BIOAG FINAL REPORT

TITLE: Breeding colored wheat and barley for nutrition and novelty for low-input integrated farms

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ABSTRACT

In western Washington growers use small grains as rotational crops to break disease and pest cycles and rest the soil between more intensive, and profitable, crops. We are breeding barley and wheat for nutritional content and end-use quality, using novel color traits to differentiate the crop and increase the antioxidant content of the grain. Unique color traits will distinguish grains grown in this region and the quality characteristics will make them desirable to bakers, chefs, brewers, and consumers. Farmers, processers, and consumers have expressed a desire for colored grains but there are not currently any varieties suited to the region. This focused on the introgression of color traits into regionally adapted lines of wheat and barley, selection of colored germplasm under organic conditions, and the characterization of the nutritional and baking profile of colored grains. This testing identified wheat and barley breeding lines that compared favorably with local check varieties for yield and some with increased antioxidant capacity, showing the potential to breed for increased levels of antioxidants. The main outcome of this work was the development of breeding lines of wheat and barley with novel seed colors and a range of grain quality traits for continued selection and eventual variety release. The next steps will involve more extensive agronomic and end-use quality testing in collaboration with farmers and bakers in the region to select lines for release.

PROJECT DESCRIPTION

Wheat and barley are both staple crops grown on a large scale world-wide and sold as commodities with distinct quality requirements and market classes. The market classes are designed to group grains with similar qualities fungible in end-use production, regardless of their origin. Outside of the commodity designations, there exists genetic variation for a range of seed colors that have not been explored because no market currently exists for them.

Breeding varieties with acceptable yield and end-use quality that have colors outside the commodity system could allow them to be grown and sold on a regional scale where pricing can reflect the cost of production and add value to local economies. The objectives of this project included: 1. Developing adapted wheat and barley lines with unique seed color combinations and known quality parameters and advancing them through the breeding program. 2. Selection of colored germplasm under organic and low-input growing conditions. 3. Characterization of the nutritional and baking profile of these colored grains.

Breeding lines were developed from diverse germplasm obtained from the USDA small grains collection and breeders that were then crossed with locally adapted varieties in different end-use categories. Selection was carried out for seed color, maturity, straw strength, disease resistance, and grain yield under organic and low-input conditions. Those lines are now at the stage of broader agronomic testing and end-use quality evaluation.

In addition, a set of breeding lines from previous efforts were tested at multiple locations over two years for agronomic and end-use quality. For wheat kernel hardness and solvent retention were used to broadly categorize the potential for baking leavened products and in barley beta glucan testing was used to quantify the soluble fiber content. A range of values exist in this material, forming a genetic base for breeding varieties suitable for a range of products.

A subset of lines were tested for micronutrient and antioxidant capacity in comparison with commonly grown check varieties to assess the potential to breed for increased nutritional quality. This evaluation identified lines that had increased levels of antioxidants that might be used in future breeding work.

Outputs

Overview of Work Completed and in Progress:

- In the spring of 2015, we planted organic hull-less colored barley trials at WSU-NWREC and at Hedlin's Family Farms. The trial had 24 entries with two replications containing advanced colored breeding lines, colored landraces, and early generation breeding material. Agronomic data were taken and selections were made. Selections were made on the F_2 and F_3 segregating material. Grain beta-glucan measurements are in progress on the more advanced material.
- Advanced generation purple wheat lines were planted in organic replicated trials at NWREC and Hedlin's to test yield and baking quality in 2015 and 2016, along with short rows of selections from 2014. F_2 breeding material of blue and purple wheat crosses was planted conventionally and heads were selected.
- In the fall of 2015, we planted two F_4 colored barley populations at WSU-NWREC for further selection. Additionally, we planted two strips of a doubled haploid colored barley blend to select pure lines out of it.
- In the spring of 2016, we planted a hull-less colored barley trial at WSU-NWREC and at Dave Hedlin's farm; both were under organic growing conditions. The trial has 28 entries with two replications containing advanced colored breeding lines, colored landraces, and early generation breeding material.
- In the spring of 2016, we planted organic trials at NWREC and Hedlin Family Farms to compare 12 purple wheat lines to three check varieties for agronomics, antioxidant capacity, and baking quality.
- Planted F_3 and F_4 seed of blue and purple wheat breeding lines at NWREC, 150 plots and 600 headrows. Promising lines will be advanced tested for antioxidant capacity and advanced.
- Wheat and barley from this project was featured as part of a Culinary Breeding Network event in October 2016 and 2017 that brings together farmers, chefs, bakers, breeders, and community.
- A small-scale sourdough baking test for whole wheat flour was developed in conjunction with Dr. Andrew Ross at OSU to be used in later generations to select for bread making quality.
- Antioxidant testing was completed on colored wheat and barley lines in collaboration with the Linus Pauling Institute at OSU.
- Mineral content and crude protein were measured on colored wheat and barley lines at Soiltest Farm Consultant, Inc. in Moses Lake, WA.
- Seed from strips grown at NWREC and Hedlin's in spring 2016 was provided to Nash Huber to plant a test acre in spring 2017 to see viability of the line in commercial production to determine whether the line will be released.

- Colored wheat and barley breeding material and varieties were planted in small plots, yield trials, and strips in the fall of 2016 and spring of 2017 to allow for continued selection and advancement of this project.

Methods:

- Five colored barley lines and seven colored wheat lines were grown in a replicated trial at two locations in the Skagit Valley of in 2015 and 2016
- Samples were ground with a Perten Laboratory Mill 3100
- The Ferric Reducing Antioxidant Power (FRAP) and Oxygen Radical Absorbance Capacity (ORAC) methods were used to measure total antioxidant capacity (Benzie and Strain, 1996; Cao et al., 1993)
- Protein and mineral content were measured at Soiltest Farm Consultants, Inc.
- Statistical analyses completed using Proc Mixed and Proc CORR in SAS version 9.3

Results and Discussion:

Table 1 shows wheat entries, Tables 2 and 3 show means separations for measured qualities and Figure 1 shows yield data from the three site years by entry.
For the FRAP assay, there were significant differences between genotype and interactions between year, location, and genotype (Table 2). For the ORAC assay, significant differences were observed between genotypes for barley but not for wheat (Table 3). For protein measurements, significant differences were observed between years and genotypes (Table 2).

The range of kernel hardness and SDS values for wheat indicate the material could be used to produce products requiring a strong gluten network (such as biologically leavened breads) and pastries or quick breads where less gluten strength is desirable. Overall yields (Fig. 1) showed that purple wheat lines could equal the commercial check varieties. This equivalency could make it possible for growers to produce a crop that could be marketed for a price in line with commodity wheat, adjusted for their expenses.

The lack of significance of the antioxidant values for most lines suggests that the colors of these grains will be most useful as a method of identity preservation for regionally produced grains and not for nutritional benefit. Exploring potential flavor differences between the different colors is another potential area of research now that there is a base of germplasm. At this point material has been developed that is adapted enough to be selected based on culinary performance and for growers to test.

Publications, Handouts, Other Text & Web Products:

- Brigid Meints (Co-PI) gave a presentation at the Organic Seed Growers Conference in 2016 and included this project in her proceedings paper. This was published on the Organic Seed Alliance website (http://seedalliance.org).
- Brigid Meints presented a poster at the International Barley Genetics Symposium (IBGS) in 2016 on colored barley and submitted an abstract: http://ibgs2016.org/resources/IBGS_POSTER_ABSTRACTS.pdf. The same poster was presented at the Organicology Conference in 2017.
- Brigid Meints presented a poster on antioxidant capacity in colored barley at the North American Barley Researcher's Workshop in 2017 (abstracts posted :http://www.barleycanada.com/wp-content/uploads/2017/04/NABRW-program-20171.pdf.

- Work from this project was presented at the WSU Mount Vernon Small Grains Field Day (June 2016 and 2017), with abstracts posted at: http://thebreadlab.wsu.edu/. Data from the field trials will be published at http://plantbreeding.wsu.edu
- Colin Curwen-McAdams presented this research at Washburn University to faculty in students as an invited lecture November 2016.
- Colin Curwen-McAdams presented on this work to the Seattle EPA office in May 2017.
- Brigid Meints and Colin Curwen-McAdams participated in the Culinary Breeding Network event (~400 attendees) in October of 2016 and 2017 where grain from this work was prepared by chefs and bakers and presented to the public for tasting.
- The WSU Bread Lab Instagram account (@wsu_bread_lab) has shared photos from this research project.

Outreach & Education Activities:

- This work has been presented at our annual Grain Gathering during July 2016 and 2017 (which draws over 300 amateur and professional bakers, millers, maltsters as well as food industry representatives from around the world).
- This research was presented at the Cascadia Grains Conference in Olympia, WA in January 2017.

IMPACTS

- Short-Term: In the short term, this research will result in nutritional values and baking quality standards that can be used as targets for future breeding and genetic work.
- Intermediate-Term: Collaborating with growers, processors, and end-users will make them aware of the potential of colored grains and give us knowledge about how novel grains might add value to their operations. Trials on local organic farms will help us shape the agronomics, and working with local millers, bakers, and maltsters will develop the network through which the grains can be sold.
- Long-Term: Ultimately, this research will result in a better understanding of the relationship between colored seed coats and nutritional content of the grain. It will also allow farmers in this region access to adapted grains that don't fit into the commodity system and thus allow for added value. Because of the novel seed coat colors, these new varieties will be distinguishable and unique to this region. Locally bred and established varieties will contribute to a strong regional grain economy that benefits farmers, processors, bakers, and chefs.

ADDITIONAL FUNDING APPLIED FOR / SECURED: $7500 from the Northwest Agricultural Research Foundation (NARF) to work on Colored Barley (Received in 2016 and 2017).

This work contributed to a successful OREI grant on breeding naked food barley for organic systems in collaboration with Oregon state University.

GRADUATE STUDENTS FUNDED: Colin Curwen-McAdams and Brigid Meints

RECOMMENDATIONS FOR FUTURE RESEARCH: Due to the nature of the plant breeding process, this research will require many more years as we continue to select for adapted colored material and better understand the nutritional aspects of this germplasm. Color will be measured using a Minolta CR-410 tristimulus colorimeter. In-depth genotype × environment analysis. Antioxidant capacity measurements on a greater number of lines grown in a wider range of environments.

Photos: All photographs taken by Brigid Meints and Colin Curwen-McAdams. A: Harvesting organic colored wheat and barley trials at Dave Hedlin's. B: Prepping colored barley lines for planting. C: Black F2 barley breeding line in the greenhouse. D: Purple and blue experimental wheat lines. E: Counter-top malting purple barley. F: Small-scale sourdough bake tests with colored wheat lines. G: Colored samples being prepped and milled for antioxidant testing. H: Sedimentation testing of purple wheat samples. I: Colored wheat and barley samples being extracted for antioxidant measurements. J: FRAP plates after microplate readings.

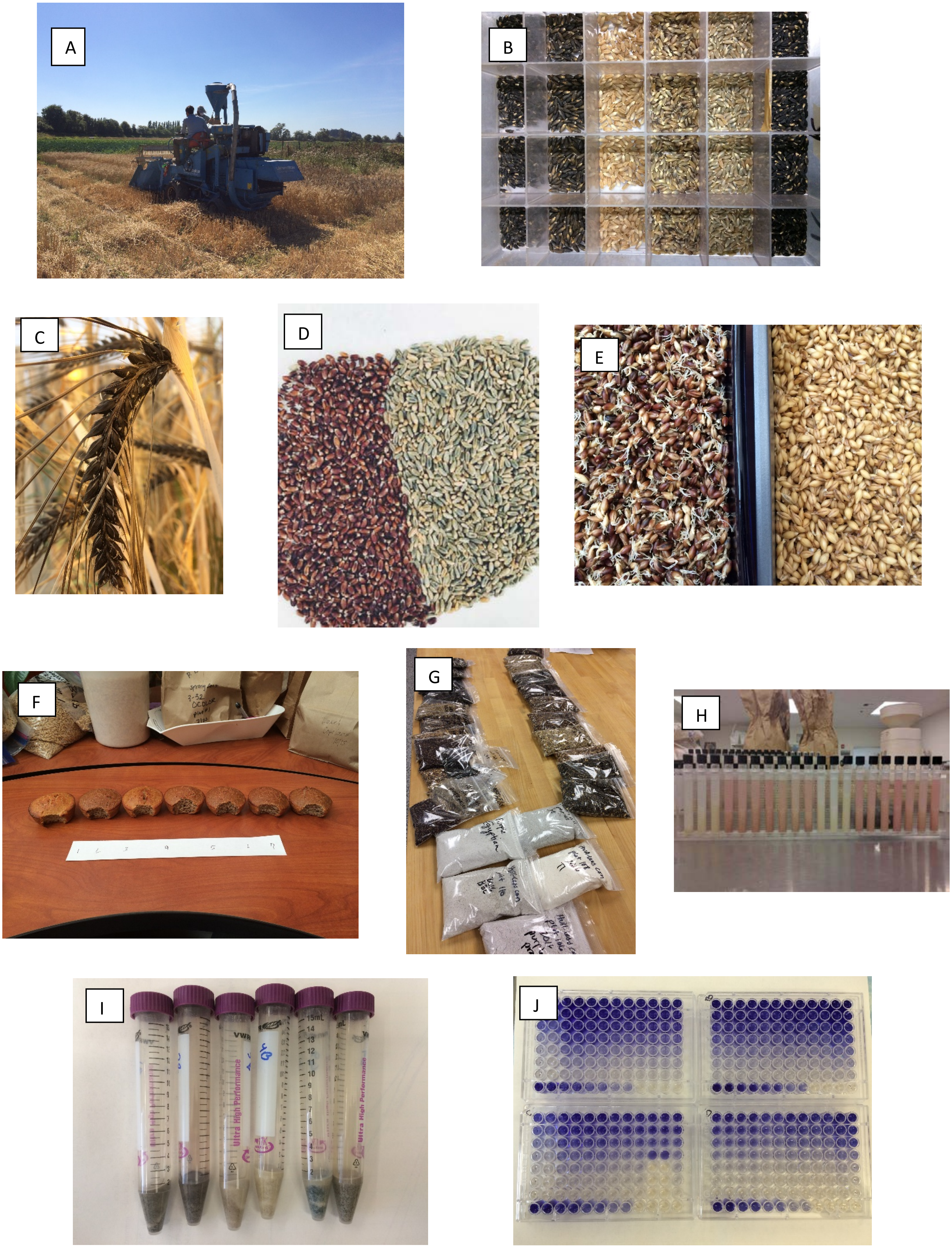
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Table 1

Wheat entries, pedigree information, seed color and kernel hardness as measured by Perten SKCS 4100.

Entry	Pedigree	Seed Color	Seed Hardness (±SD)
18	Edison	White	41.95 ± 15.99
28	Expresso	Red	75.19 ± 15.12
224	Dacke × H86-701	Purple	52.87 ± 22.34
225	Dacke × Indigo	Purple	54.42 ± 22.74
227	Dacke × 1159.288.18b.1.2	Purple	69.65 ± 17.06
228	CI 14952 × Dacke	Purple	61.22 ± 20.88
229	Nardo × Konini	Purple	62.19 ± 21.43
230	Konini	Purple	54.59 ± 18.61
552	AHR-2 (Nardo x 1159.288.18b.1.2)	Purple	72.93 ± 14.71
564	AHR-14 (Nardo x 1159.288.18b.1.2)	Purple	67.13 ± 12.53
565	AHR-15 (Nardo x 1159.288.18b.1.2)	Purple	39.06 ± 13.67
572	AHR-22 (Nardo x 1159.288.18b.1.2)	Purple	71.88 ± 13.08
573	AHR-23 (Nardo x 1159.288.18b.1.2	Purple	65.55 ± 13.18
579	Nardo × Indigo	Purple	37.35 ± 14.22
737	Dacke	Red	71.38 ± 15.95

Table 2

Means separation for pooled data of entries grown at all three location-years (except 552, 564, 565, 572 and 573) with least-squares means in bold letters underneath values. Entries that share at least one letter in common are not significantly different at p<0.05.

Entry	18	28	224	225	227	228	229	230	552	564	565	572	573	579	737
ORAC	10316	10161	11653	10359	11706	12256	11941	11986	9823	10767	11020	12380	11621	10739	10342
(µMol TE/Kg)	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**
Protein	10.38	13.46	11.25	11.64	12.12	12.00	11.99	12.18	10.56	12.56	11.53	12.85	13.79	11.05	11.56
(%)	**e**	**a**	**cde**	**bcd**	**bc**	**bc**	**bc**	**b**	**de**	**abc**	**bcde**	**ab**	**a**	**de**	**bcd**
SDS	4.6	6.3	4.3	4.5	5.4	4.7	4.3	4.2	4.6	5.8	4.5	4.7	6.6	3.7	5.6
(cm)	**d**	**a**	**d**	**d**	**bc**	**d**	**d**	**de**	**d**	**ab**	**de**	**cd**	**a**	**e**	**b**
Fe	90.10	89.41	80.33	66.03	73.02	85.11	80.81	72.64	62.58	63.64	70.64	92.42	93.25	67.90	80.74
(mg/kg)	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**
B	3.51	1.87	2.14	1.17	0.59	1.73	1.72	2.01	0.08	3.12	0.53	0.18	0.09	0.95	1.21
(mg/kg)	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**
Zn	36	43	41	42	41	43	43	44	37	44	41	49	52	39	39
(mg/kg)	**c**	**abc**	**abc**	**abc**	**abc**	**abc**	**abc**	**abc**	**bc**	**abc**	**abc**	**ab**	**a**	**bc**	**bc**
Cu	4	5	4	4	4	4	4	4	4	4	4	5	4	4	3
(mg/kg)	**cde**	**a**	**cde**	**bcd**	**bcd**	**de**	**ab**	**ab**	**abcde**	**abcd**	**abcd**	**ab**	**abcd**	**abc**	**e**
P	0.43	0.46	0.45	0.45	0.46	0.45	0.47	0.48	0.45	0.48	0.47	0.49	0.50	0.46	0.43
(%)	**c**	**abc**	**abc**	**abc**	**abc**	**abc**	**ab**	**a**	**abc**	**abc**	**abc**	**a**	**a**	**abc**	**bc**
K	0.42	0.44	0.47	0.46	0.46	0.45	0.50	0.50	0.49	0.49	0.50	0.51	0.47	0.51	0.42
(%)	**e**	**de**	**abcde**	**bcde**	**bcde**	**cde**	**ab**	**abc**	**abcd**	**abcd**	**abcd**	**abc**	**abcde**	**a**	**e**
S	0.14	0.16	0.13	0.12	0.12	0.14	0.15	0.14	0.11	0.14	0.16	0.14	0.14	0.14	0.13
(%)	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**
Ca	0.069	0.067	0.061	0.059	0.061	0.059	0.065	0.063	0.062	0.060	0.062	0.054	0.057	0.057	0.055
(%)	**a**	**ab**	**bcde**	**cde**	**bcde**	**cde**	**abc**	**abcd**	**abcde**	**abcde**	**abcde**	**de**	**bcde**	**de**	**e**
Mg	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.15	0.18	0.17	0.18	0.18	0.16	0.16
(%)	**c**	**ab**	**abc**	**abc**	**ab**	**abc**	**ab**	**ab**	**bc**	**abc**	**abc**	**abc**	**a**	**bc**	**bc**
Na	0.007	0.006	0.006	0.006	0.005	0.006	0.006	0.006	0.004	0.006	0.004	0.004	0.004	0.005	0.005
(%)	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**

Table 3

Means separation by year and location for variables with Entry*Location, Entry*Year interactions or both with least-squares means in bold letters underneath values. Entries that share at least one letter in common are not significantly different at p<0.05.

		Entry	**18**	**28**	**224**	**225**	**227**	**228**	**229**	**230**	**579**	**737**
Yield	NWREC 2015		737.5	516.0	587.4	562.1	552.1	524.9	605.5	510.9	438.8	661.6
(g/plot)			**a**	**ab**	**ab**	**ab**	**ab**	**ab**	**ab**	**ab**	**b**	**ab**
	Hedlin 2015		1895.6	1349.4	1371.8	1421.1	1333.1	1287.7	1563.5	967.5	1492.1	1120.6
			a	**bcd**	**bc**	**bc**	**bcd**	**bcd**	**ab**	**d**	**bc**	**cd**
	Hedlin 2016		1151.7	913.6	870.2	980.2	888.2	880.6	792.9	857.1	942.1	786.8
			ab	**bcd**	**bcd**	**abcd**	**bcd**	**bcd**	**d**	**bcd**	**abcd**	**d**
Test Weight	NWREC 2015		417.8	431.2	428.8	427.5	421.0	425.1	424.8	425.8	423.2	430.0
(g/0.5hL)			**b**	**a**	**a**	**ab**	**ab**	**ab**	**ab**	**ab**	**ab**	**a**
	Hedlin 2015		422.2	441.0	430.0	432.6	429.6	425.9	431.2	429.4	436.1	424.0
			d	**a**	**bcd**	**abc**	**bcd**	**bcd**	**abcd**	**bcd**	**ab**	**cd**
	Hedlin 2016		444.2	449.8	443.5	444.1	436.7	434.2	435.7	442.2	446.6	442.1
			abc	**a**	**abc**	**abc**	**bc**	**c**	**c**	**abc**	**ab**	**abc**
FRAP	NWREC 2015		1803	1772	2035	1990	1952	2226	2039	1964	1967	1675
(µMol TE/Kg)			**cd**	**cd**	**ab**	**bc**	**bc**	**a**	**ab**	**bc**	**bc**	**d**
	Hedlin 2015		1591	1505	1904	1774	1944	2097	1980	1873	1879	1622
			cd	**d**	**abc**	**abcd**	**abc**	**a**	**ab**	**abcd**	**abc**	**bcd**
	Hedlin 2016		2056	1913	2236	1984	2075	2331	2029	1880	2049	2130
			bcd	**d**	**ab**	**cd**	**bcd**	**a**	**cd**	**d**	**bcd**	**abc**
Mn	NWREC 2015		19	22	16	17	17	18	19	20	15	15
(mg/kg)			**ab**	**a**	**ab**	**ab**	**ab**	**ab**	**ab**	**ab**	**b**	**b**
	Hedlin 2015		21	24	21	20	25	23	24	26	30	20
			b	**ab**	**b**	**b**	**ab**	**ab**	**ab**	**ab**	**a**	**b**
	Hedlin 2016		19	25	20	19	18	22	18	21	18	19
			a	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**
K	NWREC 2015		0.43	0.44	0.49	0.47	0.46	0.48	0.52	0.49	0.54	0.44
(%)			**e**	**de**	**abc**	**cd**	**cde**	**bcd**	**ab**	**bc**	**a**	**de**
	Hedlin 2015		0.39	0.43	0.43	0.45	0.44	0.46	0.49	0.49	0.51	0.42
			c	**bc**	**abc**	**abc**	**abc**	**abc**	**ab**	**ab**	**a**	**bc**
	Hedlin 2016		0.43	0.46	0.47	0.45	0.48	0.42	0.51	0.50	0.49	0.39
			ab	**ab**	**ab**	**ab**	**ab**	**ab**	**a**	**a**	**ab**	**b**

Figure 1

Boxplots for yield of each entry, grouped by location/year. Lines show median values, boxes encompass the 1st through 3rd quartile and lines extending from the box denote minimum and maximum values.