OPERATIONS MANUAL FOR DR. KLATT'S

BREEDING PROGRAM:

(OH NO, SARAH'S GONE TO KANSAS)

By

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Submitted to the Students of the
Wheat Improvement Team of
Oklahoma State University

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Preface

If you're reading this, a few things have happened. One, I'm gone. I've either finally graduated or kicked the can trying. Two, you're working for Dr. Klatt, congratulations, enjoy it! And three, you're new, stuck, confused, or bored out of your mind. All of these I will try to remedy through this solution manual.

Before this lovely manual begins, here's a little information about me. I come from a farming family in Hennessey, OK. We have a cow/calf operation with winter forage provided by wheat which was really my inspiration to start working with wheat. When I first came to college my father told me to study anything but agriculture, which I did for a couple of years, but was not completely happy. After searching around campus for a while I came to the OSU Plant and Soil Sciences Department and found a great interest in wheat breeding. Thus, I began working for Dr. Klatt in January, 2008. Now, here we are, fall semester of 2011 and I hope to impart with you some of what I have learned about wheat breeding and Dr. Klatt's program.

This manual is dedicated to Dr. Klatt and the "Whamming Wombats" of 2008 to 2011.

Dr. Klatt has been a tremendous pleasure to work with these last three years. I would like to take this time to thank him for all the time he has taken teaching me about wheat breeding, agronomy, organization, prioritization, human resource management, and life. Dr. Klatt has been a true inspiration to me personally and professionally, and he has challenged me to be better at everything I do. Thanks Dr. Klatt!

I have spent countless hours working alongside Dr. Klatt's student workers. We have developed a great community, and I am very proud of my co-workers. I have learned lots from each of you, and wish you all the best personal success.

Graduate students:	*Undergraduate students:*
Dr. Safeer Hassan	Michael Reinert
Dr. Mario Rodriguez-Gutierrez	Jessica Squires
Zhiyong "Cody" Wang, MS	Hollie Delmedico
Berhanu Andarge, MS student	Clay Hattey
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	Jared Crain
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CHAPTER I

INTRODUCTION

History of wheat breeding

Plant breeding and improved agronomic practices have resulted in significant yield increases in wheat (*Triticum aestivum* L.) over time. Wheat evolved as a cross between three separate grass species at least 10,000 years ago. Since that time humans have helped in the evolutionary process by domesticating wheat plants and harvesting types that could be replanted. Modern breeding efforts began in wheat during the early 1900s using the early knowledge of modern genetics and selecting for advanced agronomic and culinary properties.

The first breeding efforts specific to the Great Plains of the USA began in the 1920s. At that time wheat in the area was mostly an introduced land race from Russia known as “Turkey” or “Turkey Red” and multiple selections made from this landrace. These varieties were tall, very susceptible to lodging with fertilizer input, and not very responsive to improved agronomic treatment. Crossing began and the first purposefully bred varieties in the Great Plains region were released in the late 1940s. In the 1960s, Dr. Norman Borlaug incorporated semi-dwarf genes into Mexican spring wheat cultivars producing shorter, higher yielding wheat cultivars, which provided significant increases in global wheat yield upon incorporation to local germplasm. The semi-dwarf characteristic was incorporated into the Great Plains wheat cultivars by the 1970s. Since that time, incorporation of diverse germplasm with varying genes for pest and disease resistance, as well as agronomic type, has been useful for making yield gains. Additionally, in more recent years breeders have had access to advanced genomic technologies to enhance their selection of modern wheat cultivars.

Throughout the past century there has also been significant progress in agronomic treatment of wheat. Fertilization methods, as well as pesticide, herbicide and fungicide treatments have been extensively studied and have given rise to higher yields. Traditionally, genetic improvements have been responsible for approximately half of the yield increases over the past century. Also, over the last few years there has been a significant increase in investment in wheat breeding from the private sector (Monsanto = WestBred, Syngenta = AgriPro, LimaGrains, Bayer CropScience, etc.). These investments have been made with the intent of possibly releasing genetically modified (GMO) or hybrid wheat within the next 10 to 20 years.

Overview of Dr. Klatt's program

At Oklahoma State University a Wheat Improvement Team was formed to give a multi-discipline approach to wheat breeding for Oklahoma. Dr. Carver leads the team as the main varietal release breeder for winter wheat in Oklahoma, however there are many specialists that assist in this process: Dr. Yan, wheat molecular genetics, Dr. Edwards, wheat extension and variety testing, Dr. Hunger, wheat pathology, Dr. Royer, entomology and Hessian fly resistance, Dr. Giles, entomology and integrated pest management, Dr. Rayas-Duartes, end user quality, and Dr. Klatt, germplasm development. Thus, Dr. Klatt's role in this system is mainly to import global material and make crosses to increase genetic diversity in the Oklahoma wheat breeding program.

As a germplasm development breeder, Dr. Klatt imports germplasm from around the world, but mainly from CIMMYT's (International Center for Wheat and Maize Improvement) programs in Mexico and Turkey. CIMMYT has a global germplasm network in which material can be exchanged fairly freely as long as participants return data to CIMMYT so they can increase their knowledge of the germplasm. Dr. Klatt normally takes a trip to CIMMYT, Mexico annually to identify potential sources of germplasm among other cooperative projects. CIMMYT ships the materials to us, which must be treated with our quarantine regimen before being tested and incorporated into our breeding program via the crossing block.

After potential germplasm passes the greenhouse quarantine procedure, it may be field tested the next year for agronomic characteristics. Field observation screening is typically done in Castroville, TX and Stillwater, OK. If a line is agronomically favorable, has good disease resistance, and looks as though it will yield well or has a documented history of high yield it can be included in a crossing block the following year.

The crossing blocks are materials we have identified as superior and acceptable parents for crosses. The crossing blocks (CB) are normally separated into these groups: winter wheat (WWCB), spring wheat (SpCB), winter synthetics (WWSYNCB), and spring synthetics (SYNCB).   Additionally, spring by winter F1's will be in the greenhouse for crossing. All crossing will be conducted in the greenhouse between February and May, which will be a busy time.

Crosses are made with a few objectives in mind. Our desired product is a cross that will have mainly winter growth habit, good disease resistance, favorable appearance, and high yield. These can be tested over the next several years, but basic crossing procedures are conducted to narrow our work by disposing of anything that will obviously not yield a favorable cross. Cross notation is made using Mendel's notation of filial generations, where the first filial generation is the F1, second filial generation is the F2, and so forth.

Spring growth habit is dominant to winter, thus most crosses have two winter parents in their pedigree in order to achieve an acceptable level of winter hardiness. Crosses to springs must be conducted as spring X winter → F1S (single cross F1), which will be spring type. Since the F1 is dominantly spring it must then be crossed again the next year, F1S X winter → F1T (top cross F1). This crossing mechanism is also used for the synthetic types, both winter and spring, because synthetic parents give such diverse and wild progeny. Lately, however, the bulk of our crossing has switched from F1T production, using synthetic or spring parents with two winter parents, to F1S production in which we cross one of our own advanced lines (originally from an F1T cross) by a locally adapted winter parent. When you are emasculating or pollinating in the spring you will be given lists of crosses to make, so you will not need to worry about this scheme on a day-to-day basis, but this is the reason that we do not make spring X spring or spring X synthetic crosses.

The F1T and F1S (winter by winter only) will be transplanted into the field in late fall. F1 crosses are transplanted and treated with great care because we do not have reserve seed from many of the crosses. The F1S populations will appear homozygous (identical) from plant to plant, since no segregation has occurred yet. In the F1T populations, segregation will have begun due to segregation caused from the F1S the year prior. Regardless of segregation or not, all populations will be harvested in bulk and either kept for F2 or discarded.

If the F1 population is kept (either F1S or F1T), it will be harvested in bulk and planted as an F2 in Castroville, TX. Beginning at the F2 stage and continuing until the F5 stage, we employ a modified-bulk breeding strategy, in which some whole populations are discarded, but mainly the best looking segregates (plants) are selected within individual populations and then bulked to make the next generation. Blue painter's tape is used to mark the selected plants and these plants are then bulked to make the next generation "modified" population. F2's are planted in TX only, F3's are planted in OK only, F4's and F5's are planted in both OK and TX.

Progeny from the F3 and F4 generations are selected the same way as in the F2 generation. The F5 generation, however, begins a different strategy in selection. Within F5 populations, selections are made the same way as in previous generations; except only one head is selected from each superior plant and care is taken to keep the head intact. This seed will then be threshed one head at a time and deposited into trays where individual head rows can be planted separately, thus creating the F6 head row nursery. Selected heads are planted as a family, always preceded by four check varieties.

F6 head rows are planted on the agronomy farm and are again treated with great care because we again have no reserve seed. From this group only the best head rows will be selected from the best populations on the basis of maturity, disease resistance, agronomic type, and seed quality. The whole row is harvested with a sickle, which will be threshed in bulk and planted in a preliminary yield trial the following year, at one or two locations (one rep per location).

First year yield trials are referred to as F7 PYT (preliminary yield trial). Following the initial yield trial, only the top 10-30% in yield and agronomic type will be continued into subsequent replicated yield trials. This reduces breeding populations down to a manageable number, in which one or two from any given year could eventually be candidates for release.

CHAPTER II

GREENHOUSE OPERATIONS

Greenhouse agronomy

Greenhouses are used in Dr. Klatt's program to protect and multiply seed, and provide a location for crossing. As a seed multiplication facility, less than 12 and up to 24 seeds may be planted for each line, but we hope to harvest at least 50 grams of seed from each pot and 100 grams of seed from each bed planted entry. Because of these high hopes, greenhouse agronomy must be practically flawless from the persepective of soil preparation in beds, pots, and flats, planting, vernalization of winter types, watering, fertilizing, pest management, and temperature control.

Seed bed preparation and transplanting

Two round-top greenhouses contain available in-ground seed beds. Each year these beds produce weeds in the summer. The weeds must be killed by herbicide and removed or hand weeded from the greenhouse in late summer. After the first round of weeding beds must be watered to allow for more weed and volunteer wheat germination and weeded again a few times. Next the beds must be tilled, watered and weeded again a couple of times to insure that all residual volunteer weeds will have germinated before transplanting. Finally, beds will be well watered and tilled up to where they are soft so we can transplant into the beds in late November or early December. When it is time to transplant, each bed will be split in half parallel to the walk ways and will have lines drawn eight inches apart from each other, perpendicular to the walk ways. The perpendicular lines are where materials will be transplanted into a row.

The spring X winter F1S and winter wheat crossing block are transplanted in the easily accessible areas of the round-tops. Synthetic crossing blocks and other pollen-only material are transplanted around the walls of the round-tops, which cannot be easily reached for emasculation.

Soil for pots and flats

Soil used for greenhouse planting must be sterile media. We do not want an introduction of a wild seed bank or soil microbes to influence our production. Thus, we use Redi-Earth and Metro Mix as our potting media.

Soil for flats is simply Redi-Earth. This soil is very porous, however, so care must be taken to insure flats are actually full. Flats are filled completely full with Redi-Earth, compacted with hands or tools, and filled again with Redi-Earth. Flats are then lightly watered a couple of times before planting to make planting easier.

Pots are filled with a one-to-one mix of Redi-Earth and Metro Mix. Wheelbarrows are typically used to mix the soil, each with one half bag at a time. Pots are stuffed with soil to the lip of the pot. Pots are then stacked and wait for transplantation.

Planting and transplanting

All greenhouse seeds are planted into flats then transplanted into beds, pots, or in the case of the F1T's, into the field. By first growing the seedlings in flats, we protect seedlings from many early diseases and harsh environments. Remember, we are multiplying seed in the early stages, so we only begin with a few seeds or a few grams of seed of each material. Additionally, we control the plant population size in pots or beds through transplanting.

Flats are actually comprised of many parts and terminology is listed here to keep everyone speaking the same language. An example flat is displayed below so you can imagine how they are arranged. Every flat is comprised of a Redi-Earth filled insert within a plastic tray for rigidity. Each insert has 12 sixlets. Each sixlet contains 6 cells (Figure 1). Two holes will be placed in each cell for seed. One seed is planted per hole, provided there are enough seed available.

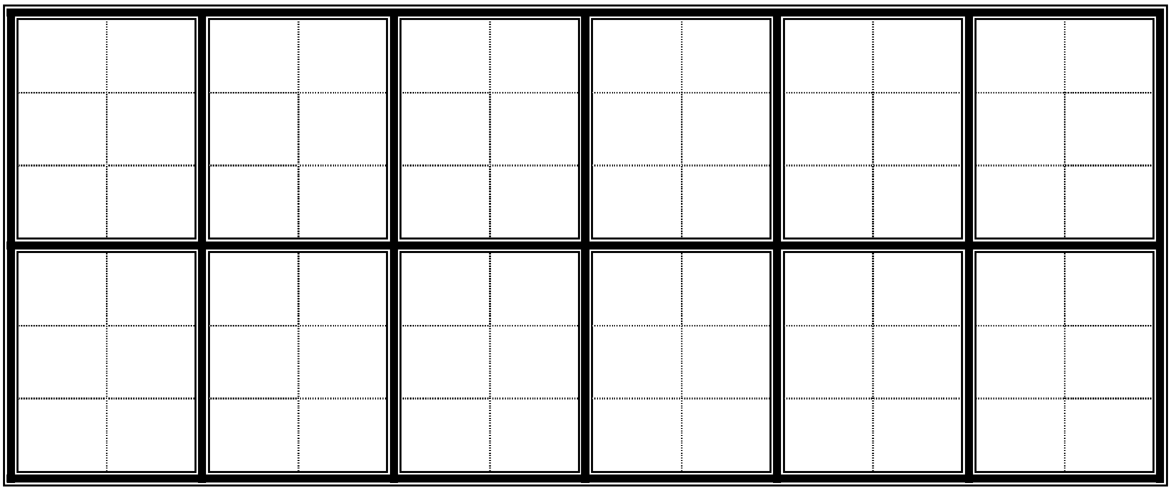


Figure 1: Arial view of flats displaying individual cells and sixlets.

Flats are normally planted when soil is moist to wet. Moist planting conditions are preferred because holes must be poked with pen, pencil, or bamboo stick ends to provide a hole for each seed. Holes are poked approximately half way down each cell. Care must be taken to make sure only one seed falls per hole, and that seed does not get planted into the wrong cell on accident. The method that I usually used for planting was to poke the holes in all sixlets first, plant one entry of seeds, cover the holes, then place the entry stake labeling that sixlet, and repeat for all entries in each flat. After all entries have been planted per flat, some additional soil should be placed on top to fill the remaining cells after they have been compacted from closure. Make sure every entry is labeled correctly!

When transplanting into pots, pick the healthiest four cells (from each sixlet or of each entry) to transplant. These four cells of the seedlings will be transplanted in a radial, evenly spaced pattern around the pot. Be sure that the cell is planted deep enough that no mounds are made from the transplant and original cell soil is completely covered. Water pots thoroughly twice after transplanting to make sure water has soaked through the pot profile.

Cells are transplanted into beds in late November or early December after winter lines are vernalized and beds are prepared for transplant. Beds are split in half lengthwise for two rows of plants to be transplanted into each bed. Lines are drawn every eight inches apart all the way across the bed to transplant into and have entries evenly spaced across the bed (Figure 2). Plants should then be transplanted on these lines with the plastic stake from the flat and a new orange stake which will identify the line more permanently. When transplanting, please remember to plant the cell deep enough that all soil from the flat is underground and do not make mounds in the bed.

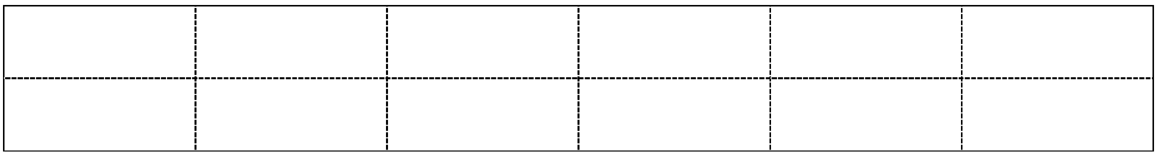


Figure 2: Arial view of beds split in half with rows on either side of the half and entries seeded eight inches apart.

Winter wheat vernalization

Winter wheat must be vernalized, or exposed to low temperatures for a certain amount of time before they can advance from vegetative to reproductive growth. Since we want to keep greenhouse temperatures regulated, it is best to put the winter materials outside in flats to vernalize. These flats are left outside from the time plants are 3-4-leaf seedlings for the next 6-8 weeks, typically mid-October to early December. During this time flats are very susceptible to drying out, so they must be watered daily or more if winds and/or temperatures are high. Because of this heavy watering protocol, nutrient deficiencies may occur more often than in greenhouses. Most yellowing or pale plants can be remedied by using Peter solution ("blue stuff"). However, iron deficiencies can be found more prevalently because of the cold temperatures. Use an iron sulfate solution if pale leaves with interveinal chlorosis ("the winter crud") are found in vernalizing plants.

Watering

Watering is very important to successful greenhouse production. In greenhouse situations available water is limited to only the water that you provide and what can be stored in the flats, pots, or beds. Additionally, potting soil used for flats and pots is very porous and does not retain much water. Therefore, water stress can occur very quickly in the greenhouse, especially in warm spring conditions.

The best way to ensure proper greenhouse watering is through scheduling. Calendars should be placed on all greenhouse doors and initialed when plants are watered. Fertilizer applications and pest management treatments can be included on the calendar as well.

When seedlings are in flats they need to be watered at least once a day because of the small soil available. Seedlings that are being vernalized outside should be checked more than once per day on very windy or very hot days to ensure they do not dry out. Plants that are in pots should be watered every other day until flowering begins, and then watered every day from flowering to maturation. Plants in beds can usually go one week without watering, but should be checked regularly.

Water should be applied to the soil surface only! Please do not water the leaves of the plant unless you are applying foliar fungicide. Watering the leaves promotes fungal disease, which can quickly become epidemic in the greenhouses. Care should be taken to not fill flats or pots past the top of their container and pressure should be low enough that soil does not shoot out of the container when watering. This type of overwatering results in soil and nutrient losses for the plant, which promote nitrification and algae growth on tables.

Fertilization

Fertilizer is needed multiple times through the greenhouse growing season. Flats and pots limit the rooting area of plants, so that roots cannot mine into rich soil profiles for nutrients. Also, the soil media used is watered so often that minerals that are available leach quickly. Finally, greenhouse situations are designed to significantly increase seed, so inputs are maximized to get maximum yields from individual plants.

Greenhouse fertilizer can be applied either in granular form or through watering with foliar Peter solution ("the blue stuff"). Granular fertilizer is used for supplying N, P, and K to soil either pre-plant or during the season. Foliar fertilizer is used for supplying a more holistic fertilizer with micronutrients as well to young plants, or plants displaying micronutrient deficiencies throughout the season.

Granular fertilizer is applied before planting in the beds to start the season with required N, P, and K. Regular soil testing of beds should occur with optimized fertilization for deficiencies. Granular fertilizer is also applied in regular intervals throughout the growing season to supply N, P, and K to the plants in pots and beds. In pots, granular fertilizer should be broadcast by hand with approximately 20 granules per pot. After granular application, plants should be lightly watered to help incorporate fertilizer into the soil solution. Care should be taken to not water over the lip of the pot where granules may immediately wash out of the pot.

The Peter solution is sprayed through a water hose nozzle with a container for the fertilizer. This solution contains many chelated, or plant available, micronutrients as well as N, P, and K. If plants are displaying odd discolorations, please look to see if this may be a micronutrient deficiency as pots and flats do not retain as many micronutrients. Vernalizing plants are especially susceptible to micronutrient deficiencies with large changes in soil temperature and pH. If a micronutrient deficiency is detected, foliar applications of that specific nutrient may be needed in which case you should contact soil fertility.

Pest management

Common greenhouse insects are greenbugs, Russian wheat aphids, and bird cherry oat aphids. These Hemipterans are very aggressive pests in the greenhouse and multiply very rapidly. These insects reproduce asexually, so the amount of time from first sightings to heavy infection is very rapid. Be on the lookout for insect pests on the stems of wheat plants regularly. These must be killed by insecticides as quickly as possible.

The most prevalent disease that is found in the greenhouse is powdery mildew. Powdery mildew should be controlled because we are attempting to maximize yields and do not want to lose any yield to disease. Powdery mildew also spreads very quickly through the greenhouses after initial infection. The best method of avoiding powdery mildew is to water carefully. However, if infection occurs chemical fungicides are available for usage.

Most greenhouse weeds are found in the beds over the summer. To control these weeds multiple attempts should be made to kill them and regerminate the seeds in the bed before transplanting. In greenhouses with tables, few weeds may emerge through the tables or ground which should be pulled whenever noticed. Weeds can be hoed, or several chemical herbicides are available for greenhouse usage.

Resistant pests come from overuse of single active ingredients or modes of action. Thus, alternating active ingredient and mode of action is vital in greenhouse pest management. Also, it is easier to control pests when there are not many of them to deal with, so be vigilant in scouting the greenhouses for pests and do your best to control any problems as quickly as they arise.

Safety is paramount in dealing with pesticides in the greenhouse. Not all chemicals are labeled as safe for use within greenhouse settings, so be sure to investigate the product before application. Take care to know what you are spraying, the dangers of what you are spraying, how you should protect yourself while spraying, and how many hours the greenhouse should be quarantined before re-entry. Please post a note on doors where pesticides have been sprayed indicating at what time a specific chemical has been sprayed and the earliest time of re-entry. Also, note the date of spray on the greenhouse door calendar whenever you spray.

Greenhouse temperatures

Greenhouse temperatures moderate growth rate of their plants. Since we want plants to mature at roughly the same time, but in a specific order for crossing, maturation must be controlled by temperature.

As stated above, greenhouse temperatures and water need go hand-in-hand. When greenhouses are warmer, take care to water more often.

Quarantine procedures

Wheat that is acquired from international sources is often subject to quarantine by the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA). According to APHIS, import permits must be provided for all seed that we receive internationally with seed and quarantine procedures subject to inspection. Common quarantines that we work with are materials from Mexico that are be quarantined for karnal bunt (*Tilletia indica*) and seed from Turkey that is quarantined for flag smut (*Urocystis tritici*).

Our quarantine procedures include growing the materials in the greenhouse for one year to inspect for signs or symptoms of the disease in the plants. Seeds must be treated by the sending agency with a seed fungicide, so take caution in opening and dealing with international seeds as they should contain chemicals. Seeds must be inspected for any signs and symptoms of the disease and can then be planted into sterile flats. Seedlings from flats can then be transferred to sterile pots only! Quarantine seedlings and soil containing the seedlings or plants cannot be used in further agricultural production in case of overlooked disease. After harvest, soil must be properly disposed, seeds must be inspected for further signs or symptoms of disease, and pots and flats to be reused must be washed in a strong bleach solution. Several pots of flats can be bleached at one time by mixing 3-4 gallons of bleach in a large trash can full of water and left to soak for 3-4 hours or even overnight.

Crossing

Wheat is a self-pollinated crop with closed flowers. Wheat flowers contain both male and female parts. Wheat flowers must be opened and manually pollinated by another line to create a cross. This process is tedious, and not everyone is well suited for this job, but with practice comes efficiency and speed!

In order to cross wheat, one must have a familiarity with wheat anatomy, which can be seen below (Figures 3 and 4). Each spike of wheat contains several spikelets (6-20). Within each spikelet are three to eight florets.

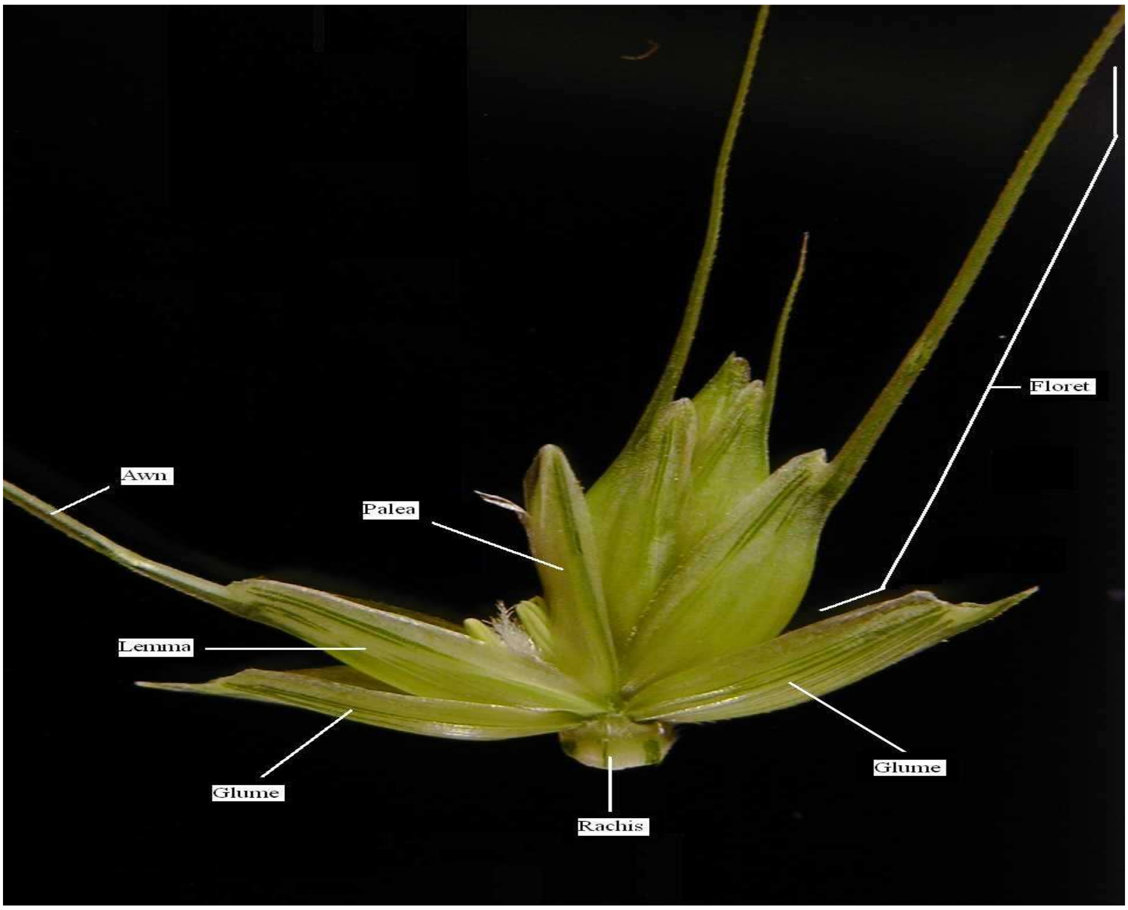


Figure 3: Wheat anatomy (Illinois State University, http://www.castonline.ilstu.edu/ksmick/150/150mflower/150whspik.JPG).

Emasculation

Emasculation is the process of removing all male parts from the flower to leave behind only female reproductive organs. When an individual floret is opened, the reproductive structures become visible to the naked eye. In immature florets, one white ovary and three green anthers are clearly visible. At this point, wheat plants are safe to emasculate without pollen contamination. If anthers are yellow, the pollen is viable, and it is not safe to emasculate this spike without self-pollination (Figure 4). Emasculation steps are described below.

Figure 4: Wheat reproductive organs (Illinois State University, http://www.castonline.ilstu.edu/ksmick/150/150mflower/150whfl.jpg)

1. Select a head to emasculate. The best heads to emasculate are still in or barely emerged from the boot.
2. To make emasculation easier top and bottom spikelets are removed from the

spike.

3. Middle florets are removed from each spikelet with tweezers leaving only the glumes and two outside florets.
4. Once all middle florets are removed, cut open the flowers with scissors through the fattest part of the glume and lemma (it is better to make this cut lower than higher).
5. When the florets are cut open ovaries and anthers are visible, check to be sure these anthers are green and not yellow. If they are yellow, remove the head and select another wheat spike that is less mature.
6. Remove the three anthers from each floret. Leave the ovary and stigma intact.
7. Repeat steps 5 and 6 for all spikelets on the spike. It should look like this when complete (Figure 5).
8. When all spikelets are emasculated, check each spikelet carefully to be sure the entire spike is free of anthers. If an anther is found during pollination, the spike will be thrown away.
9. Write your initials and the date on a glassine bag and cover spike. Close bag with paperclip being careful to not cover any plant parts with paperclip or snap peduncle of plant.
10. Write emasculation on list to make sure it gets pollinated later.

Figure 5: Emasculated wheat plant ready for pollination (Tyler Van Arsdale).

Pollination

Wheat flowers are ready to be pollinated anywhere from three to five days after emasculation depending on the temperature of the greenhouse and availability of pollen. Pollinations are done by collecting pollen donor spikes around 10-11 a.m., since this is the time that wheat pollen is most active in nature. Spikes that are ready to be pollen donors can be identified by finding spikes with a few yellow anthers extruding from the spikelet, preferably in the middle of the spike. These are then positively identified and cut approximately eight inches from the spike. They are marked with a tag indicating the row number from which they originated and placed in a cup of water.

Once the day's pollen is collected, pollen donor plants are assigned to previously emasculated plants by pairing optimal characteristics from the information listed about the history of the lines in the field books. Once the pollen is assigned, a tag will be made with the female parent on top and the male parent listed below. This tag will be wrapped around the heads to be used for pollen in order to keep a record of the cross that will be made, and the pollen heads are returned to the water until pollination.

Physical pollination is conducted by inducing anthers to shed pollen. For this task florets of pollen donors are cut just above the widest part of the glume on every spikelet and the peduncle is placed in dry sand. The spikes in the sand can be placed in direct sun or the heat can be increased in the greenhouse to further increase rate of pollen shed. Once anthers are fully exuded (Figure 6), the glassine bag of the emasculated plant is opened (cut top off) and the pollen spike is carefully picked up and twirled inside the glassine bag. Yellow pollen should readily fall into the glassine bag while twirling. Once pollination is complete, close the glassine bag with a paperclip and attach the cross identifying tag to the peduncle (stem) of the emasculated spike. Be careful that the paperclip is not put across the peduncle. The string of the cross identification tag is wrapped around the peduncle and the tag is attached to the clip at the base of the glassing bag.

Figure 6: Wheat plant ready to be used as pollen (Tyler Van Arsdale)

Harvesting

Crosses may be harvested 45 days after the date on the emasculation bag, or when the rest of the plant has dried. When harvesting crosses, double check that the female listed on the tag is actually the plant from which the spike originates. Once the plant is double checked, cut the stem 6-8 inches from the bottom of the glassine bag and bundle it in a rubber band with all the other emasculations from that female.

Let the crossed plants dry down for a few days before threshing them by hand. When crosses are threshed, count the seeds and write that number on the bottom, right corner of the envelope. Staple the cross identification tag to the top of the envelope without stapling it shut. Put the seeds of the cross into the envelope and paperclip the envelope closed.

Plants are ready to be harvested when almost all of the wheat plants are golden and dried, as in Figure 7. In potted plants remove the bamboo sick and center ring supporting the plant and place heads in sack labeled for that entry. Discard remaining biomass. Pile bamboo sticks and rings so that they can be returned to their places for summer storage. For bedded plants cut mesh netting holding plants up and pull the plants out from the roots (note: water a few days in advance of harvest to loosen soil around base of plants). Place whole plants in trash while still holding on to heads of wheat. Cut off spikes and leave remainder of plants in trashcan for disposal. Place spikes in bags labeled for their entry.

Figure 7: Wheat plant ready to be harvested (Tyler Van Arsdale).

CHAPTER III

FIELD WORK

Planting

Planting breeding populations is mechanized, but still requires human labor and careful attention. Two people are required for planting operations, and an extra person can assist. One person drives the tractor. The tractor operator must drive straight and within or beyond their last tire track to ensure proper row spacing and take notes on how the field is planted to make a field map. One person operates the planter. Their job is detailed below. A third person can prepare trays or packets for the people on the tractor to keep the operations moving as quickly as possible.

For both planters the plot length must first be set with the zero max. The zero max controls plot length so that plots are consistently the same length as in the beginning of planting. The zero max on both planters are very old and distances are relative, so actual distance must be checked before planting every time. Checking distance can be done by turning the wheel that turns the zero max and cone. A spot is marked on the wheel and rotations of the wheel are measured and compared to revolutions of the cone. Once plot distance is set and machinery is working properly, planting may commence.

Cone planter

A cone planter is used for segregating populations and yield trials. For this type of planter, seed is dumped into the cone and a lever is pulled to release the seed into the spinner. The spinner is connected to the zero max which dictates plot length. Once seed is out of the cone and into the spinner another packet can be dumped into the cone. After seed has been out of the spinner for a few seconds, equating to a couple feet, the lever can be pulled releasing the next set of seed into the spinner.

Cone planting is made easier if paperclips are removed from envelopes immediately before planting. Also, arranging envelopes in the most ergonomically feasible manner to the planter allows for quicker planting speed. While planting it is best to get a rhythm or count for dumping the packets and pulling the lever. If you keep a mental rhythm, the plots will likely be a very similar length, and you will know how long it has been since your last plot if you do happen to not pull the lever when it is time.

At the end of each row the tractor driver will say, “Last one!” At this point you should finish the packet that is currently in the spinner of the planter. Once the seed is finished in the spinner, the planter replies, “Ok!” At this point, the planter should hold on to something. The driver will lift the planter out of the ground and turn back the way they came. The driver will align their large tire track in its last pass to keep the alley spacing the same throughout the field. The planter will tell the driver what number they finished, and what number will begin the next row. This information is noted on the field map. The planter also needs to inform the driver if there were any mistakes or quirks in the previous pass at this time. Border rows of check varieties are planted around the whole field after planting of breeding materials is completed.

Head row tray planter

Head row trays are used for head rows, obviously. Head rows are grown for selecting advanced lines (F6) or for purifying populations (F8 head rows), or for taking observations of larger nurseries, like field observations of crossing blocks. F6 and F8 head rows are made during whamming (individual head threshing). Observation head rows are made later, which may lead to greater accuracy in the observation head rows than whamming head rows, thus it is very important to check the accuracy of tray numbers during planting.

While planting head rows, the planters must make sure the tray is loaded correctly and that the planter runs without mixing seed. To load the planter place the tray metal side down with the label tape nearest to the planting end. Roll the planting wheel slightly while pushing the tray to lock tray into place but not push out any seed. The weighted place-holder is then lowered onto the tray and locked into place so that the tray slides in a straight manner through the planter. Once the tractor starts to go and the planter wheel spins, the planter wheel will spin and pull the head row tray across planting four separate plots and leaving a space that is predetermined by the zero max setting.

When the tray ends the planter should leave a small space of a couple feet and yell, “Tray!” to the driver. The driver will then stop the tractor and the planter will have time to take off the old tray and situate the new tray. The planter then yells, “Ready!” and the tractor driver begins moving again. At the end of the row, the driver will simply hydraulically lift the planter and get aligned for the next row. The planter must tell the driver what tray and row number they ended on and which will begin the next row. This information is entered into the field map.

Along the way there may be problems with the head row planter. Some tray lids are bowed or cracked seed gets between the tray and the lid which causes the lid to raise and seed to mix on the tray. If this happens the planter should yell, “Stop, stop!” to the driver until the driver stops. At this point the driver and planter should try to rectify the problem by flipping the tray over to stop mixes and exchange the lid for another one that has previously worked. The driver should note any potential mixes in the field plan. The planter should keep the lid separate and note it as bad, making sure it does not return with the other trays for storage.

Border trays should be planted around the field. These are simply trays filled with check varieties and they should be noted on the map.

Field observations and notes

Field notes are commonly observed by one researcher and dictated to another researcher to write them in the field book. A general set of notes with few exceptions are used for all plots. These notes must be kept consistent for ease in understanding and making inferences from the notes later. Please use your best handwriting when taking field notes so anyone may be able to read them later!

Modified Cobb Scale for leaf rust

All adult leaf rust and stripe rust readings are taken using the Modified Cobb Scale, which allows a reading for type of reaction and percentage of the leaf impacted by the fungus. This rating scale is preferred because it gives us a good idea of intermediate reactions, which may indicate coming race changes, or presence of multiple-gene resistance.

Rust readings should be taken in the presence of a high level of infection. Heavy leaf rust infections, and sometimes stripe rust, are found mainly in our Castroville (LS), TX location. This area receives optimal moisture, temperatures, and relative humidity for heavy rust infection. We further increase the available inoculum by planting spreader rows of susceptible cultivars as the border for each trial to increase disease pressure within the trial. Additionally, the susceptible border rows show us what the most susceptible cultivar will look like under the current disease pressure, and readings can be altered to this value if necessary.

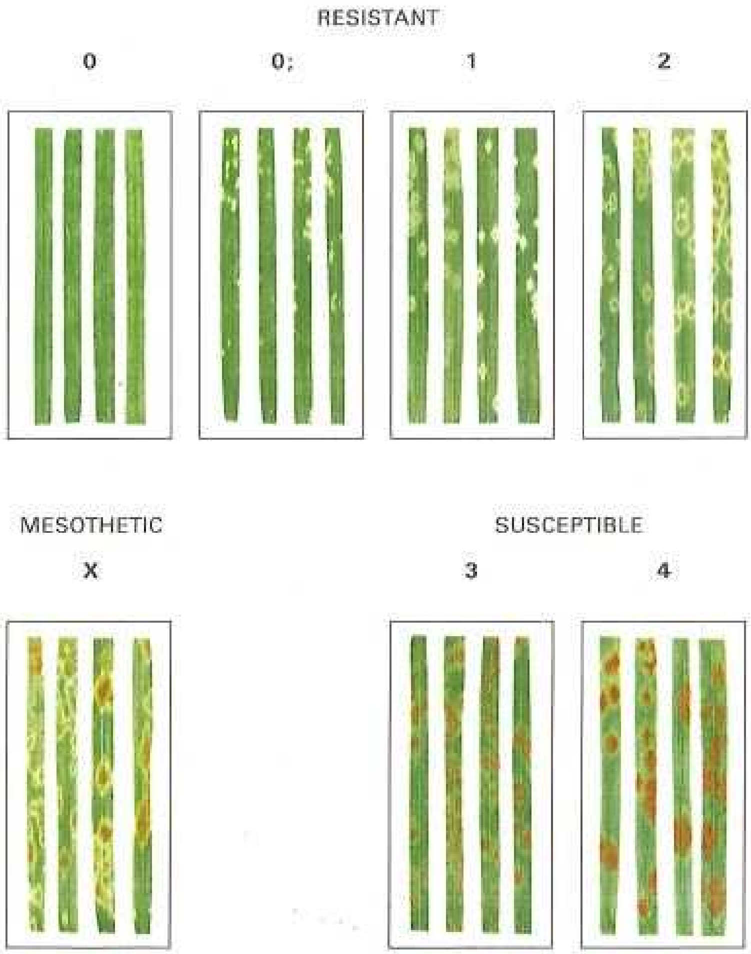
Pustule type reactions are classified as either R for ‘resistant’, MR for ‘moderately resistant’, MS for ‘moderately susceptible, or S for ‘susceptible’. Reactions are classified as resistant when leaves have small chlorotic flecks and no pustules. Moderately resistant reactions are indicated by very small pustules surrounded by a chlorotic ring. Moderately susceptible reactions are indicated by larger pustules with some chlorosis or a cholorotic ring surrounding the pustule. Susceptible reactions are categorized as those in which the pustule is large and with no chlorosis surrounding the pustule. Additionally, plants can receive the notation of FTD, or 'flecked to death', which means the leaves have many chlorotic spots that heavily impair leaf function, but little or no pustules.

A severity percentage is included with each pustule type reaction. Severity percentages, of course, are read from 0 to 100 % of the leaf area impacted by the reaction type. Thus, a number (severity percentage) and reaction type (R, MR, MS, or S) will be given for each line. Some examples of data entry for Modified Cobb Scale readings are 60S, 10MR, 5R, etc. Field identification examples are given in the appendices with the USDA Cobb Scale publication.

Rating scale for other diseases

Leaf rust is considered to be the most economically important pathogen for Oklahoma wheat production. Other diseases are commonly found, and should be included in breeder data if applicable. When general observation notes are being taken, diseases are only marked if they are particularly virulent among a line or population. Thus, if an entry is overtaken with a specific disease, one would note it as indicated in the general disease notations above.

There are scales for the diseases of lesser economic importance. Most commonly we use the Stakman severity scale where 0 indicates no evidence of disease and 4 indicates heavy infection. This type of scale is also used in seedling leaf rust screening. In this screening procedure, 0 means no flecking or pustules, 1 and 2 are very small and slightly larger pustules, corresponding to R and MR in Modified Cobb Scale, 3 and 4 are larger, moderately susceptible or susceptible type pustules, and ; represents flecking. Examples of this type of rating are given below.


RESISTANT
0
0;
1
2
MESOTHETIC
X
SUSCEPTIBLE
3
4

Common notations

Field Notes	
Notation	**Meaning**
General Observations	
P	Poor
FP	Fair to Poor
F	Fair (note, very fair not an actual observation, write F)
FG	Fair to Good
G	Good
*	Better than normal for category
Agronomic Observations	
PS	Poor stand (note, differentiate between Ps)
Lg	Lodging
W	Weak
T	Tall
Sh	Shattering
S	Short
CD	Cold damage
H2O	Water damage
No Hay	Pronounced “no I”, literal translation "not there"
Locations	
CC	Cow Creek
PK	Perkins
PP	Plant Pathology farm
LS	Castroville, TX, literal translation "Lone Star"
LCB	Lake Carl Blackwell
STW	Stillwater
Disease Observations	
Pt	*Puccinia triticina*, leaf rust
Ps	*Puccinia striiformis*, stripe ruse (note, differentiate between PS)
Pgt	*Puccinia graminis* f. sp. t*riticini*, stem rust
PM	Powdery mildew
BYDV	Barley Yellow Dwarf Virus
SBMV	Soilborne Mosaic Virus
WSMV	Wheat Streak Mosaic Virus

Harvest

Most of the harvest for Dr. Klatt's program is conducted by hand, but some of the yield trials are harvested with a small test plot combine. Hand harvesting can be done by breaking off individual heads with your hands, using scissors, or using sickles depending on the objective of the materials being harvested. Specific harvesting techniques are given for each group of materials.

Modified bulk method

A modified bulk strategy is employed when harvesting F2, F3, and F4 populations. Remember that within these segregating populations we are selecting from a group of plants that will have 50%, 75%, or 87.5% homozygosity for each plant. The goal of modified bulk selection is to select the plants within the population that appear to have good disease resistance, acceptable agronomic type and good traits of interest. Within the best populations, the modification consists of selecting the best plants, while weeding out or eliminating most of the bad plants over successive generations.

To achieve our modified bulk strategy, the best plants in a population are tagged with blue painters' tape sometime before senescence. By tagging the plants before senescence (normally about 2-3 weeks after heading), we can observe the full plant and its tillering ability, the green flag leaf and whether it is susceptible to any disease, and also the head. The tagged plants are then harvested by scissor upon maturation. Ideally, we hope to cut heads only from the plant that was tagged, but more likely we will harvest a group of heads (mostly from the selected plant) near the one tagged. At this point, we can also choose to not harvest a tagged plant if it looks like it was tagged in error, or if it has not matured well, or if it has become susceptible..

The tagged plants from each selected population are harvested to obtain seed for the next generation. After harvesting each location, the heads will be threshed and the seed graded. If seed is poor at one location, it may individually be discarded, or if seed is poor at all locations, the whole population may be discarded. Seed of one population is then bulked from all locations and the next successive generation is made. The F2 is planted at one location (LS). The F3, F4, and F5 populations are generally planted at 2or 3 locations each year.

F5 harvest method

The F5 harvest represents a shift from a segregating population to a purified or advanced line. From this point forward, the cross will not be viewed as a population with plants of varying genetic type, but as a group of advanced lines derived from the same cross, i.e. sister lines. F5 plants in each population are tagged just like the earlier segregating populations, except every good plant should be tagged in the F5. Heads from the tagged (best plants) will then be snapped off at harvest. NOTE: Be very careful not to smash the bags or burlap sacks containing the F5 heads. These heads will be threshed individually with the single head thresher or "whammer" as it is referred in this program. The F6 is planted in individual head rows, where the best individual, mostly homozygous lines, can be identified for yield testing.

Bulk method

Bulk harvesting methods are simply harvesting material (generally advanced lines), or a sample of the material, with no selection pressure. Bulk harvesting is done for individual head rows and yield trials. Head row bulk harvest is conducted with a sickle. NOTE: Be very careful when holding the head row while using the sickle so that you do not accidently slice your finger off. Yield trial bulk harvest is done with a plot combine. NOTE: Be very careful when using the combine that the machine has finished threshing the plot before starting another.

Threshing

Threshing is the separation of grains from the rest of the plant. The goal of threshing is to finish with only grain from the specific plot of interest with no chaff or debris, and no seed from any other plot. This is a pretty straight forward process, but here is a basic explanation of how it is done.

Before threshing, make sure you have all of the supplies you will need for a day of threshing. For the thresher you will need fuel and a vehicle to haul the thresher. Logistically, you will need several trashcans for the paper bags that the plots (heads) are currently contained in, seed pans to catch the grain, a few flats to prop up the seed pans for catching the grain immediately out of the thresher, envelopes for the threshed grain, markers to write on the envelopes, and boxes in which to put the packaged grain. It is also a good idea to bring sunscreen, insect repellant, and water for everyone.

To begin, get the thresher from storage. Be sure to raise the stand before driving off (this has happened before). Park the thresher where the wind is blowing the chaff away from everyone working.

Before starting the thresher everyday it must be fueled. Use gas, not diesel or any other type of fuel. Also, make sure the thresher is out of gear. The thresher is then ready to start. To start, wind the rope around the starter in a clockwise direction. Pull hard, but consistently to start the motor. Then put the thresher in gear by threading the belt and pulling the lever up to where the belt is tight and running.

Threshing must be done in a precise and safe manner. Operators of the thresher should be shown how the equipment operates while the machine is off to know which pieces to avoid. There should be at least three separate roles within the threshing crew: a feeder, an envelope writer, and several grain cleaners. The feeder first shows the bag to the envelope writer so the number can be accurately re-written onto an envelope. At this point a tray must be present at the mouth of the thresher to catch the grain (make sure there are absolutely no seeds from the previous entry in the tray!). After that, the feeder can rip open the bag and allow all of the wheat to go through the thresher, pushing the flap several times to make sure all grain from that entry has been threshed. The grain cleaner can then take the tray and remove all of the chaff and debris by shaking, picking, and blowing on the grain to make the debris easy to remove. The clean grain is then put into an envelope and into a tray. The process of writing, feeding, catching, and cleaning continues all day. Later, when time permits, the trays must be sorted and envelopes of each particular set put in order.

CHAPTER IV

SEED ROOM TASKS

Seed grading

Seed grading is basically the last step in deciding to keep or toss a particular line. Up to this point you have information on agronomic characteristics of a plant, field observations, and possibly yield, as well as the seed which will be evaluated. If the seed is bad, the line will probably be thrown away, even if the field observations were favorable.

Seed type of 1 is large. Seed type 2 is average size. Seed type 3 means the seed is very small and shriveled. A sign of - can be included to mean worse than normal. A sign of + can be added to a 1 rating to indicate unusually large seed. The star or asterisk (*) is included with a rating if the seed is very good for the given category. Also, 'b' or 'B' is added to the seed type if it is white (blanco).

Considerations for putting up seed

After seed is graded and Dr. Klatt has made the nursery list for the next year, seed can be put up to ship or plant. On each list there will be notations for how much seed to include in each packet. If the seed is very large, include more grams of seed per packet, but if the seed is very small, include fewer grams of seed per packet. This ensures that the resulting plant density will be approximately the same for all packets.

In several nurseries copies or sets of the exact nursery will be made. The only consideration here is to make sure that you stay on number and insert checks as they are indicated on the list. In some nurseries, a few copies of the exact number will be made, as well as some nurseries with seemingly random entries excluded or included. This is done to allow for an extra location depending on amount of seed available and needs of the cooperator or location.

If you determine that an error has been made in putting up seed, try to solve the problem logically. First, figure out what kind of error is anticipated. Is an entry missing? Or do you have too many entries? Are some entries in one set, but not in the other set? Then, investigate the last checks that were put in the nursery. If the last checks match, then you know you were right then, if not, your mistake is prior to that point. Keep doing this until you narrow a range where the error occurred. Once you know where your error occurred, check if any envelopes were double numbered, or one was skipped. A frame shift of envelope number is quite easy to resolve once found. If the error is still evasive, ask for Dr. Klatt's assistance and compare seed to find which entry is added twice or missing.

Whamming

Recalling the breeding scheme, F5 populations are advanced to F6 head rows. The best individual heads are taken from the best individual plants in each selected F5 population. The heads are individually threshed. Heads with acceptable seed from the F5 will then be planted into individual head rows, where each head represents a different genotype from the population.

The F5 heads will be stored through the beginning of the summer in brown paper sacks within burlap bags in the greenhouses. Whamming is time consuming and messy, so, this material will not be used until late in the summer once seed is graded and all other nurseries are packaged to plant or ship. When it is time to wham first all locations of the F5 must be put in order side-by-side. This side-by-side assortment of the F5 should then be checked against the book to make sure there are no missing entries. Then the first few of the entries for all locations can be brought into the seed room and prepared for whamming.

The single head thresher, or whammer as we refer to it, requires a bit of skill to use well. There is a power button and throttle on the left side of the machine above the motor. The throttle may need to be adjusted to prevent seed grinding, but generally there is a speed for each of the machines which does not crack or grind much seed. There is also a lever which blocks the air flow to the reservoir of the whammer. The air flow can also be controlled by covering or uncovering the opening with your hand. There is a hole in the bottom of the reservoir which will release grain, and a bag on the end of the unit which will catch chaff and any seeds blown out of the reservoir.

Once you have figured out how all of the components work and played around with the throttle and air flow, you can start whamming. To wham, turn on the unit and adjust throttle appropriately. Make sure the hole in the bottom of the reservoir is covered. Toss a wheat head into the whammer stem first. Do the majority of cleaning by alternating high, low, and no air flows to blow out most of the chaff. Collect the seed from the reservoir into a small plastic cup. The seed in the cup must then be cleaned by hand to remove ***all*** cracked and broken pieces, as well as any chaff that may have gotten in the cup. Once the seed is completely cleaned, it can be graded and put into head row trays.

Be careful with the F5 selections as some of them may have long stems or blue tape. Remove all blue tape and stems prior to whamming a group of material. Also, make efforts to stay on the correct number by threshing the locations in the same order every time and keeping entry numbers together. Additionally, whammers must communicate with the person putting up the head row trays to make sure everyone knows when one entry ends and another begins.

Head row trays

Seed from whammers must be planted as individual heads, so it is vital that there is no mixing of seed from one entry to the next or of two heads from the same population. Therefore, seed cleaners must be very careful to not spill or tip any seed. The person actually working on the tray should pour the seed into individual cells with caution. And everyone must know where one population stops and another starts!

A series of four checks must precede every population (new entry number) for field identification purposes. Seed of the entry is then placed in individual cells following the four checks. Any seed that is poor, shriveled, appears to be non-viable, or otherwise damaged can be excluded from the F6 head rows. Individual cells will be filled until there are no more entries from the population. Do not leave dangling rows. Plan to not run out of heads before the end of the row, but if you do, throw out the worst ones or put in a check at the end of the row.

A planting plan is made while whamming is conducted. This plan will identify tray and row number in which the checks for a population begin, origin of the population (F5 or other), and the tray and row number in which the population ends. The row number of the tray is the number of rows from the end of the tray. Please count these accurately every time (the easiest way to do so is to use your fingers). Please Note: Each tray holds 100 entries, i.e. 25 rows times 4 entries per row. From this plan we will be able to design a field map and F6 head row entry book. The plan must be completely precise and accurate!

CHAPTER V

DATA MANAGEMENT

Data management and organization are very important aspects of plant breeding. We need to have an accurate way of taking notes, record keeping, and making sure information and data survive year-to-year. Field books keep a physical record of all notes made through the year, but digital records will live forever. By this, I mean physical records are great for making decisions within a year, but if they are not entered into the computer correctly, the physical note will essentially be lost. Currently, we are using a shared dropbox.com folder for information sharing through data entry and field book preparation. This technology may not be employed for years to come, but there must be some organization indicating who has which files at any given time.

Preparing field books

Field books are an integral part of our research. They allow researchers to have pedigree and cross history side-by-side with current notes. It is important to include the most recent relevant history in the field book, which will normally be designated by Dr. Klatt, as well as enough space for multiple field and seed notes.

Field books are normally printed on light blue card stock paper and bound in three ring binders. Paper must be heavy enough to withstand field treatment for a whole season. If not, binder hole reinforcements should be used around the ring holes. Light blue paper is used because it is easier to read in high sunlight conditions, easier to read for those with learning disabilities, and does not attract bugs as much as white.

Field books should be grouped into binders by common locations. Examples of individual field books include: Crossing blocks (WWCB, SpCB, WWSYNCB, SYNCB), International nurseries (all quarantine material), F2/F4 (Book for south Texas), F3/F5, LS head rows (often more than 1 book), LS spring head rows, yield trials (F7 PYT, F8YT, ALYT, etc.).

To begin making field books, you must first save three copies of the data file (original, side a, side b). The original copy will be kept pristine in case data loss or major errors occur later. It is best to keep originals, working copies, and completed copies in separate folders on the computer. Each nursery is split into two data files to make one field book because they will contain the same header and footer information, but different margins. Side A (left side) contains the entry number, pedigree, cross number, and origin. Side B (right side) contains the entry number, relevant observation history, and blank spaces for new notes.

Do not be discouraged when first learning to make field books. Quickly and accurately making field books on the first try takes a few attempts. Even after much practice, quick might not be a good description of field book making, second nature, however, may. A few example book pages are given at the end of this manual. Directions for making the field books with Microsoft Excel 2007 instructions are below:

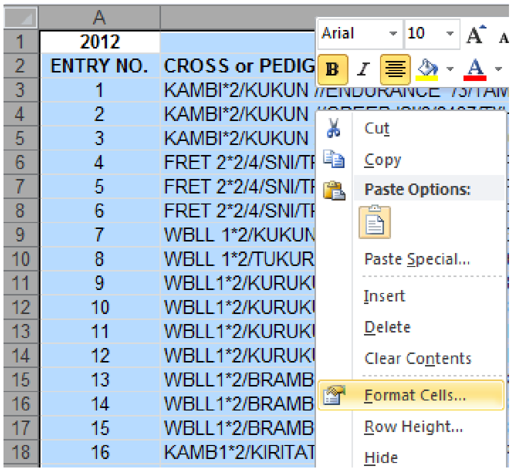
1. Save file in “Original” and “In Progress” folders.

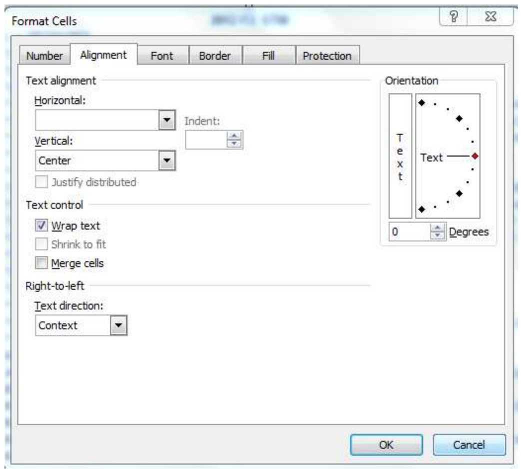
2. Format and align entries:

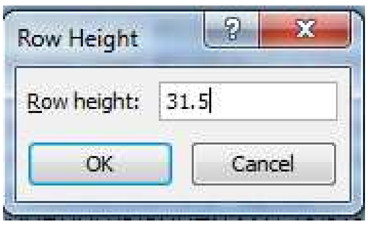
   Highlight all cells, click top left button immediately above row 1, and immediately left of column A

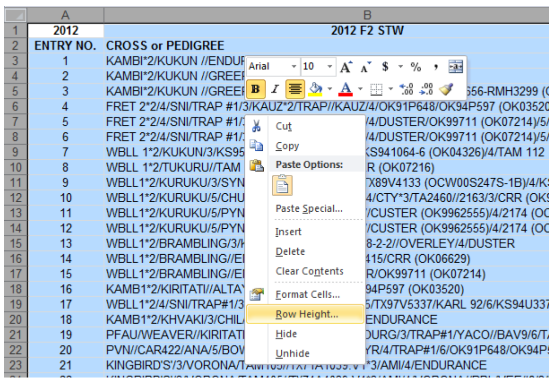
   a. Right click highlighted area, format cells, select alignment tab: Vertical alignment- center, and select “wrap text”

   b. Right click highlighted area, row height, enter 31.5 (this will give room for column headers and 20 entries per page)

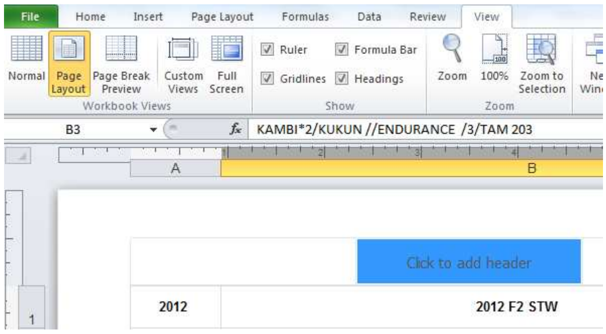


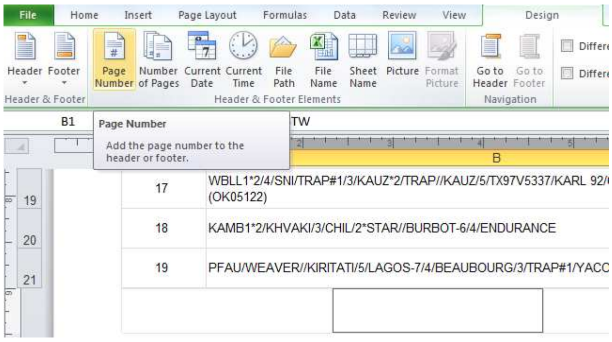






3. Header and footer: View, Page Layout (view), click in header or footer area to type (note: must click back within the body of the spreadsheet to return to normal view)

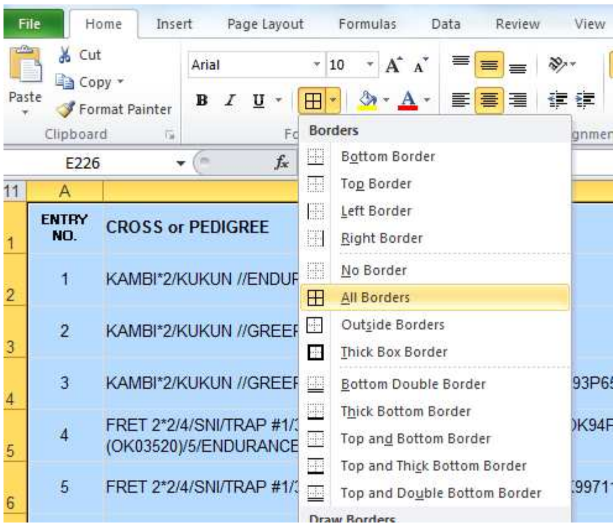




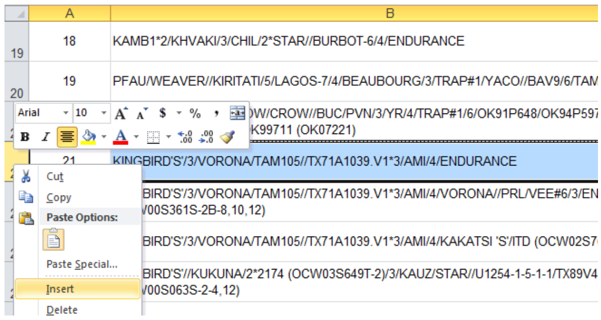
a. Header: Name of population (center, bold, 12 point font)

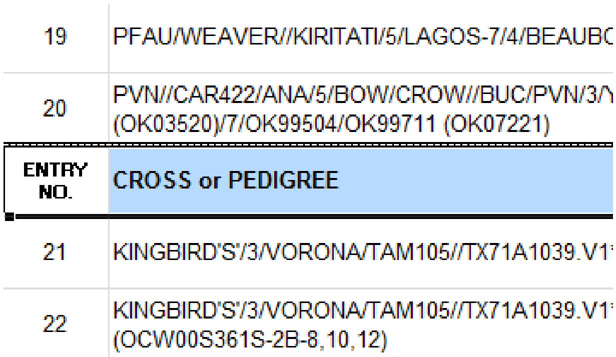
b. Footer: Page number (center)

4. Border on all cells: Highlight all necessary cells, on home tab click borders shortcut and select all borders

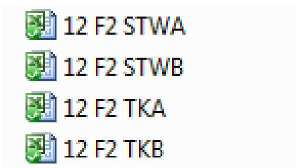


5. At the first row and after every 20 rows insert the column headers (should be the beginning of every page)

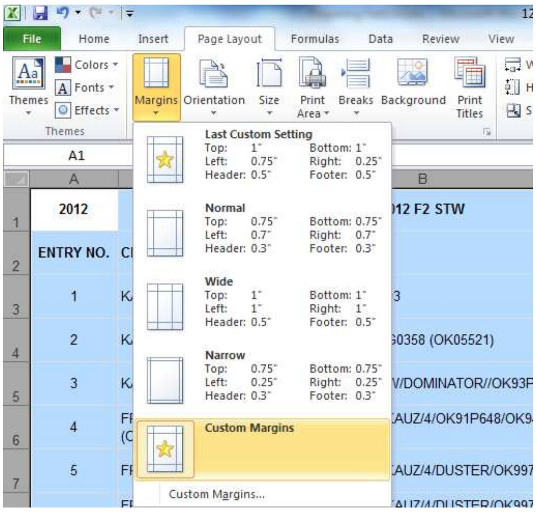


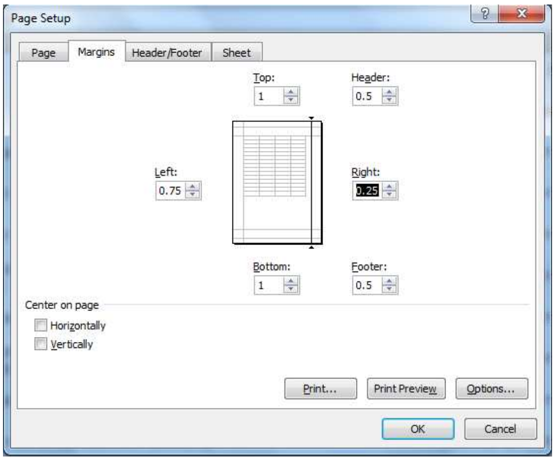


6. Copy the spreadsheet into different files: original (should already be done), A (right), and B (left).

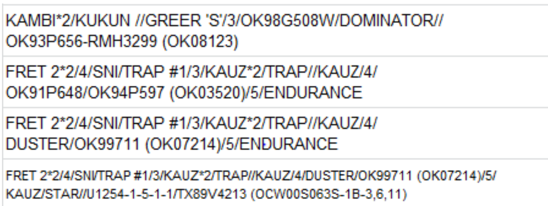


7. Set margins: Page Layout, Margins, Custom Margins
    a. Side A- top/bottom 1”, left .25”, right .75”
    b. Side B- top/bottom 1”, left .75”, right .25”

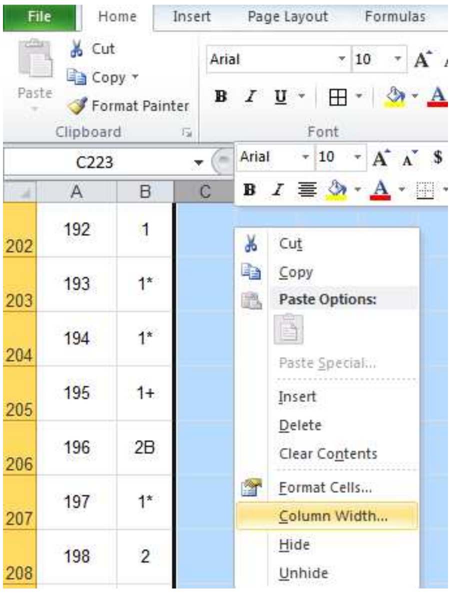


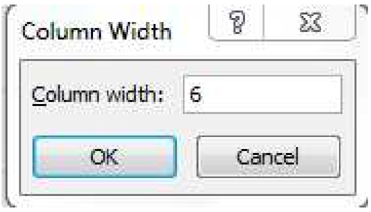


8. On the A side, the pedigree column, font should be Arial size 10, which can be made smaller if needed (7.5 point, minimum)
    a. Shrink font only certain cells typically
    b. Use ‘alt’ and ‘enter’ simultaneously to wrap pedigrees if they are too long before or after cross symbols (//, /2/, /3/, etc.)



9. On the B side, all remaining empty cells of the page should be 6 point wide.
    a. Highlight remaining columns
    b. Right click
        i. Column width 6





Entering field notes

Field note acronyms were previously described in the section on taking field notes, on disease readings, and seed grading (pg., pg. and pg.). Each column of notes should be entered as a separate column of data with year, location, and observation types listed (2010 LS Pt or 2010 PK Obs, etc.). If multiple listings are entered in the same cell, separate them with commas (T,FG* or Lg,40S, etc.). Make sure all data entries get double checked using the field books, as this will become the permanent record for each cross.