and rosemary and fresh parsley and oregano, same mixture steeped in hot water	spicy	spicy, prickly

Table A.8. Visual and mouthfeel characteristics for matzah cracker and cooked soft wheat grain evaluation.

Characteristic	Product	Range
Visual surface texture	matzah	(1) smooth (10) heavily textured, rough
Visual texture shape	matzah	(1) rounded, smooth particles (10) sharp, jagged, angular particles
Surface roughness	matzah	(1) soft, smooth (10) very rough
Graininess	matzah	(1) when chewed, smooth, silky (10) coarse, rough
Cohesion of mass	matzah	seconds of chewing to break down to swallowable mass
Firmness	matzah	(1) no or slight resistance to bite (10) very hard to bite through
Texture	matzah, cooked grain	(1) fine, easy to chew (10) coarse, difficult to chew

Table A.9. Visual and mouthfeel characteristics for pasta and cooked emmer grain evaluation.

Characteristic	Product	Range
Shininess Rate the sheen of the exterior of an individual piece of pasta.	pasta	(1) There is no surface sheen. The piece looks matte. (10) The piece is very shiny, appearing slightly glossy.
Surface stickiness Take twopieces of pasta and touch them together, pressing them gently until they give from the force. Slowly pull them apart. Rate the stickiness of the pasta.	pasta	(1) The two pieces do not stick at all; when gently pressed together they glide slightly. (10) Two pieces stick together easily. They need force to pull apart, which appears as a peeling action.
Surface roughness Place a piece of pasta in your mouth, running your tongue along the piece describe the roughness quality.	pasta	(1) smooth, pasta glides quickly over tongue (10) Pasta is rough and coarse, prohibiting a smooth motion across the tongue.
Graininess While chewing, evaluate the graininess of the sample.	pasta	(1) smooth, silky (10) coarse and/or rough, very grainy
Cohesion of mass Count how many seconds it takes for the piece of pasta to break down to a swallowable texture with normal chewing.	pasta	seconds
Firmness Bite a piece of pasta with your front teeth. Rate the force required to bite through.	pasta	(1) no resistance- falls apart upon hitting teeth (10) very chewy, minor pulling force from the hand is required to separate the piece in two
Texture Bite a piece of pasta with your teeth, then eat the piece of pasta. Describe the overall texture in terms of starchiness.	pasta	(1) very starchy, chewy and clumpy (10) not very starchy: firm, clean, structured, brittle
Dryness	whole grain	(1) very dry; takes moisture from tongue (10) very moist; exudes moisture
Texture	whole grain	(1) delicate, easy to chew (10) very chewy, difficult to chew
Flavor intensity	whole grain	(1) No taste present (10) Intense taste, very noticeable

Table A.10. Codes and variety names for variety evaluation

Code	Variety Name
ACBA	AC Barrie
ACMO	AC Morley
ACSP	AC Surprise
ADA	Ada
ARAP	Arapahoe
ARRW	Arrow
ARS9	ARS09-173
CERE	Ceres
EXPE	Expedition
FALL	Faller
GLEN	Glenn
HARV	Harvard
JERR	Jerry
LOUI	Louise
MAGO	Magog
MAXI	Maxine
MIDA	Mida
MILL	Millenium
MN61	MN00261-4
MN78	MN06078W
NUEA	NuEast
OVER	Overland
PRGE	Pride of Genesee
RB07	Rb07
RDEM	Redeemer
RDFE	Red Fife
SABI	Sabin
STEE	Steele
SUSQ	Susquehanna
TOM	Tom
WART	Warthog
YKST	Yorkstar
ZORO	Zorro

Table A.11. Codes, Best Linear Unbiased Predictors (BLUPs), and rankings of farmer selected lines among testing sites

Code	Entry	Ratio of wheat biomass to weed biomass								Yield					
		BLUP	Rank	BLUP	Rank	BLUP	Rank	BLUP	Rank	BLUP	Rank	BLUP	Rank	BLUP	Rank
		Adirondack		Butterworks		Essex		Old Town		Borderview		Grange Corner		Old Town	
AKFT	AdirondackFaller/TigreF7	-0.05	11	-0.15	15	NA	NA	0.22	7	8.40	12	-208.45	16	206.28	6
AKKW	AdirondackKelse/AC WaltonF7	-0.30	13	-0.13	14	-1.53	14	0.12	11	-119.13	20	NA	NA	253.76	5
AKKH	AdirondackKelse/HeliosF7	0.52	1	NA	NA	-1.01	12	0.14	9	10.55	10	-91.40	14	-13.38	14
AKSM	AdirondackSurprise/MaconF7	0.14	7	-0.23	18	NA	NA	0.60	2	40.14	5	301.54	2	301.06	3
AKTF	AdirondackTigre/FallerF7	0.25	2	-0.09	13	1.96	3	-0.04	13	-4.64	14	NA	NA	278.78	4
BWDO	ButterworksDefiance/OtisF7	-0.23	12	0.35	1	-1.14	13	-0.43	18	-163.37	21	NA	NA	-412.45	22
BWKW	ButterworksKelse/AC WaltonF7	0.06	8	0.31	2	NA	NA	0.46	5	-12.14	15	296.10	3	112.59	8
BWKT	ButterworksKingsey/TigreF7	NA	NA	0.09	10	1.49	5	0.13	10	NA		32.16	9	9.30	13
BWSM	ButterworksSurprise/MaconF7	0.52	1	-0.16	16	-0.92	11	-0.13	15	-58.36	18	NA	NA	318.14	2
BWSO	ButterworksSurprise/OtisF7	NA	NA	0.22	5	0.56	7	-0.19	17	-75.26	19	-316.14	18	-133.40	19
BWTF	ButterworksTigre/FallerF7	0.17	6	-0.05	12	NA	NA	0.50	3	37.70	6	140.13	5	102.78	9
GCFT	Grange CornerFaller/TigreF7	NA	NA	-0.20	17	-0.39	9	0.01	12	127.43	1	303.16	1	131.15	7
GCKW	Grange CornerKelse/AC WaltonF7	0.25	3	0.28	4	NA	NA	0.68	1	83.83	2	239.04	4	91.88	10
GCKT	Grange CornerKingsey/TigreF7	-0.36	14	0.13	7	NA	NA	0.48	4	65.57	3	-52.94	11	363.06	1
GCSM	Grange CornerSurprise/MaconF7	NA	NA	0.09	9	0.10	8	0.20	8	34.20	7	92.14	7	-19.19	15
GCSO	Grange CornerSurprise/OtisF7	0.02	9	NA	NA	-0.90	10	NA	NA	NA	NA	-72.89	12	NA	NA
EXFT	EssexFaller/TigreF7	-0.03	10	NA	NA	1.96	4	-0.14	16	30.14	8	11.91	10	29.21	11
EXKW	EssexKelse/AC WaltonF7	NA	NA	0.04	11	0.61	6	NA	NA	59.93	4	69.48	8	NA	NA
EXKH	EssexKelse/HeliosF7	0.23	4	0.19	6	-2.11	15	-0.08	14	29.27	9	NA	NA	10.65	12
EXKT	EssexKingsey/TigreF7	0.21	5	0.12	8	4.26	1	NA	NA	NA	NA	NA	NA	NA	NA
EXSO	EssexSurprise/OtisF7	NA	NA	0.31	3	2.42	2	0.38	6	NA		131.74	6	-19.90	16

Table A.11. Continued

Code	Entry	Early vigor														Lodging			
		BLUP	Rank	BLUP	Rank	BLUP	Rank	BLUP	Rank	BLUP	Rank	BLUP	Rank	BLUP	Rank	1 to 9	Rank	1 to 9	Rank
		Adirondack		Borderview		Grange Corner		Essex		Helfer		Ketola		Old Town		Borderview		Grange Corner	
AKFT	AdirondackFaller/TigreF7	-0.29	8	-0.03	6	-0.28	3	NA	NA	1.34	22	0.01	10	0.78	15	4.67	8	5.83	8
AKKW	AdirondackKelse/AC WaltonF7	1.16	13	-0.03	7	NA	NA	0.60	14	0.13	7	-0.14	9	1.46	20	4.00	7	NA	NA
AKKH	AdirondackKelse/HeliosF7	-0.07	9	0.30	17	0.33	12	0.65	15	-0.13	5	0.26	16	0.51	10	5.67	13	4.83	6
AKSM	AdirondackSurprise/MaconF7	1.11	12	0.33	19	0.07	9	NA	NA	0.34	14	-0.15	7	1.14	19	6.67	15	7.43	15
AKTF	AdirondackTigre/FallerF7	1.46	14	0.09	12	NA	NA	-0.23	4	0.32	13	-0.42	3	0.88	16	5.17	10	NA	NA
BWDO	ButterworksDefiance/OtisF7	-1.34	1	-0.52	1	NA	NA	-1.36	1	0.22	10	-0.27	5	-0.95	2	8.00	20	NA	NA
BWKW	ButterworksKelse/AC WaltonF7	-0.64	4	0.15	16	0.24	11	NA	NA	0.74	19	0.13	13	0.35	7	3.33	5	3.50	3
BWKT	ButterworksKingsey/TigreF7	NA	NA	NA	NA	-0.41	1	0.07	7	0.84	20	0.26	15	-0.06	5	NA		7.17	13
BWSM	ButterworksSurprise/MaconF7	1.89	17	0.06	9	NA	NA	0.16	10	0.71	18	0.13	14	0.66	14	7.00	18	NA	NA
BWSO	ButterworksSurprise/OtisF7	NA	NA	-0.27	3	0.45	14	0.12	9	1.47	23	0.90	22	0.35	8	6.67	15	7.33	14
BWTF	ButterworksTigre/FallerF7	0.60	11	-0.01	8	0.05	8	NA	NA	0.46	16	0.36	17	-0.25	4	7.33	19	8.17	19
GCFT	Grange CornerFaller/TigreF7	NA	NA	-0.18	4	1.06	17	1.11	17	1.28	21	1.03	23	0.99	17	2.00	1	2.83	1
GCKW	Grange CornerKelse/AC WaltonF7	1.74	16	0.31	18	0.57	16	NA	NA	-0.31	3	0.46	19	1.13	18	5.33	11	6.00	9
GCKT	Grange CornerKingsey/TigreF7	-0.64	3	0.08	10	-0.04	6	NA	NA	-0.02	6	-0.14	8	0.64	13	5.33	11	6.83	10
GCSM	Grange CornerSurprise/MaconF7	NA	NA	0.08	11	0.35	13	0.30	11	0.22	8	0.42	18	0.53	11	6.17	14	7.00	12
GCSO	Grange CornerSurprise/OtisF7	1.67	15	NA	NA	0.05	7	-0.25	3	-0.23	4	-0.31	4	NA	NA	NA	NA	7.50	16
EXFT	EssexFaller/TigreF7	0.12	10	0.14	14	0.19	10	0.60	13	0.41	15	-0.26	6	0.47	9	4.67	8	6.83	10
EXKW	EssexKelse/AC WaltonF7	NA	NA	0.15	15	0.47	15	-0.16	5	0.31	12	0.54	20	NA	NA	3.33	5	5.00	7
EXKH	EssexKelse/HeliosF7	-0.53	6	-0.04	5	NA	NA	0.03	6	0.53	17	0.04	11	-0.02	6	6.67	15	NA	NA
EXKT	EssexKingsey/TigreF7	-0.61	5	NA	NA	NA	NA	0.80	16	0.22	9	0.69	21	NA	NA	NA	NA	NA	NA
EXSO	EssexSurprise/OtisF7	NA	NA	NA	NA	-0.07	5	0.33	12	0.26	11	0.13	12	0.56	12	NA	NA	7.50	16

Table A.11. Continued

Code	Entry	height												Test Weight					
		cm	Rank	cm	Rank	cm	Rank	cm	Rank	cm	Rank	cm	Rank	BLUP	Rank	BLUP	Rank	BLUP	Rank
		Adirondack		Borderview		Butterworks		Grange Corner		Essex		Old Town		Borderview		Grange Corner		Old Town	
AKFT	AdirondackFaller/TigreF7	90.00	11	87.56	4	88.11	11	104.00	13	NA	NA	114.67	12	0.41	5	0.32	6	0.10	11
AKKW	AdirondackKelse/AC WaltonF7	79.67	4	90.44	8	81.22	8	NA	NA	80.00	4	111.67	8	0.69	1	-0.05	9	2.36	3
AKKH	AdirondackKelse/HeliosF7	91.33	13	92.11	12	NA	NA	99.67	6	86.67	7	115.67	14	0.17	10	NA	NA	1.32	5
AKSM	AdirondackSurprise/MaconF7	88.00	9	90.22	7	76.89	3	95.33	3	NA	NA	112.33	10	-0.87	21	-0.79	14	0.00	13
AKTF	AdirondackTigre/FallerF7	91.67	14	87.56	4	78.67	5	NA	NA	96.67	13	114.33	11	0.01	13	NA	NA	1.12	6
BWDO	ButterworksDefiance/OtisF7	80.00	5	85.33	3	89.33	12	NA	NA	83.00	5	109.67	6	-0.57	18	NA	NA	-2.18	21
BWKW	ButterworksKelse/AC WaltonF7	86.00	7	91.22	10	92.44	18	99.33	5	NA	NA	115.33	13	0.36	7	-0.52	12	1.05	7
BWKT	ButterworksKingsey/TigreF7	NA	NA	NA	NA	79.33	6	102.00	10	94.00	11	108.33	4	NA	NA	-1.37	16	-0.40	16
BWSM	ButterworksSurprise/MaconF7	90.00	11	95.00	18	78.11	4	NA	NA	91.67	9	110.67	7	-0.74	20	NA	NA	-4.08	22
BWSO	ButterworksSurprise/OtisF7	NA	NA	92.44	14	92.11	17	106.67	15	99.00	15	109.00	5	-0.43	16	-0.02	8	-0.23	14
BWTF	ButterworksTigre/FallerF7	91.67	14	89.78	6	80.56	7	112.00	17	NA	NA	116.00	15	-0.31	15	0.81	4	0.97	8
GCFT	Grange CornerFaller/TigreF7	NA	NA	93.56	17	95.17	19	100.33	7	84.00	6	117.00	16	0.36	8	-0.35	11	0.15	10
GCKW	Grange CornerKelse/AC WaltonF7	89.67	10	103.44	19	90.11	16	103.33	11	NA	NA	118.33	18	0.19	9	0.15	7	0.26	9
GCKT	Grange CornerKingsey/TigreF7	77.00	3	92.56	15	89.44	13	100.67	9	NA	NA	117.33	17	-0.11	14	-0.58	13	-1.36	19
GCSM	Grange CornerSurprise/MaconF7	NA	NA	90.56	9	89.56	14	98.00	4	87.33	8	105.00	3	-0.69	19	-2.24	18	-1.78	20
GCSO	Grange CornerSurprise/OtisF7	96.33	17	NA	NA	NA	NA	104.33	14	95.33	12	NA	NA	NA	NA	-3.35	19	NA	NA
EXFT	EssexFaller/TigreF7	92.33	16	92.78	16	NA	NA	103.67	12	98.67	14	111.67	8	0.16	11	-0.11	10	-0.90	17
EXKW	EssexKelse/AC WaltonF7	NA	NA	91.56	11	85.33	10	109.00	16	93.33	10	NA	NA	0.11	12	NA	NA	NA	NA
EXKH	EssexKelse/HeliosF7	86.33	8	92.22	13	89.78	15	NA	NA	72.00	2	120.67	20	0.37	6	0.56	5	1.71	4
EXKT	EssexKingsey/TigreF7	81.00	6	NA	NA	84.11	9	NA	NA	103.33	17	NA	NA	NA	NA	NA	NA	NA	NA
EXSO	EssexSurprise/OtisF7	NA	NA	NA	NA	97.44	20	100.33	7	102.33	16	119.67	19	NA	NA	-0.84	15	0.01	12

Figure A.1. Early vigor of genotypes included as parents in the participatory breeding program for weed-competitive ability. Over five site-years, varieties were rated visually for early vigor on a scale of one to nine, with nine being the most vigorous. Parental varieties included a range of early vigor, with 'AC Walton' tending to have low vigor and 'Faller,' 'Helios,' and 'Kelse' displaying higher vigor compared to other varieties. Sites include Alburgh, VT in 2010 and 2011, Old Town, ME in 2010, Sidney, ME in 2010, and Willsboro, NY in 2011 (indicated as 'WB 2011').

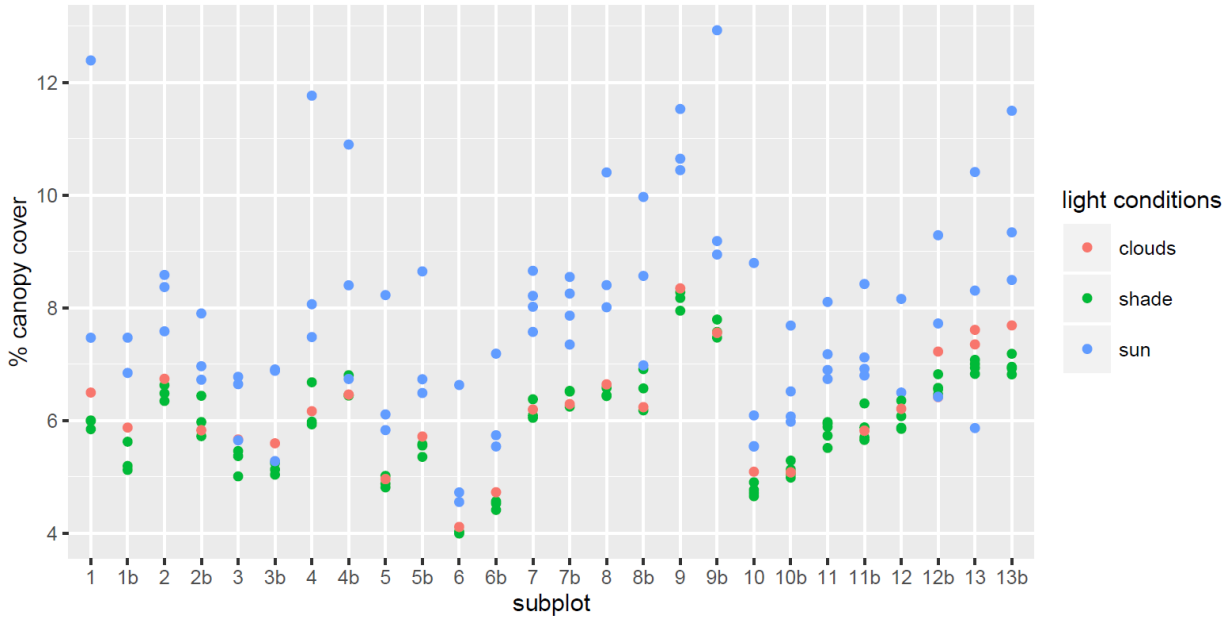

Figure A.2. High variance under full sun conditions in the Canopeo program. Ground cover measurements of two subplots (*e.g.*, "1" and "1b"), within 13 genotype plots, were taken on the same day in full sun, cloud cover, and under artificial shade cover. Large variation in measurements in full sun prompted the use of shade cloth.