The primary goals of the Colorado State University (CSU) Wheat Breeding and Genetics Program are to: a) develop improved hard red winter (HRW) and hard white winter (HWW) wheat cultivars and germplasm adapted for the diverse production conditions in Colorado and the west-central Great Plains, and b) conduct research to improve understanding of genetic and environmental factors that affect wheat yield and end-use quality. Our program is staffed by extremely dedicated and experienced researchers and overall we have the necessary funding, facilities, and equipment to ensure success.

In summer 2013, we harvested breeding trials at eight locations in eastern Colorado (Akron, Burlington, Dailey, Fort Collins, Julesburg, Lamar, Orchard, Yuma). While yields were generally very low, adequate data were available to inform selection decisions in the breeding program. Due to emergence problems from the drought, severe drought after emergence, or spring freeze injury, we abandoned breeding trials at Arapahoe, Genoa, Roggen, Sheridan Lake, and Walsh.

No lines were on Foundation Seed increase in 2013 and no new cultivars were released in fall 2013. Three HRW and two HWW lines were on Breeder Seed increase in 2013, yet all but one of these was discarded from further consideration. The one line retained, a HWW designated as CO09W293, carries the glutenin trait that is present in Snowmass. Because CO09W293 was mixed for this trait, the Breeder Seed increase was done in Yuma, Ariz., using a headrow progeny purification facilitated by DNA marker analysis for the glutenin trait. Foundation Seed is currently being produced in Yuma, Ariz., to enable release of CO09W293 in fall 2014. In two years of testing in the CSU Elite Trial (18 locations), dryland grain yield of CO09W293 was 102% of trial average, compared to 109% for Byrd, 103% for Brawl CL Plus, 101% for Antero, 97% for Hatcher, 92% for Thunder CL, and 88% for Snowmass. CO09W293 has average test weight and straw strength, and is moderately susceptible to stripe rust and susceptible to leaf rust.

In fall 2013, three HRW and two HWW lines were advanced for Breeder seed production to enable potential release in fall 2015. These lines have all shown good field performance, good test weight, stripe rust resistance, and average or better milling and baking quality. The three HRW lines (CO11D174, CO11D346, CO11D446)
are all doubled haploid (DH) lines while the two HWW lines (CO09W009, CO09W040-F1) are conventionally derived. A decision on retention and advancement to Foundation Seed production will be made after harvest in 2014.

CWRF royalty funds and our partnership with ConAgra Mills are providing for a significant expansion of experimental HRW and HWW line development through the use of doubled haploid (DH) breeding technology. Since 2010, we have utilized the DH capacity at Heartland Plant Innovations (HPI) and Washington State University (WSU).

In 2012, we began to develop our own DH laboratory capacity at CSU, first using the microspore embryogenesis method (which didn't work very well) and now focusing exclusively on the wheat-maize wide hybridization method (which appears to work very well). Funding secured from CWAC and various internal CSU sources is being used to renovate a lab in the CSU greenhouse complex for DH production. We expect the lab to be fully operational by early December 2013.

In 2013, 231 DHs (mostly HRWs) were tested in partially replicated trials at 8 field locations. Based on available data, 31 of these were advanced to the 2014 CSU Elite Trial (along with 25 conventionally derived lines from our Advanced Yield Nursery). In 2013, we also had 1,889 DHs on increase in Yuma, Ariz., originating from materials generated by HPI (funded by ConAgra Mills) and Washington State University (funded by CWRF). Visual selection in Yuma, Ariz., together with DNA marker data generated on the lines, reduced this number to 629 DHs that were planted in multi-location, partially replicated trials in Colorado in fall 2013. Among these are 324 HWW DHs (254 of which carry the Snowmass glutenin trait) and 305 HRW DHs.

An order placed with HPI in July 2012 returned 3,065 new DHs in September 2013. Each of the 38 crosses carries Snowmass in the pedigree, and several also carry Clearfield® or low PPO traits. Due to variability in seed amounts, 2,502 DHs were planted in the field at Fort Collins and 563 DHs will be increased in the greenhouse during winter 2013-14. An order placed with WSU in July 2012 for 1,150 DHs has not yet been completed but we hope to receive these in time for planting in spring 2014. Several of these crosses were single-backcrosses with Bearpaw (from Montana, for solid-stem trait) and both Byrd and Antero. DNA markers for QTLs associated with the solidness trait were used to identify backcross-F1 plants for DH production.
We continue to utilize a wide variety of diverse germplasm sources in our crossing program, including elite materials from other regional programs, new sources of leaf and stripe rust resistance, solid-stem varieties from Montana State University for wheat stem sawfly resistance, winter wheat lines introduced from Turkey and other areas of eastern Europe, and germplasm carrying various novel traits from our program and other programs.

Our crossing program has been especially aggressive in recent years, producing many thousands of different crosses for our program (averaging over 2,600 per year), perhaps beyond our capacity to efficiently manage and evaluate in the field. To efficiently accommodate our DH effort, and utilize personnel and facility resources more effectively, starting in fall 2013 we have modified our crossing strategy such that single crosses and top-crosses will no longer be made in both the fall and winter greenhouse crossing cycles. Our spring cycle will focus on elite single crosses and top-crossing will now be done exclusively in the fall cycle using F1s made the previous spring. We will continue to utilize the spring-planting capacity at Fort Collins for the top-crosses that are made in the fall cycle, as well as DHs that are produced and harvested from our greenhouses.

In summer 2013, Craig Beil joined our breeding program as a PhD graduate student. The focus of Craig's dissertation research will be to leverage next-generation sequencing technologies (i.e., genotyping by sequencing, GBS) to more efficiently exploit winter wheat germplasm obtained from the CIMMYT-Turkey program through the Facultative and Winter Wheat Observation Nursery (FAWWON).

Two of our other PhD students are making excellent progress on their dissertation projects. These include Sue Latshaw, who is working on genomic selection for nitrogen use efficiency, and Jessica Cooper, who is working on characterization of agronomic and quality effects of different glutenin subunits (related to our work with Snowmass) and genomic selection for pre-harvest sprouting tolerance and other aspects of end-use quality.
In fall 2013, we planted over 17,000 yield trial plots across 14 trial locations in Colorado, in addition to three dryland CSU Elite Trial locations in Kansas in collaboration with Kansas State University (KSU) and one under irrigation in Nebraska in collaboration with a private company. We also planted over 30,000 headrows, over 1,300 early-generation (F2-F4) populations (including 218 two-gene CLEARFIELD populations), and over 1,400 new cross combinations at Fort Collins in fall 2012 and spring 2013.

We added one field trial location for our CSU Elite Trial in fall 2013, at New Raymer, located in a field that will likely be subject to wheat stem sawfly infestation in 2014. While we don't yet have our own advanced solid stem materials ready for field testing, and we don't expect that our hollow-stem materials will provide any substantive protection, we are preparing ourselves for solid-stem germplasm evaluation. We are also collaborating with Frank Peairs on a study with solid-stem blends.

We continue to collaborate with KSU on evaluation of their advanced trials (KIN, KS AYN) at three locations in eastern Colorado (Sheridan Lake, Burlington, and Julesburg). We also initiated a trial exchange with a private company (Limagrain), involving our CSU Elite Trial grown at Imperial, Neb., and their advanced trial grown at Burlington. We continued this arrangement for the 2014 season.

The project leader of the wheat breeding program will be taking a six-month sabbatical leave in Europe from December 2013 to May 2014. The focus of the sabbatical will be to learn new ideas and new techniques at the interface of plant genomics and wheat breeding.
Our objective is to develop and validate novel traits in wheat using advanced, functional genomics-based techniques, such as TILLING or other technologies. TILLING (Targeted Induced Local Lesions In Genomes) has shown promise in wheat for novel trait development using mutation induction rather than transgenics (GM). Therefore, TILLING-derived traits are non-GM and may provide new and beneficial technologies for wheat. Our current trait emphases include drought stress tolerance, insect resistance, and human health related traits.

In 2013, we successfully integrated next-generation DNA sequencing with TILLING in a novel technique called “TILLING-by-Sequencing” (TBS). Mutations in all three genomes of nine different genes were identified using TBS.

A total of 34 different plants carrying mutations were identified and nine of the most promising plants have been confirmed via DNA re-sequencing of individual mutant plants and cDNA sequencing from RNA isolated from the plants.

A publication is being finalized to document TBS and we expect that it will be the first report of high-throughput, multiplex mutant screening using next-generation DNA sequencing technology in wheat.

A new Linux computer system, software, and upgraded hardware were installed to accommodate the bioinformatics computing requirements of TBS.

Collaboration based on the TBS technology is being explored with other faculty members, such as Dr. Courtney Jahn (BSPM Department) and Dr. Christie Peebles (Chemical and Biological Engineering Department). We are also exploring a new method known as ‘TALEN’ for site-directed mutagenesis of specific genes.

In a collaboration led by Dr. Jahn, using TBS, we are working to identify mutations in 30 different genes related to the stem solidness trait for wheat stem sawfly resistance. Libraries of DNA samples from our TILLING population were prepared and sent for sequencing to identify plants with mutations in these genes. The raw
sequencing data have been returned to us and we are now processing the data. Dr. Jahn led the preparation of a USDA-AFRI grant proposal to help fund and expand upon this research, but the proposal unfortunately was not funded.

In 2013, we developed methods to identify native variants in candidate genes using genotyping by sequencing (GBS) data already being generated for genomic selection (GS) in the breeding program. We have confirmed the potential of this method and have modified the GBS pipeline to accommodate screening for functional variants in 16 different genes. This methodology will potentially streamline marker screening of experimental lines in the breeding program.

In 2012, we identified mutants on all three genomes for a gene related to expression of gliadins involved in Celiac disease (DME) and a gene related to production of a chemical in the wheat plant that attracts the wheat stem sawfly (CHAT). For both traits, we made crosses with the mutants in spring 2013 and crosses were made in fall 2013 to combine two mutations in a single plant.

Seed from crosses to be made in spring 2014 between two-gene plants will be used to produce doubled haploids (DH) for trait validation. Screening of plants during crossing/backcrossing and of DHs will be done using KASP marker assays developed by research associates Tori Valdez and Emily Hudson-Arns.

Mutations identified in genes for high amylose (SBEIIa, developed by UC-Davis) and drought stress tolerance (Era1, developed at CSU) continue to be transferred to elite wheat germplasm for trait validation and potential commercialization. The three high amylose traits were backcrossed to Snowmass and 685 single-head selections made from 12 segregating populations were planted in the field in fall 2013. The three Era1 mutants were also backcrossed to Snowmass and in January 2013 we submitted an order to Heartland Plant Innovations (HPI) to generate 390 DHs for validation purposes. KASP marker assays will be used to confirm the presence of the traits among lines developed for purposes of trait validation.
The objective of this program is to implement “genomic selection” (GS) in the CSU Wheat Breeding Program. Genomic selection takes advantage of next-generation DNA sequencing technologies and statistical models to predict the trait potential of breeding lines prior to, or as a complement to, evaluation in the field or lab. For our breeding program, we are working toward selecting experimental lines partly on GS-based predictions which will reduce the amount of time required before returning promising lines to the crossing program. If successful, the value of GS will be realized through shortening of the breeding cycle time, in conjunction with doubled haploid (DH) technology, and increased rates of genetic improvement for yield, quality, and other traits. Dr. Eric Storlie is coordinating the development of genomic selection models in the breeding program.

In 2010-11, we assembled a group of 400 lines and varieties from our program to form our initial “training panel” for GS model development. In 2012-13, we built upon this panel through addition of 1,900 new lines. Some of these (30%) were breeding program discards from prior years while most (70%) were under advanced or preliminary lines under field-testing in 2013.

In 2012-13, we were successful in adopting techniques for dense, genome-wide marker analysis using “genotyping by sequencing” (GBS). Our use of GBS involves DNA sample preparation at CSU (using protocols from the Jesse Poland Lab, USDA-ARS), outsourcing of the DNA sequencing to the University of Missouri core facility, and processing/marker data extraction of the raw sequencing data by Jesse Poland. We are exploring the possibility of in-house data processing at CSU using the bioinformatics capacities developed by Harish Manmathan.

In 2012-13, we carried out GBS on the set of 1,900 new lines mentioned above. In 2013-14, we are planning to conduct GBS on an additional set of 2,806 lines. These materials include all of our mid- to late-generation breeding lines (986), preliminary lines (1,314), a group of eastern European winter wheat breeding lines (371, as mentioned below), and 135 lines from a Snowmass/Antero DH population. Due to the increasing numbers of individuals we have genotyped, the number of marker datapoints per individual obtained via GBS has increased from 22,000 with our first run to over 45,000 with our last run. Thus, since we implemented GBS we have obtained a total of around 233 million marker datapoints (not counting missing data).

Using 2006-2012 trial data on our GS training panel (2,368 individuals), and the GBS markers obtained on the panel, we have developed GS models for grain yield and test weight. Through this process we have a much better logistical and technical understanding of how GS will fit into the breeding program after working through all the steps in one cycle. Predictions obtained were not used in line selection in 2013 though they were used in selection of crossing parents for the spring 2014 crossing program. Cross-validation accuracies (correlation
between observed and predicted phenotypes) within the panel are above $r=0.60$ for both traits while accuracies with independent validation using lines tested in 2013 were generally lower, sometimes drastically so. We have learned that some training sets (i.e., years) were more predictive than others, suggesting that genotype x environment (GxE) interaction will be an important consideration that must be addressed in model development. We are exploring a factor analytic (FA) model recently reported by CIMMYT to adjust the GS models for GxE interaction.

We currently have three PhD graduate students focusing on different aspects of GS model development and implementation in the breeding program. Sue Latshaw is focusing on nitrogen use efficiency (through GS modeling of “grain protein deviation”), Jessica Cooper is focusing on GS modeling of end-use quality traits (including pre-harvest sprouting tolerance), and Craig Beil is focusing on GS- and GBS-facilitated exploitation of eastern European winter wheat germplasm for diversity enhancement and yield improvement.
The objectives of this program are to incorporate Russian Wheat Aphid (RWA) resistance into our germplasm and ultimately develop improved varieties carrying RWA resistance.

Due to much lower RWA incidence in recent years and inexpensive chemical control options, RWA resistance now occupies a somewhat lesser position among our program’s breeding objectives. We will continue to address RWA resistance, however, as uncertainties exist with regard to the future importance of RWA in Colorado and the economics and availability of chemical control options. All of our RWA resistance effort is done in close collaboration with Frank Peairs and his team (BSPM Department).

Over the last few years we have developed and tested many experimental lines carrying RWA Biotype 2 resistance. None of the lines have been yield-competitive with our best varieties (Byrd HRW or Antero HWW) and thus none have been advanced toward larger scale seed increase. In order to move the “RWA yield curve” forward a bit more rapidly, we are generating a limited number of doubled haploid (DH) lines from crosses with different sources of the Dn7 resistance gene. The first of these is a group of DHs that are planted in multi-locaional trials in 2014.

Our most advanced RWA-resistant line was CO10091, which carries an unmodified source of the Dn7 gene. Grain yield of CO10091 in the 2013 CSU Elite Trial was only average and it was thus discarded from further consideration. Many lines in the 2013 CSU Elite carry RWA Biotype 1 resistance in their pedigree and these lines were screened for RWA Biotype 1 resistance. Several of the lines that showed resistance were among the lines advanced for further testing.

In 2013, four lines in the Advanced Yield Nursery (AYN) and 85 lines in the Preliminary Yield Nursery (PYN) carried a source of RWA Biotype 2 resistance in the pedigree. None of the lines were advanced from the AYN and only six were advanced from the PYN. In 2013, several headrow populations derived from crosses with various RWA resistance sources were grown in Fort Collins. A total of 49 selections were made from these populations for inclusion in the 2014 PYN. We will conduct RWA Biotype 2 screening on all of these materials in winter 2013-14.

In 2013, we conducted field trials of a group of near-isogenic lines derived from backcrossing a modified version of Dn7 into Bill Brown. When we made the line selections in Yuma, Ariz., visual observations suggested that isolines carrying the modified Dn7 were not as adapted/productive as those lacking the modified Dn7. Field trial data at Fort Collins and Julesburg in 2013 countered these observations as the isoline group with RWA resistance (n=18) averaged about 2 bu/a higher yield and 1 lb/bu higher test weight than the group without RWA resistance (n=20). We have completed backcrossing this source into Byrd and Antero, and resistant selections
were planted in the field in fall 2013 for line selection in 2014. Depending on their performance, and the level of interest on the part of producers and the Colorado wheat industry, we could fast track these materials for further testing and potential seed increase and subsequent release.

We have completed backcrossing of different RWA Biotype 2 resistance sources (Triticale, CI2401) into a Yuma background, to complement the Yuma-derived Dn7 source we released as germplasm (CO08RWA050). These materials will undergo purification in greenhouse screening in winter 2013-14. Once purified, these materials may be useful for RWA biotype monitoring.

In addition to RWA, we are also pursuing wheat stem sawfly (WSS) resistance as a formal breeding objective. With one month of salary support from our program, Terri Randolph from Frank Peairs’ team coordinated solid stem evaluations of segregating populations growing at Fort Collins. We are also generating a group of DHs with the solid-stemmed variety Bearpaw from Montana and both Byrd and Antero. Using conventional and DH methods, we hope to rapidly develop semi-solid or solid-stemmed varieties that provide some protection against WSS and show better performance in Colorado than the Montana varieties.
The objectives of this program are to apply DNA molecular marker-assisted selection (MAS) as a tool in selection for traits of interest in the breeding program. On a limited basis, we would hope to be able to identify and validate our own markers for new traits and also optimize marker-trait associations identified by other programs.

Beginning in 2012, we increased the number of preliminary lines that we develop and test in our program (1,013 in 2011, 1,815 in 2012, 1,903 in 2013). In 2013, we significantly expanded routine marker testing of these lines, including those developed via single seed descent (SSD) in prior years. Of the group of 1,903 preliminary lines tested in 2013, 1,710 had one or more marker assays done for key markers associated with various traits. We also conducted marker analyses for the two Clearfield® traits on 118 of the 231 lines in an advanced trial of doubled haploid (DH) lines being tested in 2013. Overall, we generated over 6,500 total datapoints on materials in these trials to aid in selection.

In 2013, we conducted marker analysis on a group of 1,780 DH lines under increase in Yuma, Ariz., in 2013. Most of the DH lines in Yuma were hard whites (HWW) developed through our partnership with ConAgra Mills. From this group, we tested 1,390 lines for one or more DNA markers, generating over 3,950 total datapoints on these materials to aid in selection. The marker data were also used to target certain lines for crossing purposes for further DH production.

We continued to utilize DNA markers for backcrossing various traits from different germplasm sources. Products of these efforts are then used in “forward crosses” for integration into the breeding program. In the fall 2012 and spring 2013 crossing cycles, we conducted over 2,500 marker tests, focusing on introgression of the following traits into various backgrounds: quizalofop and Clearfield® herbicide resistance, drought stress tolerance (TILLING mutants), high amylose (TILLING mutants), polyphenol oxidase, stem solidness (wheat stem sawfly), UG-99 stem rust resistance, and leaf and stripe rust resistance.

We have succeeded in implementing a new marker analysis method known as KASP for screening for several target traits. Using CWRF royalty funds, in 2012 we purchased a new piece of lab equipment (real-time PCR head; $20,000) for KASP and the equipment is working very well for us. The KASP assays are useful...
for those traits with a known DNA sequence, as is the case for TILLING-derived traits. After they are optimized and validated, KASP assays are extremely rapid and inexpensive. The USDA-ARS genotyping laboratory in North Carolina has assisted us with optimization of several assays. We have implemented KASP assays for several traits of interest: quizalofop herbicide resistance, high amylose, high grain protein content (GPC-B1), rye translocations, several disease resistance traits, and a common glutenin trait. We are currently optimizing KASP assays for PPO genes and for the glutenin traits in Snowmass. The KASP assays were used for genotyping over 2,000 plants in two rounds of validation experiments with the quizalofop-resistant mutants.

In 2013, we tried to correlate genotyping by sequencing (GBS) data with DNA markers for key genes among a set of over 600 preliminary lines. Due to the vast amount of missing data that is common with GBS, we have realized that we need to find other approaches. In 2013-14, we will work to modify our GBS library preparation by “spiking” the libraries with PCR products from known marker-gene combinations. Using this method, detection of key markers would then be accomplished through high-throughput sequencing and bioinformatics analysis of the sequencing data, thus allowing us to combine genomic selection and marker-assisted selection into a single step.
The objectives of this program are to conduct end-use quality evaluations on experimental lines in our breeding program and those collected from the state dryland (UVPT) and irrigated (IVPT) variety trials. We are also engaged in several other research areas that are not focused directly on line selection but are complementary to the quality improvement efforts in the breeding program.

End-use quality evaluations are done annually on samples from a variety of different field trials and research studies. In 2012, 6,950 total samples went through our lab for one or more different tests. From breeding trials in 2012, we conducted comprehensive milling and baking quality tests on five locations of the CSU Elite Trial, two locations of the AYN, and two locations of the PYN, and a subset of headrows from Fort Collins, which is pretty similar to the numbers of analyses done in recent years. Our quality analyses of these materials include the full spectrum of quality tests, including NIR protein, single kernel characterization system (SKCS), Mixographs, polyphenol oxidase (PPO), and Quadrumat Senior milling and pup-loaf baking tests. Our overall strategy in breeding line evaluation is to properly characterize experimental lines in order to inform the line selection and seed increase decision-making processes.

Comprehensive milling and baking quality evaluations (as described above) are done annually on selected locations of the state dryland (UVPT) and irrigated (IVPT) variety trial program. From trials in 2012, grain samples were collected from four dryland (UVPT) variety trial locations (Akron, Lamar, Orchard, Yuma) and two irrigated (IVPT) variety trial locations (Fort Collins, Rocky Ford). Preliminary small-scale quality analyses were done to determine suitability of the samples for full-scale analyses. In this process of sample selection, two of the dryland locations (Akron, Yuma) and one of the irrigated locations (Fort Collins) were excluded from analyses beyond protein content. All of the data were reported in the Making Better Decisions booklet. The data are also used to develop and update the milling and baking quality scores that are reported in the “Variety Characteristics Table” in the Making Better Decisions booklet. Other variety-trial related activities from trials in 2012 include NIR protein on 214 samples from the Collaborative On-Farm Testing (COFT) program and 140 samples from the western Colorado trials at Hayden and Yellow Jacket.

From trials in 2012, we determined NIR protein content from every plot of five locations of the CSU Elite Trial (750 total samples) and from every plot of our genomic selection training panel grown at Akron and Fort Collins (3,500 total samples). From trials in 2013, we are doing NIR protein on over 1,800 samples from six locations of an advanced doubled haploid trial. Our objective is to begin to use “grain protein deviation”
(protein content adjusted for grain yield) as a measure of nitrogen use efficiency (NUE) and eventually develop genomic selection (GS) prediction models for NUE selection in the breeding program. This research forms the basis of Sue Latshaw’s PhD dissertation. Sue expects to finish her degree in 2014 and presented some of her results at the American Society of Agronomy meetings in November 2013.

From trials in 2012, we evaluated 920 samples from a study to evaluate the effect of different glutenin allele combinations on agronomic properties and end-use quality. This study is being repeated with samples from the 2013 season. This research is part of Jessica Cooper’s PhD dissertation. Jessica is also working on genomic selection model development for end-use quality traits, including pre-harvest sprouting tolerance. Jessica presented her work on pre-harvest sprouting tolerance at the American Society of Agronomy meetings in November 2013.

John Stromberger participated as an evaluator for the PNW Wheat Quality Council program and meeting in Scottsdale, Ariz., in January 2013. John is also participating as an evaluator for the Hard Winter Wheat Quality Council program beginning with the 2012 crop-year samples.
Wheat virus research/demonstration

In 2010, CSU participated in a Great Plains survey of the mite-transmitted viruses wheat streak mosaic virus (WSMV), High Plains virus (HPV), and Triticum mosaic virus (TriMV). TriMV was discovered in 2006 in Kansas and was subsequently shown to be transmitted by the wheat curl mite. These viruses are estimated to cause 3-5% annual yield loss with greater losses occurring in certain years. Survey results indicated that TriMV was widespread throughout the Great Plains and usually was found in co-infections with one of the other viruses.

As a result of this work, the USDA-NIFA grant program awarded the University of Nebraska a $3M grant in 2013 to continue research on mite-transmitted viruses. The plant pathology program of Colorado State University is a co-investigator in this grant. Thus, CSU was able to leverage funds provided by CWAC for the original survey into a regional program with national support. As part of this project we established a research/demonstration plot in the fall 2013 at the USDA facility at Akron. This trial will be conducted during three consecutive years to show effects of management strategies on the development, severity, and impact of these viruses. WSMV-resistant and -susceptible winter wheat cultivars will be planted in two locations on three different dates: 1) two weeks before the normal planting date, 2) normal planting date, and 3) two weeks after the normal planting date. Plots will be monitored for development of virus symptoms in the fall and during the growing season the following year. Field days will be held each year to show the effects of planting date, cultivar resistance, and volunteer wheat control on the development and severity of diseases caused by these WCM-vectored viruses.

Bacteria associated with Russian Wheat Aphid

The Russian wheat aphid (RWA) is a major insect pest of winter wheat in Colorado. It is managed with resistant varieties and in some cases insecticide applications. Resistant varieties ‘recognize’ molecules called effectors that are injected by the aphid and then mount a defense response. Unfortunately, the effectors in RWA saliva responsible for the resistant reaction are still unknown. Their elucidation is a promising way to identify resistance mechanisms and perhaps to speed the process of identifying resistant plants. Previous work by L. van Eck and N. Lapitan at CSU showed that among 1700 proteins identified in RWA saliva, 78% have a very strong similarity to gram-negative, rod shaped bacteria proteins belonging to the family enterobacteriaceae, a common family of many plant pathogens, particularly plant pathogenic enterobacteria like Erwinia sp. (6) (Figure). Our lab has isolated several Erwinia spp. from surface sterilized aphids, artificial diets on which aphids fed, and from wheat leaves infested with RWA. As we were unable to isolate these bacteria from aphid-free wheat, they are likely injected into the plants during aphid feeding. We are continuing studies to determine whether these bacteria are pathogenic to the plant, are contributing effectors that are recognized during a resistant reaction, or may be affecting the health of the RWA.

Early response and diagnostics support for wheat diseases

Each year I supply up-to-date information on the status of wheat pests, particularly diseases, during the growing season. Information on potential disease problems, gathered from plant pathologists in adjacent states and from extension agents and crop consultants in Colorado are summarized and sent via email in a timely manner to Colorado wheat producers. In 2013, drought and spring freezes damaged much of the wheat in the southern part of the state. Disease pressure during the remainder of the season was light with no significant development of stripe rust.
The Colorado State University (CSU) wheat variety trials and Collaborative On-Farm Tests (COFT), represent the final stages of a wheat improvement program where promising experimental lines are tested under broader and more and more variable conditions. Trials are planted in different soils and in areas with different disease and insect pests. Producers make better variety decisions because varieties are tested in many diverse locations. In addition, the CSU breeding program can more reliably select and advance the most promising lines toward release as new varieties.

2013-14 Wheat Variety Performance Trials

The CSU variety testing program is built on 3 pillars: many locations, many varieties, and three years of results. There were 44 entries in the 11 dryland performance trial (UVPT) locations and 28 entries in the 3 irrigated performance trial (IVPT) locations. We are proud to have had a wide array of different varieties in the dryland trials, including 7 CSU experimental lines, 11 varieties from private seed companies, 13 varieties from surrounding states, 10 PlainsGold varieties, and 3 older CSU released varieties. The large number of varieties and diverse testing environments leads to better variety choices. The 28 irrigated entries came almost equally from CSU experimental lines, seed company varieties, other university entries, and PlainsGold varieties. We believe in the value of uniform variety trials, where all entries are tested in all UVPT or IVPT trials, whereas some neighboring states are carving their wheat growing areas into small regions and courting the seed companies to grow their programs. There were excellent planting conditions at all dryland performance trial locations in fall 2013 due to consistent rains in September and October, resulting in good to excellent emergence and plant stands.

Winter Wheat Variety Testing Program: Crops Testing Program Update
Jerry Johnson, Extension Specialist - Crop Production

Jim Hain and Jerry Johnson planting a wheat variety trial near Orchard, Colo.

Wheat field day at the test plot at Yuma, Colo.
Wheat variety trial results are posted online soon after harvest. The Crops Testing Program summarized the results from the 2013 wheat variety trials and provided one, two, and three-year yield summary tables for both the dryland and irrigated trials. These tables were published on our website, in the 2013 Wheat Performance Ag Experiment Station Technical Report, and in a special edition of the High Plains Journal that has very wide circulation in Colorado. The top three varieties from the dryland 2011-13 three-year trial average were Byrd, a newer variety from CSU in 2011, Antero, a new release from CSU (2012), and Ripper, an older variety also released from CSU (2006).

Project funds received from the Colorado Wheat Administrative Committee are used for the UVPT and IVPT (vehicle costs, equipment parts and repairs, and fuel for visiting trial sites.)

**Collaborative On-Farm Tests (COFT)**

The objective of the 2012-13 COFT was to compare performance and adaptability of popular and newly released CSU varieties: Byrd (HRW and high yielding), Brawl CL Plus (HRW 2-gene Clearfield®), Denali (HRW marketed especially in Kansas), Hatcher (HRW planted on significant acreage in Colorado), Snowmass (HWW high quality), and Antero (HWW high yielding) under unbiased, field-scale testing conditions. In the fall of 2012, thirty-three eastern Colorado wheat producers planted COFT in 14 counties across eastern Colorado. Each collaborator planted the six varieties in side-by-side strips at the same seeding rate as they seeded their own wheat. Yield and test weight results from fifteen of the thirty-five total locations showed Byrd yielded significantly higher than four of the other five varieties, with Antero yielding the second-highest. Brawl CL Plus had the highest test weight of the six varieties.
2013 Weed Research Related to Wheat
Phil Westra, Weed Scientist

Kochia

There was a great increase in reports of glyphosate resistant kochia in 2013, often discovered in meandering streaks of kochia across fields where other weeds and most kochia were controlled by a labeled of glyphosate which was often applied in combination with dicamba and 2,4-D. Our weed science graduate students were able to get to 5 geographically distant sites (Denver, Sterling, Byers, Yuma, and Cheyenne Wells) to collect plant material and conduct molecular tests that showed that the resistant plants exhibited increased EPSPS gene copy number which resulted in overproduction of the EPSPS enzyme (which is normally bound up by glyphosate). Because of this large amount of extra enzyme, a labeled rate of glyphosate cannot tie up all the enzyme and the plant survives. Interestingly, at 1 site, we were able to apply a 6X rate of glyphosate (4.5 pounds or 1 gallon) over top of perhaps 50 resistant plants, and all except 3 were killed by this treatment. However, such high rates are not labeled, are impractical, and would likely select for plants with even higher levels of resistance.

We now have nearly 200 accessions of kochia collected in the falls of 2011 and 2012 from all eastern Colorado that we are testing for kochia response to multiple herbicides including glyphosate, dicamba, fluroxypyr, and other products.
**Feral rye response to Beyond herbicide**

Numerous feral rye collections were made across eastern Colorado in 2012. These were hand threshed at the CSU weed lab, and samples were planted in flats for a replicated study where Beyond herbicide was applied at 3, 6, and 12 ounces to all accessions.

Although most accessions were controlled by the 6 ounce rate, several of the feral rye accessions had plants that survived the 12 ounce treatment in the greenhouse. The survivors were damaged, but would likely set seed in the field and put additional resistant seeds into the soil. This research suggests that some cases of commercial failure to control feral rye with Beyond herbicide may not be due to poor herbicide application or adverse environmental conditions or feral rye growth state, but rather, may be due to tolerance to Beyond herbicide that has built up in some populations.

![Image of feral rye response to Beyond herbicide](image.png)

This image shows resistance of feral rye at the 3-, 6- and 12-ounce per acre rate. The highest labeled field rate is 6 ounces per acre. Each flat included the same 6 accessions, as each accession was planted every two rows.

**Mutagenesis of wheat to develop novel traits including herbicide resistance**

Additional research in this on-going project included the use of EMS (ethane methyl sulfonate) to mutagenize 2 new CSU generated elite winter wheat varieties and 1 spring wheat variety. The mutagenized seed was field planted, allowed to produce seed, and the M1 seed was then planted for use in field selection studies.

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this type of research is largely a numbers game, large numbers of seeds must be planted to produce large numbers of wheat plants that vary for many genetic traits due to the mutagenesis treatment. Research is ongoing to select for plants that survive treatment with herbicides that normally would kill wheat. Proof of concept research is being conducted with several advanced wheat mutants, and in some cases, we have the novel DNA sequence that appears to provide resistance. We have mutant winter wheat survivors to both Assure II and to Select herbicide. For the Assure II trait, we have 3 mutant lines, each of which carries two copies of the novel ACCase gene. Eventually we may try to get all 3 of the genomes carrying the novel resistance trait in a single line. Despite considerable research effort, it has been more difficult to develop and fully characterize Select mutants.

M1 seed of the two new elite varieties (Byrd and Antero) was planted in September 2013 on approximately 3.5 acres of land, and in the spring of 2014, these plants will be subjected to selection pressure with a field application of the appropriate chemicals. All of this mutagenesis novel herbicide resistant wheat trail research has been largely funded by CWAC and CWRF.

**Pyroxasulfone: a potential new herbicide for wheat**

Several companies are looking at a new soil applied herbicide for potential weed control in winter wheat. Dale Shaner and I had a graduate student conduct a MS thesis research project on this new, low use rate soil applied herbicide. Pyroxasulfone provides quite good control of downy brome and small seeded broadleaf weeds, but does not control jointed goatgrass or feral rye. In 2012-13, a field study was conducted with Byrd, Brawl CL, Hatcher, Above, Thunder CL, Bond, Yumar, Akron, and Ripper. These were planted in the fall of 2012 and within 2 days, the herbicide was soil applied to ½ of all the plots. A single rate of herbicide was used to evaluate winter wheat variety safety as well as weed control, with all combinations set up in a split plot design with ½ treated and ½ not treated. Control of spring germinated common lambsquarter, kochia, and wild buckwheat was excellent with no injury observed on any of the varieties. Currently, it is not likely that growers would invest in a fall, soil applied herbicide, but with the development of more herbicide resistance in weeds of wheat, such technology may be useful in the future.
Brown wheat mite traditionally has been a sporadic pest of wheat, with widespread damage limited to periods of drought. For unknown reasons, possibly due to changes in production practices, this pest has become more a more consistent and widespread problem over the past few years.

Research priorities include mass rearing, infestation methods, host range and resistance screening techniques.

We have taken two approaches to the problem of egg dormancy, which is a major barrier to mass rearing. The first is to manipulate the egg environment to get dormant eggs to hatch prematurely. Many insects and mites use photoperiod (relative length of day and night) as an environmental cue for life history events such as breaking dormancy. We have found that interrupting the night with several brief periods of light, in combination with relatively cooler temperatures and higher humidity, does hasten egg hatch by at least one month. The other approach is to prevent eggs from going dormant in the first place. We also are evaluating photoperiod as a means to accomplish this. Less progress has been made with the second approach. Much needs to be learned before we are able to efficiently mass rear brown wheat mite for plant resistance screening and other studies.

Field studies of brown wheat mite movement from crested and intermediate wheatgrasses to winter wheat support the idea that cool season grasses play an important role in brown wheat mite infestations. Such studies improve our knowledge of brown wheat mite field ecology, as well as our techniques for field infestations and damage evaluation, which will be necessary for screening for resistance once mass rearing methods have been developed. In addition, an observation from these studies has suggested a third approach to the egg dormancy problem. Samples from these grasses during the summer months occasionally contain a small number of brown wheat mites, which raises the question as to whether these mites have just lived longer into the summer or whether they represent a subpopulation that breeds actively year round. A colony will be established with mid-summer adults to determine which is the case.
A study examining treatment timing (fall or fall + spring), miticides, (dimethoate, Oberon and Onager) and varieties (Hatcher and Snowmass), was conducted. Treatment timing and variety did not affect the total number of mites observed, and, among the miticides, only dimethoate provided any control. Yield benefit from miticide use was not observed. A simplified version of this experiment, focused just on yield protection, has been planted. While dimethoate clearly is an effective brown wheat mite control, measuring the benefit of its use has proved quite difficult because of variable infestations. This is another example of our need to be able to efficiently mass produce and infest with brown wheat mites to produce uniform infestations.

**Russian wheat aphid**

We have found Russian wheat aphid on noncultivated grasses in montane environments for the past several growing season. We have biotyped 80 aphid clones collected from higher elevations as well as from three eastern Colorado locations. Most of the clones were closely related to the original Russian wheat aphid biotype (RWA1) or to the more virulent RWA2. Two clones, one from a higher elevation and one from Briggsdale, were clearly different and likely are new biotypes. We are trying to determine the origin of new biotypes, and it seems that both prairie and montane environments are important sources.

We completed an evaluation of the effectiveness of resistant varieties with the Dn7 source of resistance against Russian wheat aphid biotype RWA2. This source provides highly effective resistance against this and all other known biotypes. We have completed two years of a similar study with a new source of resistance in barley.

We continue to evaluate insecticides for Russian wheat aphid control. Lorsban Advanced (and other chlorpyrifos products) remains the most effective treatment. However, newer products such as Cobalt Advanced (chlorpyrifos + lambda-cyhalothrin) and Endigo ZC (thiamethoxam + lambda-cyhalothrin) are promising alternatives, if priced appropriately.

**Wheat stem sawfly**

The wheat stem sawfly, Cephus cinctus Norton, has long been considered a key pest of wheat in Montana, North Dakota and the Prairie Provinces. Since the beginning of the last century the sawfly has spread south out of Canada into northern Great Plains spring wheat. In the last three decades, it has become more abundant on winter wheat and has spread into southeastern Wyoming and the Nebraska Panhandle. It has been found damaging winter wheat along Highway 14 in Weld County, CO from 2010 to 2013. It is unknown how far into Colorado the problem will spread.

We surveyed 107 fields in eastern Colorado wheat producing counties in both 2012 and 2013 to help determine the distribution and rate of spread of the infestation. Results are given in Table 1. Results in 2013 from the
southeastern part of the state were limited because of the severe drought conditions. A total of 29 and 37% of the 107 sites sampled were infested with wheat stem sawfly adults and/or larvae in 2012 and 2013, respectively. Perhaps more importantly, the percentage sites where larval infestations were observed increased from 14% to 36% from 2012 to 2013. This information will be used to help the wheat breeding program prioritize this issue among the many important breeding objectives it is trying to meet.

We again evaluated chemical control of adults to prevent egg laying. As in 2012, significant adult mortality was observed, however, there was little reduction in damage. This could be due to poor timing or because the insecticide used was not fast acting enough to kill females prior to egg laying. We also monitored adult sawflies in 10 fields in the New Raymer area. The flight was more similar to 2011 than 2012. Temperature seems to influence adult emergence and we are interested to adapting a degree day model to help predict emergence and other stages in the sawfly life cycle. We also started testing a pheromone, observing that an inexpensive water trap was as effective as several more expensive commercially available pheromone traps. Having an effective pheromone could improve management decisions such as insecticide treatment timing and variety selection.

Table 1. Eastern Colorado wheat stem sawfly survey results: 2012-2013.

<table>
<thead>
<tr>
<th>County</th>
<th>Locations</th>
<th>% Infested locations¹ (Number of locations with larvae)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adams</td>
<td>7</td>
<td>14 (1)</td>
</tr>
<tr>
<td>Arapahoe</td>
<td>3</td>
<td>33 (1)</td>
</tr>
<tr>
<td>Baca</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Boulder</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cheyenne</td>
<td>8</td>
<td>25 (0)</td>
</tr>
<tr>
<td>Kiowa</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Kit Carson</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Larimer</td>
<td>3</td>
<td>33 (1)</td>
</tr>
<tr>
<td>Lincoln</td>
<td>6</td>
<td>33 (0)</td>
</tr>
<tr>
<td>Logan</td>
<td>6</td>
<td>83 (0)</td>
</tr>
<tr>
<td>Morgan</td>
<td>3</td>
<td>100 (2)</td>
</tr>
<tr>
<td>Phillips</td>
<td>6</td>
<td>12 (1)</td>
</tr>
<tr>
<td>Prowers</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Sedgwick</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Washington</td>
<td>13</td>
<td>69 (8)</td>
</tr>
<tr>
<td>Weld</td>
<td>7</td>
<td>71 (0)</td>
</tr>
<tr>
<td>Yuma</td>
<td>5</td>
<td>20 (1)</td>
</tr>
</tbody>
</table>

¹Adults and/or larvae

Wheat stem sawfly larva in wheat stem.
2013 was a challenging year for Agronomy Foundation Seed (AFS). The fall of 2012 was extremely dry and there was little snowfall or rain during the winter. A couple of spring storms brought some welcomed moisture into our region that benefited the wheat crop immensely. Even with the weather struggles that we experienced we still had a very successful sales year. With an increasing demand for its Foundation seed, AFS decided that it should also contract additional Foundation seed production as an assurance that an adequate supply of seed would be available for the Colorado Wheat Research Foundation (CWRF) grower participants. This approach to ensure an adequate pure Foundation seed supply worked very well. It proved valuable as there was adequate supply of most varieties with the exception of Hatcher.

As I reported last year, managing Foundation seed supplies has always been a challenge, either in producing too much seed or failing to have enough seed to meet the demand. Many certified seed growers frequently experience this same production issue. The Foundation Seed Committee at Colorado State University (CSU) that oversees AFS policies addressed this issue in 2013 with the implementation of the Year in Advance (YIA) Contract as an option for seed growers. The YIA allows a seed grower to order his seed a year in advance with a deposit. This planning tool allows AFS and seed growers a mechanism to plan for future seed requirements. We have promoted the YIA contract option throughout the year and have found it to be embraced by many seed growers. YIA contract agreements are helping us to effectively manage the AFS seed production.

The growth in demand for Foundation seed of CWRF varies is phenomenal. CWRF has created a program that has successfully marketed the newest varieties from CSU in-state as well as to several neighboring states. This increased demand for seed has been instrumental in leading AFS to make some efficiency changes. This year was the first year that two combines were used at harvest time. The additional machine sped-up the process immensely when it was not raining. The second machine not only saved time, but it made a more efficient use of labor. There is a plan to make improvements inside the seed plant and to add two more hopper bins for seed storage in 2014. These improvements once completed will improve operations.

In addition to his regular duties in 2013, Aaron Brown assists the wheat breeding program with needed repairs and maintenance of planting and harvesting equipment. He also assisted with the University greenhouse with the wheat crossing block last spring. This cooperative arrangement with the wheat breeding program over time has benefited the program. On behalf of AFS, I would like to extend our sincere appreciation to all those who support the AFS program at CSU.