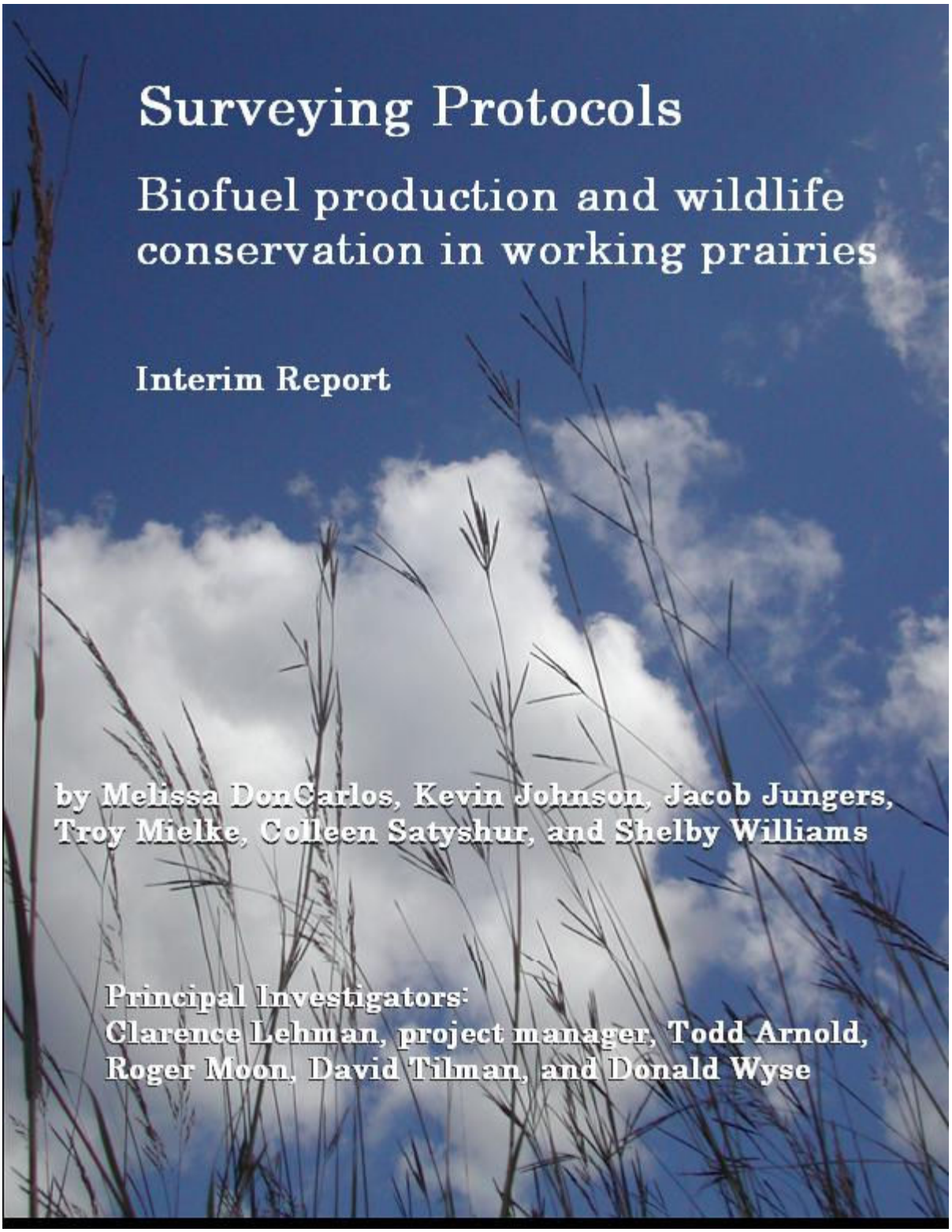

Surveying Protocols
Biofuel production and wildlife conservation in working prairies
Interim Report
by Melissa DonCarlos, Kevin Johnson, Jacob Jungers, Troy Mielke, Colleen Satyshur, and Shelby Williams
Principal Investigators:
Clarence Lehman, project manager, Todd Arnold, Roger Moon, David Tilman, and Donald Wyse

Preface

This document is an interim report of the sampling protocols used in the first three years of the project, "Biofuel production and wildlife conservation in working prairies," funded by the Environmental and Natural Resources Trust Fund under recommendations of the Legislative-Citizen Commission on Minnesota Resources, with supplemental funding by the National Fish and Wildlife Foundation, the US Department of Agriculture, and the University of Minnesota College of Biological Sciences. The project is detailed on its website, http://www.cbs.umn.edu/wildlife.

These protocols are being extended into the next three years of the project and will be adapted as required during that time. A final report of these protocols will be submitted in 2014. In the meantime, what is documented here may be disseminated and used in other surveys of wildlife and prairie. In the interest of achieving the best possible methods, please contact the project manager, Clarence Lehman (lehman@umn.edu), if these are useful to you, or if you find ways to improve them.

—C.L.L., November 15, 2011

Table of Contents

Sampling harvested biomass and recording harvested area

Objective: To collect biomass samples from cut and baled biomass for chemical analysis. To measure the area cut by harvester and the residual plant material remaining after harvest to determine precision and accuracy.

Methods:

Bale Coring Protocol – Sample approximately 50% of the bales in each plot. When approaching a plot that has been cut and baled, start at the first bale that was ejected from the baler. At that bale, flip a coin (or other means of random selection) to determine if that will be the first bale to be sampled. After starting at the first bale, sample every other bale in order of ejection from the baler. For instance, after starting at bale one, the second bale should be skipped and the third to be ejected would be sampled next.

Sample the first bale by drilling the bale corer into the end of the bale (curved side of round bale, not flat face). Flip a coin to determine which end to drill. The corer should be drilled at a 90 degree angle in the center of the end of the bale. The corer should be drilled all the way to the white holding section of the apparatus. When the corer is removed, drive the core into the holding section with the ram-rod. When the holding section is full, or if all the samples have been collected from the plot, empty the holding section into a brown paper bag and label the bag with the date, plot number, and corer initials. Weigh the paper bag with biomass and record on bag and data sheets.

Biomass Handling in Field-Bags of bale cores should be stored in a refrigerator or freezer until they can be dried to cease all respiration. Bags should be stored with tops open or holes punched into upper section. Samples should not sit in ambient temperatures for longer than two days after collection. Once the samples can be delivered back to the drying ovens on campus, they should be dried at 140 for three days, then weighed, and processed for analysis.

Stubble height Protocol - After the bale core is sampled, use a meter stick to measure stubble height at that location. Stubble height will be measured approximately 10 meters in one direction from the bale. Flip a coin twice to determine which direction walk from the bale to measure stubble height. Walk 10 paces in the direction away from the bale. Stop 10 paces away and set the end of the meter stick straight down. Kneel down and measure the height of the stubble directly behind the meter stick. Record this value, along with the direction from the bale it was sampled on the data sheet.

Recording sampling locations on GPS – At each bale that requires a core, mark a waypoint in the GPS for that location. The waypoint should be labeled using this format: P74 B01 Use the letter "P" followed by the plot identification number to indicate where the sample was collected, then the letter "B" followed by the sample number in order of collection to indicate which bale the sample is from. The bale number should be labeled in order of samples taken, NOT which number was ejected from the baler. Example: B03 is the third bale sampled but could be the sixth bale on the field. Record these labels on the data sheet.

Labor: Two people will work as a team to collect bale cores, measure stubble height and record harvesting perimeter.

Equipment:

- Forageurs Hay Probe – Forageurs Corp., PO Box 564, Lakeville, Minnesota, 55044. Phone: (952) 469 2596. Stainless steel tube 14 or 24 inches long with re-sharpenable, hardened steel tip that cuts 0.6-inch in diameter cores. Multi-core canister holds 20 to 30 cores. Corer can be used with hand brace or a 0.5-inch electric drill. Price: $171 (including shipping) for 14 inch probe; $181 (including shipping) for 24 inch probe.
- cordless drill with ½" chuck and extra batteries
- cordless drill battery charger
- paper bags
- markers
- compass
- meter stick
- handheld GPS
- ATV with storage tote

Figures:

Data Sheet:

Initials	Harvest Date	Core Date	Plot	Bale ID	Stubble Height	Orientation	Comments	Number of Bales	Core Weight

Sampling plant composition and richness

Objective: To measure species richness, relative abundance of each species, and biomass in grassland bioenergy plots. To determine changes in plant biodiversity and productivity from biomass harvest.

Methods: Measure species richness, relative abundance, and biomass in 12 randomly distributed sample points per 20 acre plot. At each random point, a 1 X 1.5 meter PVC frame (quadrate) will be used to delineate the sample area. For plots with partially harvested areas, proportionally distribute the sample points equal to harvest proportions (i.e. a plot harvested at 75% should have 9 points in harvested area and 3 in refuge).

Randomly generate 12 sample points per 20 acre plot using ArcMap 9.3 and load onto hand-held GPSs. Each surveyor will be assigned a list of points in each plot and travel to each point using the GPS. If a surveyor approaches a point that is inundated or does represent the plot (cattail pot-hole), he/she should move to the next point. Managers should train technicians to determine the conditions that warrant this action. Each surveyor will have extra points in case of this situation. Upon approaching the sample point, the surveyor will dedicate all his/her attention to the GPS and avoid noticing the surrounding vegetation. When the survey has reached the point, he/she will turn 180 degrees from the walking direction and toss the quadrate over his/her head. Vegetation under the quadrate frame should be shifted to either side of the frame to allow the PVC to be in contact with the litter. Stems of plants that are rooted within the frame should be shifted to inside the frame. Once the frame is in place, a digital picture will be captured and coded on the data sheet.

Within each quadrate, the surveyor will identify each species and list those on the data sheet. The surveyor will then estimate the percent of quadrate that that each species covers. The coverage estimate is of the light intercepted by the herbage of the species. Reference shapes of one, three and five percent of the quadrate should be cut from cardboard and laminated and provided to each surveyor. Bare-ground and litter should be counted separately. Species that cover less than 1% should be labeled "Trace". All percent cover values will sum to 100.

The entire area within the quadrate will be cut using a hand sheers to a height of 2.5 cm above the ground or at the litter layer. All biomass will be placed in paper bags and labeled with the plot, point, cut or uncut area, date and surveyor's initials. Each bag will be weighed using a portable scale at the field vehicle before returning to the lab. Bag weights will be recorded on the provided data sheets. Bags of biomass will be dried at 140 degrees F for five days before being weighed. Sub-samples of empty dry bags will be weighed and averaged to be subtracted from the total biomass bag weight.

Labor: Three people will work on a team, sampling one plot in 1.5 hours.

Equipment:

Individual Items

- 1 meter X 1.5 meter PVC quadrate
- PVC frames
- reference shapes
- clipboard
- data sheets
- pencils
- GPS
- watch
- radio
- sunscreen
- bug repellent
- clippers + blades
- paper bags
- markers
- large plastic bags

Group Items

- extra Clippers + blades
- clipper charger
- AC converter
- scale
- batteries
- duct tape
- weighing data sheets
- extra percent cover data sheets
- first aid kit
- laptop with sample points
- GPS cables
- large plastic bags
- truck bed net
- bungee cables

Data Sheet:

Species Richness and percent cover (abundance) record

Date	Initials	Plot	Point	Species	Cover	Comments	Picture

Harvested Biomass Weights (g)

Plot	Point	Bag #	Bag Type	Wet Weight	Sample Date	Dry Weight	Weigh Date	Initials

Surveying phenology

Objective: To study the effect of harvesting grasslands on the timing and amount of plant growth.

Methods: Select three species of plants (one from three separate plant functional groups) that are prevalent in the experimental plots. Randomly generate GPS points in each quadrant of each plot (using ArcMap). These points will be used to randomly locate plants to monitor. These points should be generated with a 15 meter buffer from the edge of the plot, as well as a seven and a half meter buffer between points within a quadrant. Use a GPS unit to navigate to the first point in a quadrant, and upon arrival, search within a 15 meter radius around the point for and individual of the desired plant species. Once a plant is located, mark it with the GPS unit and with two marker flags, one 0.3 meters north and one 0.3 meters south of the plant. Repeat this process so that there are two individuals of each plant species in all four quadrants of each plot. There will be 6 individual plants, two of each species, in each quadrant for a total of 24 marked plants in each plot. Then monitor the phenology of these plants; visit each plant twice a week during the spring and summer months (April-September). Record the date, plot number, plant ID (GPS point name), phenophase and a basic visual assessment of the plot near the plant. Measure the blade and flower stem length and record on the data sheet.

Labor: It takes one person roughly 1 to 1.5 hours per plot to conduct this phenology survey.

Equipment:

- clipboard
- data sheets
- pencils
- black Sharpie marker (for labeling flags)
- GPS unit & spare batteries
- marker flags

Datasheet:

The following symbols were used to record phenophase:

E = Emergence, Greening
B = Budding
F = Flowering, Pollen release, emergence of anthers in Grass
D = Fruit or seed development, end of the presence of anthers in Grass
R = Fruit or seed maturation, the beginning of seed dispersal
L = Leaf fall /Senescence

Surveying bloom phenology – 2010 pilot study

Objective: To test a method of measuring the effect of mowing on the diversity, abundance and timing of blooming forbs which provide food for pollinating insects.

Methods: Bloom estimates were started in summer 2010 and were performed while walking between phenology points as a part of weekly phenology surveys (see above) on 8 plots in the southwestern site. The spacing of the phenology points meant that the observer covered most of the plot. The remaining plots in the southwest site were surveyed twice during the summer via 4 walked transects across the plots. While walking a plot, any species of forbs in bloom was recorded and an estimate made for the total number of "blooms" of each species on the plot. For the purpose of this study a "bloom" referred to a "distinguishable flowering part." For example, on a sunflower this meant an individual flowerhead in bloom. In the case of Milkweeds "bloom" referred to one cluster of flowers. The first sighting of a blooming forb on any plot was also entered into the master checklist. Attempts were made to record pollen presence or absence in blooms via a touch test. This became difficult to do or to verify in many plants and was not continued through the season.

Results of this study, along with notes on methods and labor requirements were used to create a full scale study of blooming forb phenology which will be performed in phase II of this project.

Sampling with insect sweepnets

Objective: To sample insects in the vegetation of grassland plots for diversity and abundance.

Methods: One sweepnet "transect" consists of 15 steps and 15 swings of the sweepnet. Each net swing should be an180° arc centered in front of the collector. The bottom edge of the net should be slightly in front of the top during the sweep. The net should sweep through the sward height (the height below which 80% of the vegetation lies) and the vegetation just below it. Height of net should vary as vegetation height varies along arc of sweep. At the end of one 180° sweep, the sampler should take a step forward, quickly whip the net up, over and down and start the next arc. After the turn, the net should be in contact with vegetation for the full 180° sweep. Continue at constant pace of one second per 180° arc for 15 arcs. Immediately whip net back and forth in air to drive insects to the bottom, then grab net to prevent escape and empty the contents into a ziplock bag. Well-labeled ziplocks should go into a handheld cooler while sampling the plot, then into a larger cooler back at the car. Stack bags vertically so that at least part of each bag touches ice at bottom. Car coolers should be emptied into a freezer at the middle and end of each day. Once insects have been frozen for 24-48 hrs, the air can be carefully let out of the bags. If possible, sweepnet sampling should always be performed by the same people to ensure consistent techniques. Samplers should practice to match techniques at the beginning of the season and then re-checked for standardization at the beginning of every week.

Collect eight transect samples per 20-acre plot. Randomly generate starting points for transects with assigned sequential numbers. Then choose the eight points with the lowest numbers according to the following criteria: the eight points should be divided proportionally between harvested and un-harvested areas and there should be two points per quarter plot. Transect start points should be 15m apart and should not fall on boarders of plot or harvest edges. When two people sample a plot, each person sweeps one point per quarter plot and equally divide transects within each harvest type. Do not walk through or near transect line when approaching start point and make sure the whole transect is within the appropriate harvest type.

There are certain constraints on when sampling may be performed. Vegetation must be completely dry before sweep-netting. Start after dew has lifted usually about 8:30–9:00 am. Transects should run into the wind, whatever direction that is for any given day. Wind speeds should be less than 25 mph. This is not always an easy condition to satisfy but do not sample on very windy days. Randomly chose the order in which to sample blocks of plots. Then, divide plots within blocks into geographic groups. Randomly chose order in which to sample these geographic groups.

Labor: This work requires the flexibility to work odd hours and weekends as weather is an important factor. It takes a crew of two approximately 1 hour to sample a plot. Drive time between plots is a factor but generally 4-6 plots can be swept in a day. It takes approximately 8 hours to pick a sweepnet sample by the methods used in this project.

Equipment:

- sweep nets (3-4)
- spare net bags (2)
- maps of random points/plot/month
- 2 GPS to navigate to transect start point

- gallon ziplocks bags, double zipper ones work better.
- labels
- 2 travel coolers
- ice packs (30 big ones)
- freezers
- meter sticks (3-4)
- thermometer
- pencils
- datasheets
- compartment clipboards
- binder
- composition book for daily notes

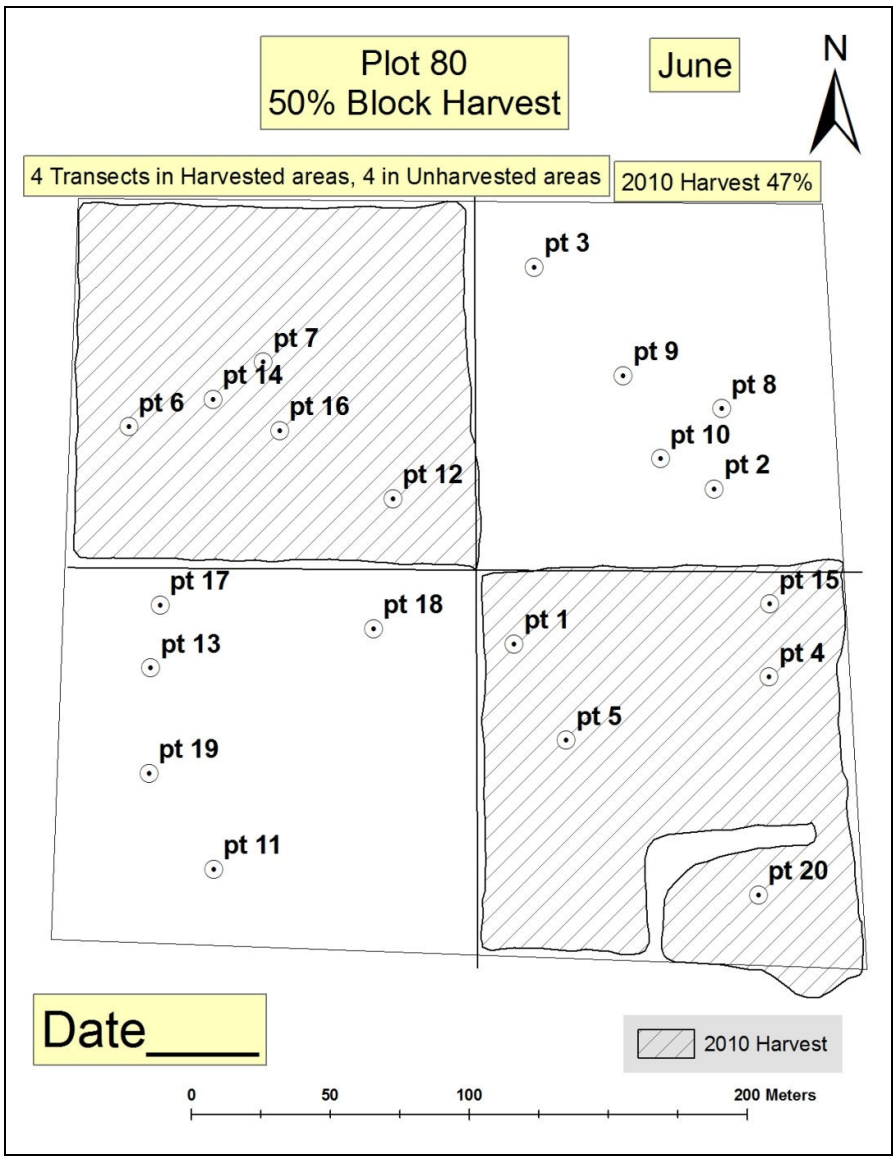
Data Collection:

Upon arrival at plot:

1. Percent overcast
2. Temperature - in the shade.
3. Approximate wind speed and wind direction.
4. Circle transects done on maps

At each transect:

1. Sward height- Measured as the height under which 80% of the vegetation appears to be growing. Sward height measuring should also be calibrated once a week along with sweep net technique.
2. Start time.
3. Note any unusual circumstances.



Datasheet:

Site-block		**Start time**	
Plot		**Start temp**	
Transect #		**Wind cond**	
Date		**% overcast**	
Collector		**Sward Height**	

Surveying with insect pitfalls

Objective: To sample ground-dwelling arthropods in grassland plots, focusing on the family Carabidae.

Methods: Set up eight pitfalls traps on each 20-acre plot (see figure below). Place two pitfalls per quarter plot, one halfway between center and the corner of the plot and the second five meters from the first towards the center. Use a GPS to find the center point and a meter tape to measure the inner point.

Traps should be dug at least two weeks before first sampling run. To install a trap, use a trowel or bulb planter to dig a hole just a little bigger than a 16oz. Solo brand cup plastic cup. Place a plastic bag over one cup and secure with a rubber band. Place this cup in another cup and set it in the hole and make sure top of the inner cup is flush with ground level. If the cup rim is above ground level invertebrates will notice the trap and avoid it. Caps can be made from 11 x 14.5cm (4.4 x5.75in.) piece of half-inch plywood. Drill a hole in each corner and secure a 4.5-5.5in bolt in each hole to make legs. Label the roof with sharpie, center it over cup and push it in flush with ground. Flag the trap so you can find it again at a later date. Leave traps closed when not actively sampling.

For each sampling run, leave traps open for 3 days (72hrs). Traps will not suffer under light sprinkles, but do not run traps during major rain events. To start the run, take the plastic bags and rubber bands off inner cups and replace them in the permanent cups. Check that the cup rims are flush with soil surface. Add two inches of soapy water to the inner cups. To make the soapy water just add a big squirt of scentless dish soap to a gallon jug of water and mix. Set the trap roofs two inches above the ground. Note the time that traps are opened on each plot. Plots should be opened and closed at the same time of day.

To collect a sample, remove the inner cup and pour the contents through the brine shrimp net so that kill solution runs into a storage container and not onto the plot. Rinse net contents with water, again into the container. Shake net so excess moisture comes off then invert net into a brown paper bag and flick all the arthropods off. Gray tape on the bottoms of the paper bags will help keep them from falling apart. Fold the top of the bag over twice and staple it shut. Label the bag and place it in a travel cooler. Close the trap by placing a plastic bag on clean dry inner cup and secure with a rubber band. Insert the inner cup into the permanent cut and push lid down. Repeat with the rest of the traps. Bring paper bags to the freezer at the middle and end of the day.

Run 3 times per season-June, July and August.

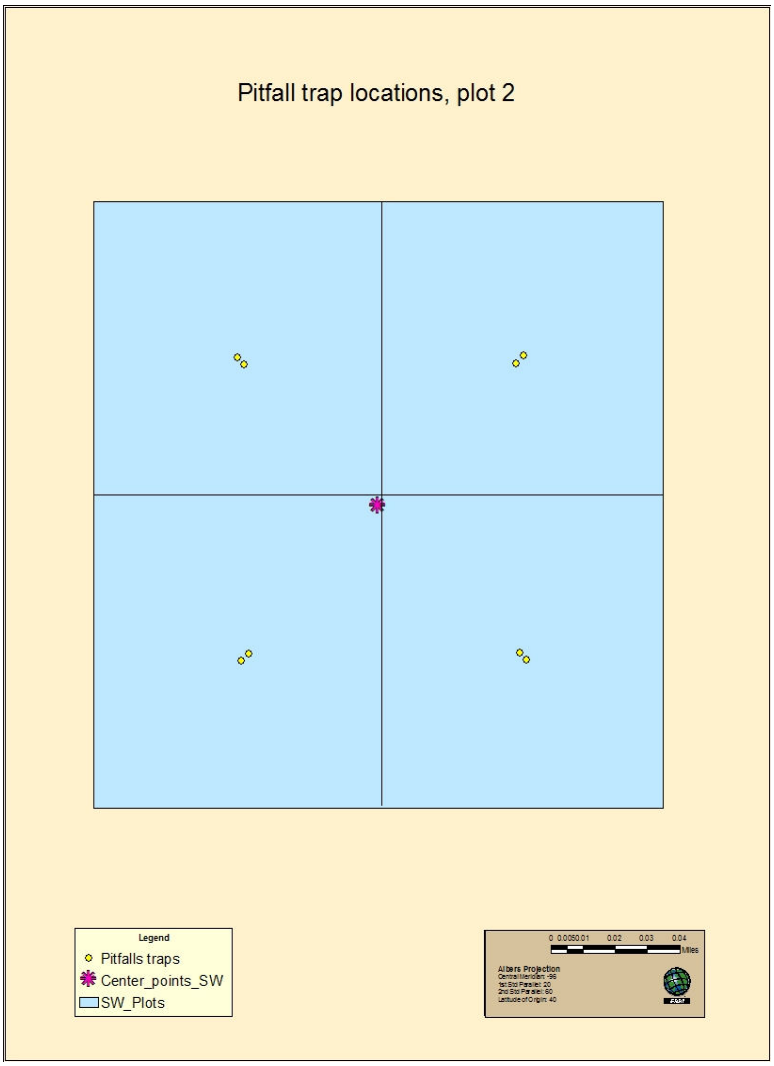
Labor:
Digging traps: Takes two people 1-1.5 hour to dig traps in each plot
Starting run: Takes two people about 20-30 minutes per plot
Ending run: Takes a little longer than starting the run. Takes two people about 45-60 minutes per plot.

Equipment:

- Solo plastic drinking cups- 16 oz = 8.2cm in diameter, 11.5cm deep with smooth insides.
- plastic wrap or baggies
- rubber bands.
- wood roofs- half-inch plywood roughly 11 x 14.5cm (4.4 x5.75in.)
- bolts and nuts as legs 4.5-5.5 in long bolts ¼ in. thick.
- meter sticks- to measure height of trap roofs.
- Sharpies
- dish soap-unscented
- jugs and pitchers for transporting and storing used kill solution
- water bottles to rinse nets
- aquarium nets
- duro size 2 ex-heavy duty paper bags.
- hand-held coolers and icepacks
- little staplers
- surveyer's flags

Data collection: Record the date and time that traps are opened and closed for each plot. Also record any rain during run and weather that affected water level in solo cups or anything else. Note any unusual circumstances.

Figure:



Data sheet:

Order entered	Site	Block	Plot	Date opened	Time opened	Date closed	Time closed	Weather (rain? Etc.)	Notes (damage, flooding, etc.)

.

Quantitative Insect Sampling Tent (QUIST)

Objective: To capture all insects in a given volume of prairie to determine absolute abundance and calibrate sweepnet samples with height of vegetation.

Methods: To use this method to calibrate sweepnets, pair QUIST sample locations with a sweepnet transect taken 1-4 days beforehand. Additional sweepnet samples may be taken nearby in order to increase accuracy. Determine sample locations using random points within a plot. For this project three QUIST samples were taken per 20-acre plot and plots of various vegetation heights were sampled.

To perform this type of sampling, a tent must be built (Figures 1 and 2). Use white cotton cloth (not too thin so to sacrifice durability) to form the sides and top of a box measuring 0.75m length by 0.75m width by approximately 1.5m high. Each side of the tent should have about 0.5m of extra cloth at the bottom to form a skirt on which sandbags will be placed in order to seal the tent to the ground and keep invertebrates from leaving or entering the tent. The tent seams should all face out, so that the inside of the tent is as smooth as possible and there are minimal folds where invertebrates can hide or get stuck. Fabricate one face of the tent with a vertical zipper that extends two thirds of the length of the side. Insert two more zippers perpendicular the first at the bottom of the first zipper. These zippers should start at the sides and zip so that all three zippers meet at the same location when fully closed. Zippers should form an inverted "T" shape. Use one-inch PVC pipe to build a frame slightly larger than the tent. Attach ties or sleeves to the outside of the tent in order to attach it to the frame so that it hangs taught. Also make four sandbags about two inches in diameter and 1m long.

Sampling a point involves several steps. First, record the sward height in front of the sample point. To do this, approach from downwind and try not to disturb insect from the sample area. About 10m downwind of the sample point, assemble frame and tie in the tent. Make sure the bottom of the tent is closed so no invertebrates can get in. Using two people, lift and carry frame upside down to sample point. Quickly place frame on ground over the sample point, arrange skirt flaps out and seal them to the ground with sandbags as quickly as possible. The area of the bottom of the tent should be 0.75m by 0.75m. Check that bottom secured. Next, vacuum all the invertebrates around the door area and from as much of the walls and vegetation as possible. For this project we used a modified leaf blower. While one person vacuums, the other must help keep the door zipped as far closed as possible and watch the door with a net or aspirator and try to catch insects escaping from doorway. After the "door vacuum," one person cuts the vegetation and places it in a pillow case. Work methodically from the door inwards, always keeping the zippers as much closed as possible and watching for escaping insects. After about half the vegetation is cut, the sampler can completely enter the tent and be zipped in to cut and collect the remaining vegetation. Before exiting the tent, the sampler should use an aspirator to remove any insects on the walls of the tent. The next step is to vacuum the ground within the tent for 2-3 minutes. Finally, use aspirator once more to get any last invertebrates off the cloth or ground.

Samples should be placed on ice as soon as possible and transferred to a freezer at the middle and end of the day. Samples will take a lot of freezer room, so be prepared.

Labor: QUIST sampling takes at least two people, preferably three. One sample can be collected in approximately one hour. Picking one sample takes approximately 40 hours.

Equipment:

- one modified hand-held leaf-blower
- gas:oil mix at a ratio of 40:1 (1 gallon gas: 3.2 oz oil) Use any 2 cycle engine oil
- fine-mesh bags to fit in leaf blower nozzle
- band with a plastic snap to hold mesh bags onto leaf blower
- gallon zip-lock bags for vacuum and aspirator samples
- pillow cases for vegetation
- electric clippers and charged batteries
- QUIST tent and frame
- sandbags
- aspirators and spares
- labels
- data notebook
- pencils
- meter sticks
- ensect net
- gloves
- grey tape for repairs
- GPS unit and random points

Datasheet:

Record plot, sample location, date, time of day and observers.

Figures:

Vacuum sampling QUIST:

QUIST sample complete:

Surveying bees — pilot study 2010

Objective: To develop a survey method to study the effects of harvesting grasslands for bioenergy on bees.

Methods: During the 2010 field season we ran a pilot project to test methods and feasibility of sampling bees in grassland biofuels plots. Sampling was performed on four plots in the southwest site in July and August. Plots were selected to represent the range of harvest and biodiversity in the area. For bowl and paint selection, as well as methods for putting out bowls and collecting and preserving specimens, we used the guidelines in Sam Droge's "Very Handy Bee Manual." http://www.extension.org/mediawiki/files/7/71/TheVeryHandyBeeManual.pdf

We used 3.25 oz clear soufflé cups and painted them white, blue and yellow. White spray paint was used to make white bowls. Blue and yellow bowls were made using Guerra Paints silica flat base with blue fluorescent pigment and yellow fluorescent pigment. Color was painted directly onto both sides of the clear soufflé cups and allowed to dry fully before use. One transect of bowls was placed on the ground in each of the four plots, with a total of fifteen bowls per transect (5 of each color). Bowl colors were alternated blue then yellow then white. Starting points were randomly determined using ARCMap. The first bowl was placed at the GPS point and marked with a green flag. Subsequent bowls were placed 5 meters apart (measured by pacing the distance), with the trajectory of the transect going toward the center of the plot. The same transects were used in the same place for both months.

Vegetation in grasslands can grow one to two meters tall. To test if tall vegetation reduced catch in the bowls, vegetation was clipped two randomly selected bowls of each color in each transect in July. Clipping was done with the goal being to make the bowl visible while doing as little harm to the plots as possible. The diameter of the area clipped was less than 50cm. Vegetation in August in all plots was seemed to have re-grown in clipped areas and was tall enough that clipping to create full visibility would result in too large of a cleared area.

To start a sampling run, each bowl was filled ¾ full of water mixed with blue Dawn dish soap. Soapy water was mixed using a gallon jug of water with a big squirt of soap in it. After 24 hours, bees were collected from each bowl using forceps and placed into vials of isopropyl alcohol. Soapy water was collected in a bucket and deposited it in the sink at the field house. For each plot, samples were combined by bowl color and whether or not the vegetation had been clipped around the bowl. Bowls were placed in all four plots between 8:00 AM and noon on 12 July 2010. On 15 August 2010 two plots were opened between 5:00 PM and 6:00 PM and the other two were opened on 16 August 2010 8:15 AM. We chose not to run bee bowls on one plot in August because the vegetation was so dense and tall that the lack of visibility would probably have been a confounding variable in the samples we collected there.

All bees collected from bee bowls were kept in isopropyl alcohol until they could be cleaned, blow dried, pinned or pointed, clearly labeled, and identified. Based on the results of this pilot project and notes on effectiveness of methods, and labor and time requirements, a full scale bee sampling protocol was developed and used in Phase II of this project.

Surveying reptiles and amphibians

Objective: To observe species richness and abundance of herpetofauna on grassland bioenergy plots. To determine changes in species richness and abundance as a result of biomass harvest on these plots.

Methods: Collect and record herpetofaunal species on each 20-acre plot using fence arrays and pitfall traps.

Array installation protocol: Install two trapping arrays on each 20 acre plot, 70 meters from the plot center, and directly opposite of each other. Each array consists of three arms made of aluminum flashing material, trenched into the ground and reinforced with wood stakes (See figure). Within this array install four pitfall trap holes, one at each arm end and one in the center. Each arm is 25 feet long and 20 inches tall, and should be spaced at even angles from each other (120 degrees), extending out from the center pitfall hole. Each pitfall hole should have a 2.5 gallon bucket so that the top of the bucket sits flush with the ground. Slits in the sides of the bucket help accommodate the arms of the array. In each of the pitfall traps, place a rope that extends from the bottom of the bucket to the top to allow small mammals to escape if captured accidentally. Also place a receptacle with a water-soaked sponge to keep captured animals hydrated. Place a small square of construction foam, to act as a floating refuge if the bucket should become inundated with water. Wooden covers should be placed on top of pitfall traps on each side of the array arms during non-survey times to prevent captures when traps are not being checked regularly. These covers should have screws in each of the corners that will be used as legs so the cover can be turned over during survey times to allow access into the bucket by animals and provide shade. Half-way along each array arm, a funnel trap is fastened to either side of the arm, totally six funnels per array. The funnel traps are made of a coated, malleable screen material, and are cylindrical with an inverted cone entrance on each end. These inverted cones can be pulled out and pinched shut during non-survey times to prevent animals from going into the traps.

Survey protocol: Surveys should be conducted during the spring and summer months (April-September). At the beginning of the survey period, open all funnel and pitfall traps and check them twice a week. Look in every bucket and funnel and record species captured. Measure the snout-vent length and record on the data sheet. Date, plot number, weather conditions and array location will also be recorded. Upon each visit to the arrays, replenish the water in the sponge/dish and make sure the rope and floats are still present in all traps; replace if needed.

Labor: Approximately one hour per plot to check all of the traps on both arrays and process captures, depending on number of captures.

Additional methods: There are two additional methods that can be tried for surveying herpetofauna. *Pitfall-only arrays:* Two pitfall arrays will be laid out in the same way as fence arrays, but will only have pitfall traps; no fence arms. Wooden covers will still be used to close the traps when surveyors are not operating. As with running fence arrays, place the covers with the screw legs down when

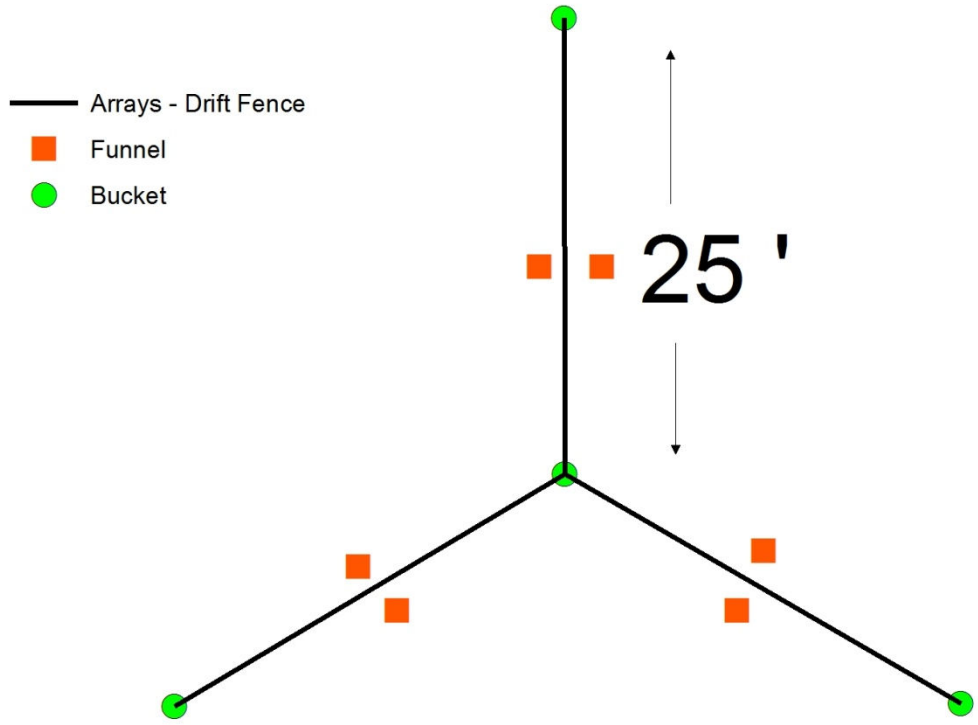
surveying; flip the covers over during non-survey times to prevent unwanted captures. Survey protocol for this method will be the same as that for fence arrays.

Visual encounter surveys: One observer will walk an S-like route through the plot and look for herpetofauna. The observer should spend two hours surveying each plot and record every encounter with an animal on a map of the plot.

Equipment:

- aluminum flashing
- funnel traps
- 2.5 gallon buckets
- wood stakes (reinforcement for flashing)
- screws (short size for screwing stakes to flashing, long for bucket covers)/drill
- shovels
- mallets
- work gloves
- eye protection
- scissors
- wood for bucket covers
- plastic weigh boats (water receptacle)
- rope
- clothes pins (for pinching funnel traps shut)
- sponges
- data sheets
- pencils/pens
- measuring tape
- scales
- camera
- zip lock bags
- water

Figure:



Surveying small mammals

Objective: To measure small mammal species richness and species occupancy in grassland bioenergy plots. To determine changes in small mammal species occupancy from biomass harvest.

Methods:

Setting up trap grids:

A 7x7 grid of traps is laid out at the center of each 20-acre plot. Each flag marks the location of one of 49 equally spaced (15 meter) trap locations and is labeled in a classic spreadsheet format: alphabetical rows, numerical columns, (e.g. D1…D7 North-South axis, A4,B4…G4 East-West axis). The NW corner is always A1, while the center post of the plot is always used as the center trap location (D4) of the trap grid (See figure).

First, using a tape measure and compass, measure out in each cardinal direction 45 meters, from the center post of the plot. A marked flag is placed every 15 meters along each 90 meter line to form a cross.

Secondly, fill in the quadrants of the grid, referencing figure one. For example starting at D3 pace 15 meters to the West, place flag C3, turn 90 degrees to the south and confirm the distance to flag C4 is 15 meters, return to C3 and adjust as needed. Note always use two known points as a reference for placing a flag. Repeat this process for all remaining points using the appropriate directions for the reference points. This entire process usually takes two hours to complete.

Setting traps:

Teams of two check and open traps on four plots each day, for a total of 196 traps. Research literature suggests that one person can handle 100 traps in a day, which is determined by worker efficiency and small mammal safety.

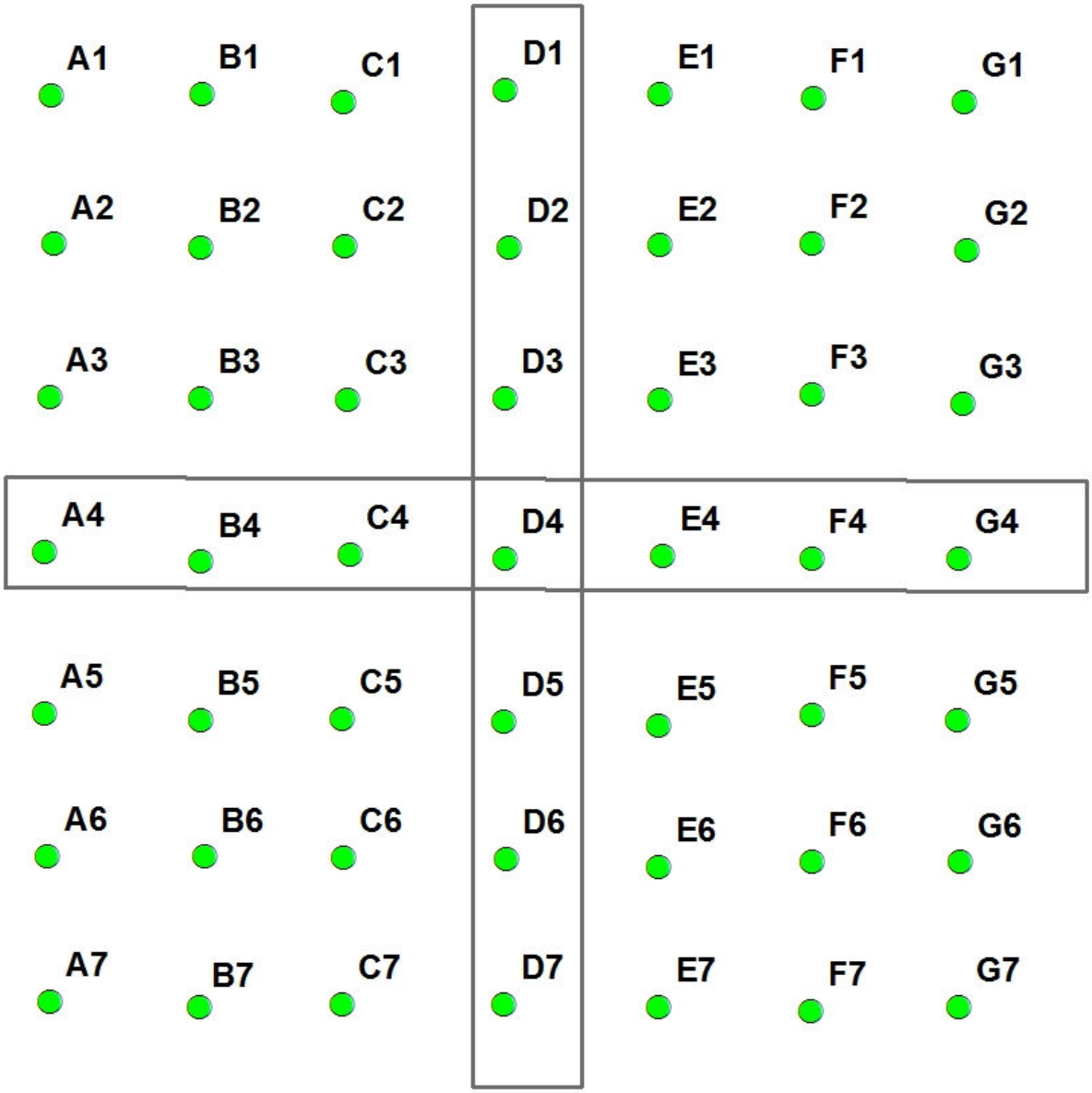
Traps are placed within one meter of each marker flag, baited, supplied with bedding and set open starting at 5pm. For bedding, use either 6-8 medium size cotton balls, or 3-4 full size cotton balls. Bait is a mixture of peanut butter and oats, mixed beforehand using an 8:1 ratio of rolled oats to peanut butter. Between one and two tablespoons of bait is distributed to the back of the each trap. Traps are left open overnight. During the second through fourth night of trapping, setting requires replenishing bait in traps that captured small mammals the previous night and/or replacing fouled bedding.

Checking traps:

Trap checking starts half an hour after sunrise. Current weather conditions and time of day are recorded. Traps are checked by walking each line of the grid, closing traps that had not been triggered over night. When a closed trap is found, it is then checked by carefully looking into one end. If the captured animal is a ground squirrel or least weasel it is released without taking measurements, as they are not of interest in our study. If the captured animal is a mouse, vole, or shrew the animal is transferred into a gallon size zip-lock bag for measuring. This is done by wrapping one end of the trap within the bag, and holding the trap door open to allow the animal out of the trap and into the bag. Weigh the bag with the small mammal in it, then weigh the bag after releasing the animal to determine weight of the animal. Record the genus, species, sex, and age. Immediately before releasing, mark the animal on its underside with a blue permanent marker to identify a recapture in the following days of trapping.

Equipment:

- Sherman live traps
- peanut butter & oats bait (mixed before going out into field)
- cotton balls for bedding
- compasses, for flagging
- flags, pin flags
- Zip-lock plastic bags
- clipboards, data sheets
- pencils/pens
- Sharpie markers (blue)
- 50g and 100g scale
- gloves


A1
B1
C1
D1
E1
F1
G1
A2
B2
C2
D2
E2
F2
G2
A3
B3
C3
D3
E3
F3
G3
A4
B4
C4
D4
E4
F4
G4
A5
B5
C5
D5
E5
F5
G5
A6
B6
C6
D6
E6
F6
G6
A7
B7
C7
D7
E7
F7
G7

Data Sheet:

Small Mammal Data Sheet								
Date:			**Plot:**					**Page _____ of _____**
			GPS Pt	**Start Time:**		**End Time:**		**Temperature: (C)**
Observers:			**Wind:**			**Precip:**		**Cloud:**
			0. None			0. none		0. <10%
			1. Light breeze			1. drizzle		1. 10-50%
			2. Mod wind, gusts			2.Mod or intermittent		2. 50-90%
			3. Strong wind, gusts			3. Heavy, thunder		3. >90%
Trap #	***Genus***	***sp.***	**Sex**	**Age**	**Total wgt (g)**	**Bag(g)**	**Recap (Y/N/U)**	**Notes**

Nest Searching

Objective: To record nesting waterfowl numbers for species richness and relative abundance in grassland biofuel plots. To determine changes in waterfowl species richness and relative abundance from biomass harvest.

Methods: Nest searches are conducted using a chain-dragging method. Each plot is searched two times, three weeks apart, by a team of two people. Two four-wheel ATVs each pull one end of a 100 foot length of ½ inch chain attached to a trailer hitch. The chain is rigged with a swivel mechanism (see image below) so that they turn freely and therefore, easily roll over the vegetation. The ATVs are driven at equal speeds through the plot parallel to each other, approximately 65 feet between them. The two ATVs drive from one end to the plot to the other, and back again over unsearched terrain (see figure).

Nesting waterfowl will be flushed off their nests as the chain passes. When a duck is flushed, the drivers should stop. One driver should proceed by foot to locate the nest with directions from the other driver who remains where he or she first observed the flush.

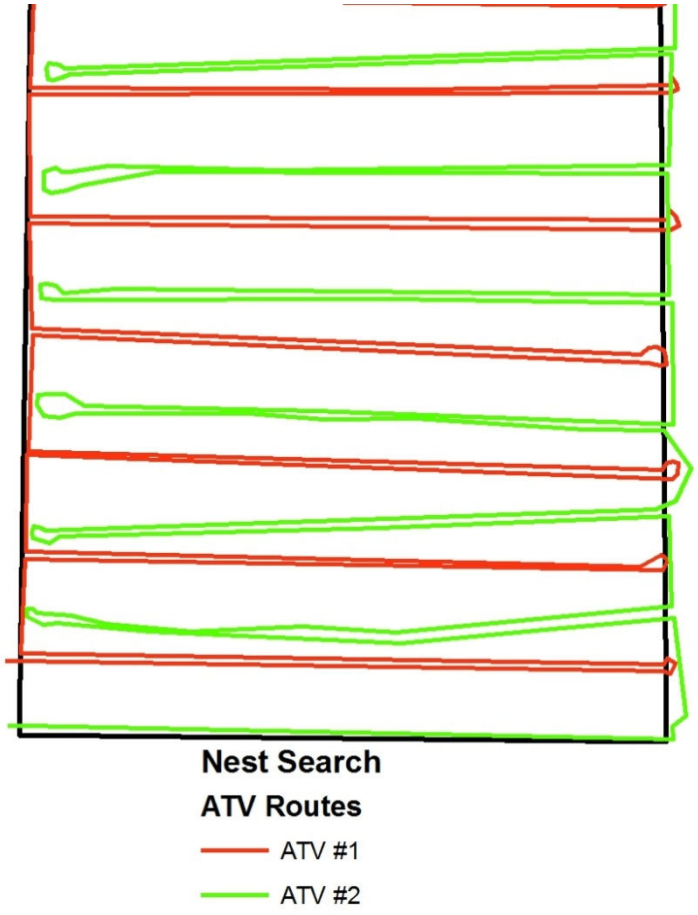
Upon locating a nest, a picture of the nest and its contents is taken. Data such as nesting species, condition of the nest, presence of the hen, number of eggs, and stage of incubation should be recorded. Stage of incubation is determined by candling (see figure) or a float test. A GPS point is taken at each nest in order to be returned to for weekly monitoring to determine the fate of each nest (see data sheet). Nests are marked by placing a flag three meters north of the nest. Each nest is revisited on a weekly basis with the same data rerecorded as before. Once the fate of the nest is determined the nest is no longer visited.

Labor: Over the course of a season it takes two researchers six hours to complete two searches and rechecking of nests on one plot.

Equipment:

- two ATVs
- 100 of ½ inch chain
- trailer hitch/swivel system (see photo below)
- hitch with no ball
- homemade swivel (Far right in photo)
- 1/2 inch swivel, carabineers
- spare bearings (inside homemade swivel, 4459-00. GBC item 607 GBC; S0698312)
- spare carabineers
- flags

Figures:



Data Sheet:

Site Data

Study Area			Year				Nest Number			STV-149, TRV-155, BST- 011 County			Apx. 10m Habitat Class		Syst.1 Incid 2	Jeep cable-2; flushed hen-6; landing hen 7 Search Method
			2	0	0	8										
6	7	8	12	13	14	15	16	17	18	21	22	23	24	25	30	33

NEST DATA

Species MALL; BWTE; PINT; SHOV; GADW				Nest Site Veg. (~1 m) 02, upland grasses		GPS Waypoint #
19	20	21	22	23	24	

Box 37 HEN STATUS	Box 38 NEST STATUS
0 absent, nest successful 1 absent, warm/covered 2 absent, warm uncovered 3 absent cold/covered 4 absent cold/uncovered 5 absent all eggs missing/destr. 6 present alive 7 present dead 8 probably dead 9 hen injured/killed by inv.	1 normal 2 invest. Damage 3 invest disturb 4. part destroyed pred. 5 totally destroyed pred. 6 other damage 7 some eggs missing 8 eggs missing/broken previously Or disturbed nest hatched.

Nest Visits

Visit		Mo	Day		Time				Status Hen	Status Nest	Whole Host Eggs No.		Whole Host Eggs Incub.		P-site eggs No.
28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	
0	1														

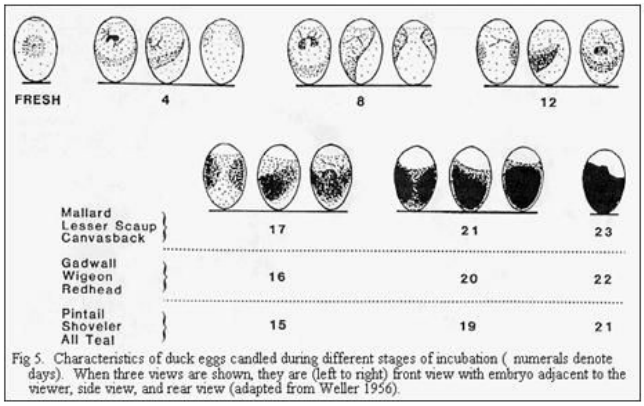


Fig 5. Characteristics of duck eggs candled during different stages of incubation (numerals denote days). When three views are shown, they are (left to right) front view with embryo adjacent to the viewer, side view, and rear view (adapted from Weller 1956).

Complete on last visit; success = one or more HOST eggs hatching	
Fate 1-succ 2-aban 3-dest 4-nonvia 5-unk	**Cause** (if Fate = 2 or 3) 1-pred 9 unk
64	65

Comments (nest location details, parasitism by pheasants etc)

_____

_____

Nest Initiation (m/day) ___ ___ **Est. Hatch** (m/d) ___ ____ **Age Found**_____**Exposure Days** _____

Surveying songbirds

Objective: To observe species richness and abundance of songbirds on grassland bioenergy plots. To determine changes in species richness and abundance as a result of biomass harvest on these plots.

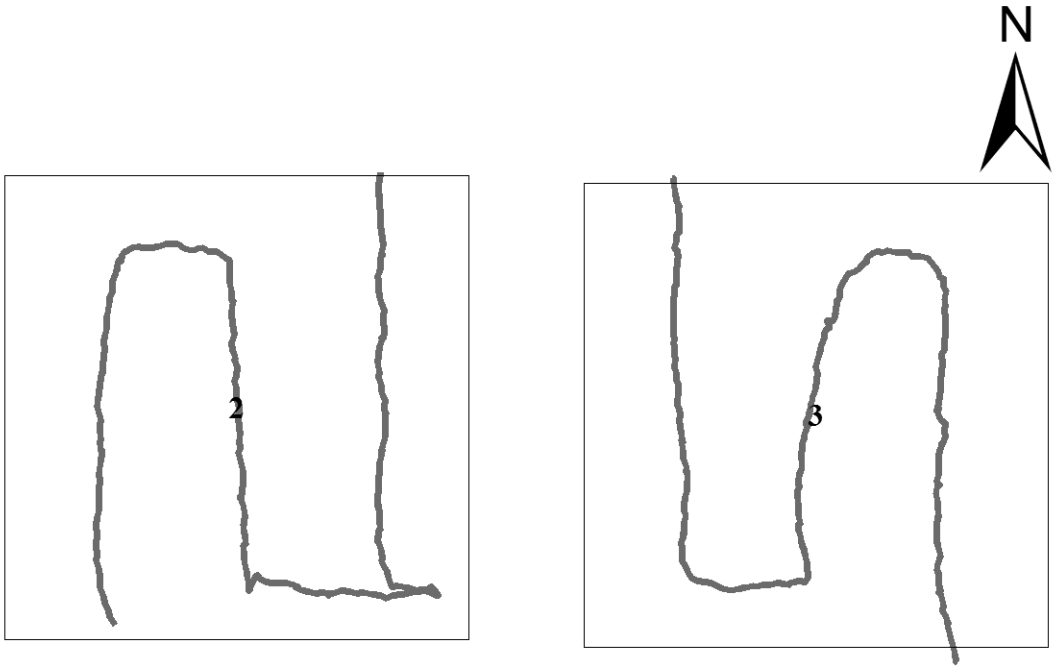
Methods: Observe bird species on each 20-acre prairie plot using the area search method. The observer will use audio and visual cues to detect and record birds in each survey. Songbird surveys start in May and may continue into mid/late June, when breeding season is at its peak. Conduct surveys during the time period between half an hour past sunrise and noon, during which the birds are most vocal and active. One person conducts a survey on one plot at a time, allowing a minimum of 15 minutes and a maximum of 30 minutes for each survey. This assures against any bias in survey numbers as a result of spending significantly more time on one plot than another. The surveyor will make three passes through the 20-acre square plot, starting at a randomly selected corner. The observer will walk 60 meters to the east or west of the starting corner before beginning the survey. The observer will be able to see or hear any bird in this distance between them and the edge of the plot as they walk through the plot. Record the date, plot, weather conditions, and start time and then begin walking the plot north to south or south to north, depending on the starting location. At the end of a pass, shift 60 meters before beginning the next north/south pass. The observer will be able to hear or see any bird within this distance from the last walking pass. This pattern results in an S-curve path that allows the observer to cover the whole 20-acre plot. As the observer walks the plot, they should be aware of the time, as they must spend at least 15 minutes on the plot, and not more than 30 minutes. As the observer walks through the plot, he or she will watch and listen for any bird within the plot. When a bird is sighted or heard, record the species on a map of the plot, showing the approximate location of the observation within the plot. The observer will record the end time when they reach the edge of the plot on the last pass.

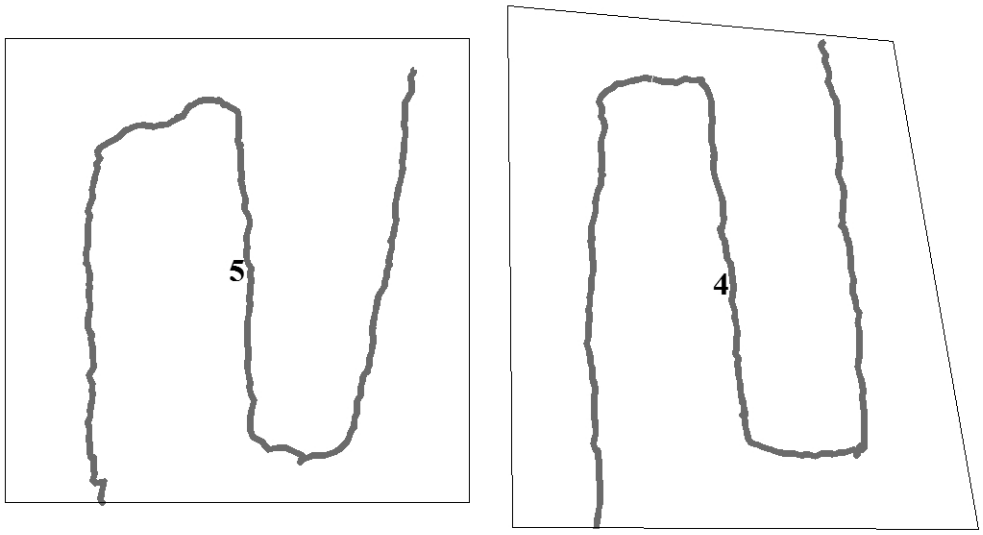
Labor: On average, two people can conduct roughly seven or eight surveys on 20-acre prairie plots in one morning during the designated time period, weather allowing.

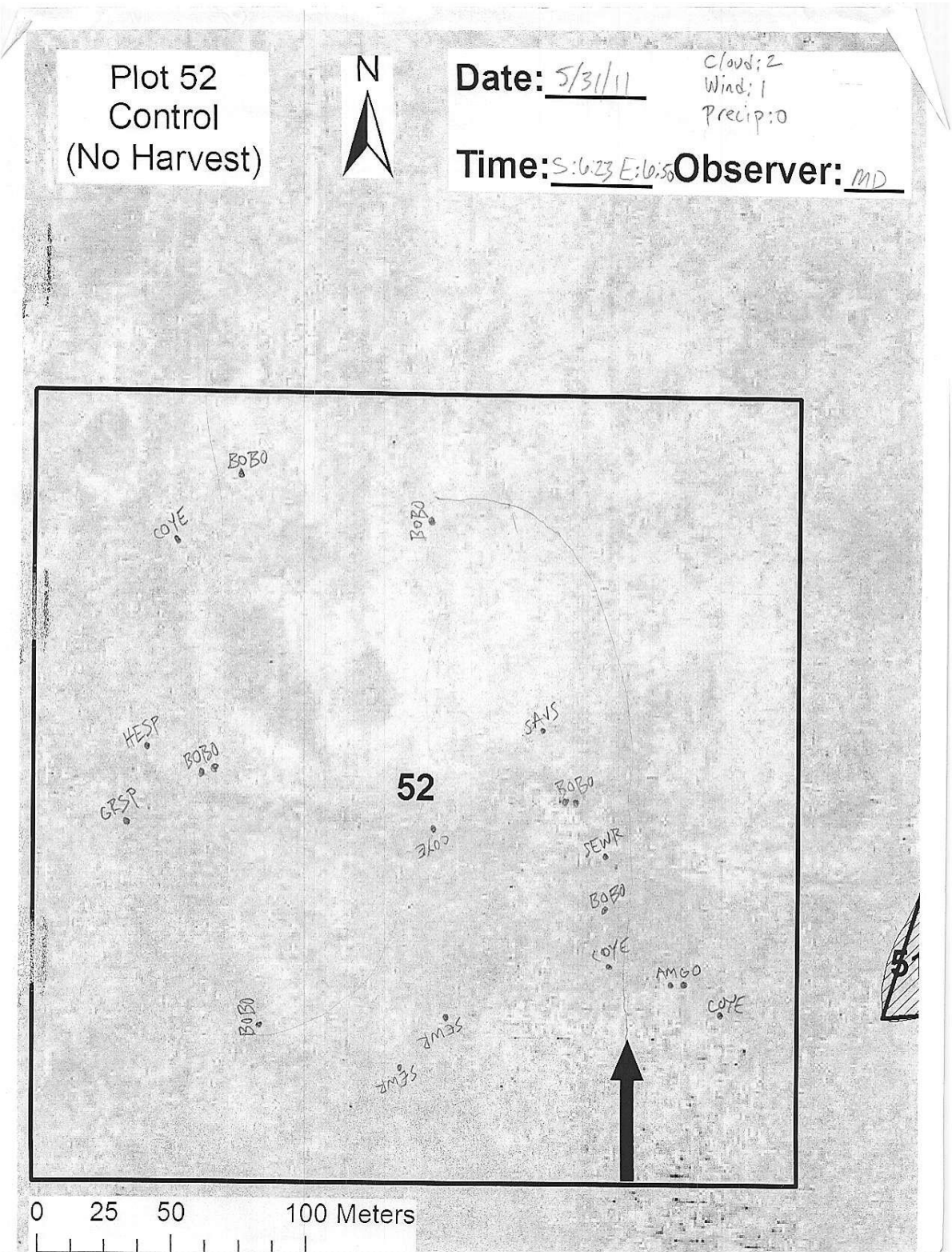
Equipment:

- clipboards
- data sheets (maps of plots with randomly selected starting points identified on them)
- pencils
- binoculars
- watch or time-telling device
- bird ID book, if necessary

Figures:






Plot 52
Control
(No Harvest)
N
Date: 5/31/11
Cloud: 2
Wind: 1
Precip: 0
Time: S: 6:23 E: 6:55 Observer: MD
BOBO
COYE
BOBO
HESP
BOBO
SAVS
GRSP
52
BOBO
COYE
JEWR
BOBO
COYE
AMGO
COYE
BOBO
SEWR
SEWR
0 25 50 100 Meters