

ELYTRON BRANDING AND PREDICTED
OCCURRENCE MODELING OF AMERICAN
BURYING BEETLE IN THE NORTHERN PLAINS
ECOREGIONS OF NEBRASKA AND SOUTH DAKOTA

By

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Abstract: The conservation of an endangered species often requires knowledge of not only the causes of the decline, but also the current distribution and habitat requirements of the species. In Nebraska, the American burying beetle, *Nicrophorus americanus* (Olivier) populations occur in three ecoregions. Previous studies have produced predictive occurrence models based on habitat suitability in the Loess Canyons and Sandhills ecoregions. This study examines occurrence of American burying beetle in the Northwestern Prairie ecoregions of Nebraska and South Dakota. Two methods were used for the construction of predictive occurrence models, a computer learning system (random forest), and generalized linear model (logistic regression). Both models indicated average minimum winter temperature was a strong predictor, and the random forest model found average precipitation and percent coverage of grassland to be positive predictors, while the generalized linear model found percent coverage of wet grasslands to be a strong positive predictor. The random forest model produced an area under the curve 0.82 and the logistic regression model produced an area under the curve of 0.83. Along with predictive occurrence modeling, population estimates are heavily used to monitor the health of known population of American burying beetles. Mark-recapture population estimates require the use of permanent marks that do not alter the survival or behavior of marked individuals. Studies have revealed problems with mark retention or damage to tested individuals associated with permanent marks. Currently used permanent marking techniques involve making a hole or removing a wedge from an ABB elytron. In this study, we tested the efficacy of elytron branding using a surgical cauterizer. The cauterizer was used to ablate one of the orange maculations on the elytron of *Nicrophorus* beetles. I found that the marking technique was rapid compared with other techniques, permanent, and easily interpreted.

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Silphidae

For over 100 years, beetles in the family Silphidae have been known to be important decomposers of small vertebrate carcasses in the environment (Fabre 1918). While all Silphidae use carrion as a food resource, species in the Subfamily Nicrophorinae are of particular importance as they entomb the carcass beneath the ground to feed and raise their brood (Ratcliffe 1996). In contrast, the Subfamily Silphinae do not bury carcasses but instead utilize the carcass by laying eggs in moist topsoil near the carrion, where their larvae will then consume fly larvae that are feeding on the carcass (Anderson and Peck 1985). The family Silphidae is a small group of beetles with 208 species identified in 13 genera worldwide, and 18 species in 6 genera in Nebraska (Ratcliffe 1996).

Beetles in the subfamily Nicrophorinae are most notable for their burial of carrion under the ground which led to the common name of the genus, "burying beetles". Male and female Nicrophorinae beetles will find and cooperatively bury an appropriately sized carcass and raise their offspring and feed on the carcass for up to two weeks (Anderson and Peck 1985).

Life History

Once a carcass is found in the environment, usually by a male, the amount of juvenile hormone (JH) in his body will rise rapidly to many times its normal level (Trumbo *et al.* 1995). If no female is present but a suitable carcass is found, the JH level of the male decreases more slowly over the course of several hours when compared to a male in the presence of a female and a carcass. In addition males release pheromones that attract conspecific females (Trumbo and Robinson 2008). Female beetles also experience a marked rise in JH levels upon discovery of a carcass and mate (Trumbo *et al.* 1995). The rise in JH in adult beetles has been hypothesized to relate to intensity of parental care and burying behavior as both males and females were observed to have elevated levels of JH in their bodies when laying eggs and caring for first instar larvae (Urbański *et al.* 2008; Trumbo and Robinson 2008).

When a pair of burying beetles are at carcass, they crawl underneath it and attempt to lift it to gauge the weight of the carrion. If the carcass is of a suitable size, the male and female will remove the soil from under the carcass until it is completely below ground level. The pair then prepare the carrion for brood by stripping away any fur or feathers and rolling the carrion into a “brood ball” (Milne and Milne 1976).

The carcass is coated with antimicrobial secretions to preserve the carrion and protect the larvae from infection by bacteria or fungi, and this protection continues throughout the brooding process (Hoback *et al.* 2004; Jacques *et al.* 2009). The amount of antimicrobial secretions maintained on the brood ball is dependent on the level of development of the offspring with third instar larvae requiring less antimicrobial secretion being deposited by the parents when compared with first instar larvae (Urbański

et al. 2008). Larvae are also able to produce antimicrobial secretions further prolonging the viability of a carcass as they feed (Arce *et al.* 2013).

Most carcasses are tended by a single male-female pair, usually the largest individuals of the largest species to arrive. Some species of burying beetle (eg. *Nicrophorus tomentosus* Weber) work cooperatively to bury large carrion resources. This type of cooperative burial is rare and only exhibited by a small number of species of burying beetles with the majority of beetles being competitive (Scott 1994; Scott 1998; Komdeur *et al.* 2013). All males, of all species, that arrive at a carcass compete for the carcass resource but eventually the largest and strongest male will succeed and force all losing males to leave. The same competition occurs among females, with the largest and strongest female prevailing and chasing off all competitors (Wilson and Fudge 1984; Otronen 1988; Müller *et al.* 1990).

Once the carcass is won, the victorious pair quickly bury the carrion to escape further competition and competition with flies and other carrion feeders. After burial, the carcass is stripped of fur or feathers. The process of stripping away fur or feathers reduces fly eggs and maggots that may be present. The parents remain with the carrion and offspring to defend the carcass and continue to treat it with antimicrobial compounds (Scott 1998). In some species an ability to visually recognize a mate versus an intruder has been noted, and would be an important way to limit invasion by members of the same species (Steiger and Müller 2010). Even without competition, it is common for parents to exhibit infanticide if the carrion is insufficient to support all the offspring to avoid the loss of the whole brood (Scott 1998).

Distribution and Habitat

The Silphidae have members found in North America, Europe, and Asia. In the Americas, members of the family Silphidae are isolated to only North America (Sikes and Raithel 2002). The distribution of species is thought to be, at least in part, because of the presence of higher numbers of other detritivore specialists, such as flies and ants in tropical climates (Scott *et al.* 1987). In the Northern Hemisphere, burying beetles can be found in managed fields, marshes, prairie, or forests (Bishop *et al.* 2002). Many aspects of burying beetle preferences and behavior vary across species including, seasonal activity, diurnal or nocturnal activity preference, and multivoltine (more than one brood per year) or univoltine (one brood per year) breeding (Scott 1998; Ratcliffe 1996).

The American burying beetle, *Nicrophorus americanus* (Olivier) (ABB), is a habitat generalist as it can utilize many types of environments (Bedick *et al.* 1999; Jurzenski *et al.* 2014). Habitat selection by ABB is thought to be primarily driven by resource availability. Because ABB is the largest of the burying beetles, it requires the largest carrion (80-100g), and in habitats where the availability of this size of carrion is limited, ABB is rare (Kozol *et al.* 1988). In contrast, where larger vertebrate carcasses are plentiful, as long as other factors do not disturb the beetle, ABBs may be found in relative abundance (Lomolino and Creighton 1996). ABBs also prefer to inhabit tracts of land that are undisturbed by agriculture or other forms of human land development (Bedick *et al.* 1999).

Although the ABB may be found in a variety of habitats, modeling in Oklahoma and two regions of Nebraska has revealed associations with certain habitat types

(Crawford and Hoagland 2010; McPherron et al. 2012; Jurzenski et al. 2014). ABBs in Arkansas are associated with litter-covered, loose, deep, and moist soils in which to bury and reside in their inactive periods (Lomolino and Creighton 1996). ABB populations in Nebraska and Oklahoma are associated with different plant communities with the Nebraska population preferring grassland habitats dominated by mostly bunchgrasses and sparse trees as well as wetlands and the Oklahoma populations are primarily found in forested environments (Lomolino and Creighton 1996; Walker and Hoback 2007; McPherron *et al.* 2012; Jurzenski *et al.* 2014). This differentiation of habitat preference between these two disjunct populations of the ABB is not well understood. ABB also tend to avoid any land tracts with more than 30% of its total area dedicated to agriculture (Jurzenski 2012; Jurzenski *et al.* 2014).

Ecological Importance

Studies have shown a beneficial relationship between burying beetle presence and increases in microbial and nematode activity in areas previously used as burial sites by burying beetles. This has the possibility of increasing beneficial microbiological activities in the mycorrhizal layer surrounding roots of rangeland plants which in turn may cause a positive increase in forage biomass available for cattle feeding and haying (Sayre 2001; Carter *et al.* 2007).

Along with potentially benefiting vegetative communities by increasing microbiological activity and increasing availability of limited nutrients, burying beetles also have the ability to act as natural enemies of pest flies in rangeland which may reduce the chance of spreading harmful pathogens (USFWS 2008). Carrion beetles have multiple modes of reducing competition with flies for carrion resources. Beetles in the Subfamily

Nicrophorinae bury the carcass and separate any fur or feathers to eliminate fly eggs, and beetles in this subfamily also carry beneficial phoretic mites that consume fly eggs further decreasing fly competition (Gibbs and Stanton 2001;Kozol *et al.* 1988).

These symbiotic mites belong to four families: Parasitidae, Anoetidae, Uropodidae, and Macrochelidae (Anderson and Peck 1985). The mites are phoretic and attach to beetles for transportation, and then disembark once beetles arrive at carrion sources. The mites feed on fly eggs present in the fur or feathers or those remaining on the carrion. The mites then reproduce and their offspring attach to the newly-matured beetles and are carried to a new carcass where the cycle continues (Trumbo 1990; Brown and Wilson 1992; Schwarz and Koulianos 1998).

Conservation Measures

ABB was placed on the federal endangered species list in 1989 due to substantial range reduction (USFWS 1991). In the previous 100 years, ABB distribution has reduced by more than 90% of its previously recorded range. The ABB is now limited to populations in Nebraska, Oklahoma, South Dakota, Kansas, Arkansas, and Rhode Island. Previously, ABB was recorded in 35 U.S. states and 3 Canadian provinces (Lomolino *et al.* 1995; Szalanski *et al.* 2000). While reintroduction and captive rearing is ongoing in Ohio, Missouri and Massachusetts, these efforts have been mostly unsuccessful, and none of these populations were self-sustaining.

The most common limiting factor cited for the decline of ABB has been the lack of appropriately sized carrion (around 80-100g in weight) (Kozol *et al.* 1988). One such animal that was present in great numbers in much of the range of ABB was the Passenger Pigeon (*Ectopistes migratorius* L.) which went extinct after the last known individual

died in captivity in 1914. As Passenger Pigeons were present in great numbers and had migratory flocks that would move around North America to obtain food, it may have provided a widespread source of carrion for ABB in the past. Even in extant populations, competition for carrion resources among flies, ABB, other burying beetles, and vertebrate scavengers remains strong. Insecticide use and light pollution have also been suggested to a decrease in ABB populations with remaining natural populations of ABB occurring in areas mostly free from agriculture and urbanization (Lomolino *et al.* 1995; Sikes and Raithel 2002; USFWS 2008). More research into the possible negative relationship between ABB, pesticides, and light pollution should be undertaken in the future to help identify the severity of such a relationship.

In most environments, ABB occurs with other burying beetle species. This further increases competition for carrion resources. In most areas where ABB has been extirpated many other *Nicrophorus* species remain (Sikes and Raithel 2002).

Protection of the ABB occurs at both state and federal levels, and these measures include land management of grazed pastures, land set aside as conservation areas, Eastern Red Cedar (*Juniperus virginiana* L.) tree clearing, and controlled burns along with intensive and ongoing monitoring of range distribution and population densities (Amaral *et al.* 1997; Bedick *et al.* 2004; Walker and Hoback 2007). These efforts help to preserve the landscapes where ABB still reside in their historically favorable conditions for ongoing use by ABB.

Several other conservation techniques have been tested for use with ABB. One technique included placing carrion in the environment along with ABB to allow a mating pair to reproduce on it (USFWS 1991). Another technique tested in the field was “bait-

away stations” where suitable carrion was placed outside of the development area to attract beetles away from the area being developed. The technique was in effective at moving beetles from, construction zones and attracted opossum (*Didelphis virginiana* Kerr) and leopard frogs (*Lithobates pipiens* Schreber) scavengers which ate ABB (Jurzenski 2012; Jurzenski and Hoback 2011). An alternative method to move beetles away from areas when development is set to occur is trap and relocate. Trapping beetles in the area to be developed and moving them to another area where ABB already exist has potential for conservation of ABB by avoiding beetle loss due to development; however, trapping still requires special permits (Conley 2014).

Trapping and Beetle Marking

Mark recapture studies are employed to monitor ABB. Trapping beetles to determine presence or absence in an area along with population monitoring by trapping beetles in areas of known ABB occurrence is one of the most important tools currently used for studying ABB (USFWS 2008; Krebs 1999). These studies require a mark to be placed on the beetle which does not increase likelihood of mortality due to injury or predation and causes a minimum of interference with beetle behaviors including movement, mating, and brood care (Kozal *et al.* 1988; Lomolino *et al.* 1995; Hagler and Jackson 2001; USFWS 2008; Butler *et al.* 2012).

Currently used marking techniques can be classified into two groups, permanent or temporary marks. Currently used permanent marks include elytron cauterization and elytron clipping. Both of these methods use an instrument to remove a piece of the elytron exposing the abdomen or wing beneath. Permanent marks of this nature cannot be lost and are easy to interpret (Butler *et al.* 2012). However, both these methods may

cause damage to underlying abdominal tissue, which may lead to infection or desiccation, and may alter flight. Insects with asymmetrically damaged or worn wings are more likely to lose control of their flight in adverse conditions such as wind, or fall victim in an encounter with a predator when compared to an individual with undamaged or symmetrically damaged or worn wings (Vance and Roberts 2014). Another detrimental effect of the current permanent marking methods is damage to the beetle's ability to communicate through stridulation, leading to lowered brood success in marked individuals (Hall *et al.* 2015).

Currently employed temporary methods include affixing a queen bee marking tag to the elytron of captured beetles with superglue and applying a dot of paint. These methods are non-invasive but maybe lost rapidly. Bee tagging was shown to be effective for a period of only five days, and after that period of time, loss of bee tags from previously captured beetles may lead to statistically significant differences in apparent recapture rates. The tags can also allow soil to adhere to the beetle affecting the mating and dispersal capabilities of marked beetles (Butler *et al.* 2012).

Distribution in the Prairie Ecoregion of Nebraska

One of the newest and most accurate modeling techniques for modeling ABB distribution is Random Forest (RF) modeling (Crawford and Hoagland 2010). Random Forest modeling denotes a type of computer learning program in which random decision trees based on a variety of variables are able to separate data points into separate groups with a relatively high degree of accuracy (Svetnik *et al.* 2003). Decision trees are a conceptual sorting device which divides the incoming data points into two groups, in this case, using environmental condition data present near trap sites and generating

coefficients for prediction. Each decision tree will include several of these divisions (branches) each representing one of the environmental condition variables modeled. After this “Random Forest” of decision trees is created, the program will then use a separate set of data that was not used in the creation of the decision trees to select the trees that were more accurate in separating the data accurately. Those trees that are more correct are then averaged to prevent overfitting the modeled dataset which ensures greater accuracy outside the data set on which the model was built (Liaw and Wiener 2002). While other related decision tree based modeling techniques exist, such as Regression Tree Analysis, Bagging Trees, and Multivariate Adaptive Regression Splines, RF models tend to have equal or higher accuracy than the other modeling protocols when compared with various means of measuring accuracy when making predictions outside the given dataset (Prasad *et al.* 2006).

Predictive models are one of the most important tools utilized in conservation planning. By being able to determine potential for ABB occurrence with a high degree of accuracy, project proponents can assess the likelihood of needing federal permits and the development of a Habitat Conservation Plan (HCP). ABB has been characterized as a habitat generalist, however, some studies have found close association between ABB and forested areas (Walker 1952; Anderson 1982; Lomolino and Creighton 1996), while other studies have found an association with prairie (Kozol *et al.* 1988; Bedick *et al.* 1995; Lomolino 1999; Jurzenski *et al.* 2014). These divergent findings in regards to habitat preference may suggest that there is not a single set of characteristics that can effectively predict ABB occurrence across all of its current range.

Due to the lack of a defined set of characteristics which predict ABB occurrence, Jurzenski *et al.* (2014) described the ABB as a habitat generalist within the Sandhills ecoregion of Nebraska. A separate model by McPherron *et al.* (2012) demonstrated different variables to be important to habitat use in the Loess Canyons of Nebraska. For this reason it may be best to model one discrete ecoregion rather than making a nationwide or statewide predictive model due to ABBs in different locations having different apparent habitat preferences (Jurzenski *et al.* 2014). This lack of consistency may be partly caused by the trapping method used which allows one trap to capture beetles effectively from 800m in any direction from the trap. This represents a large coverage area and thus many traps sample widely varying habitat conditions which are within the reach of a single trap (USFWS 2014; Leasure *et al.* 2012).

Because ABB have been documented to move more than a mile each night, trap coverage area greatly increases the ability to determine the physical presence or absence of beetles with relative efficiency. The large trap area of each ABB trap reduces costs when compared to small area trapping techniques (Butler *et al.* 2013). It would be best to gather location data on each trap area by remote sensing of the entire area being sampled by the trap rather than trying to base predictions on the conditions immediately around the trap. It is unlikely that, given the predicted area of attraction from traps currently used (800m radius), that the beetles originated in the area immediately adjacent to the trap (Crawford and Hoagland 2010; Jurzenski *et al.* 2014).

Variables shown to be correlated with ABB occurrence in Nebraska include: precipitation, temperature, soil type, vegetation community type, geology, and soil texture close to the trap site (Lomolino *et al.* 1995; Crawford and Hoagland 2010;

Jurzenski *et al.* 2014). There is a positive correlation between an increasing percentage of sand in the soil and probability of ABB occurrence in Oklahoma (Creighton and Schnell 1998). Arranging and weighting each of these parameters by priority and ability to accurately separate presence/absence, and checking this accuracy with existing or future presence/absence data sets may produce an accurate model.

Objectives

The objectives of this study were to develop an alternative and novel system for marking *Nicrophorus* beetles for use in future mark recapture studies. Congener beetles were used for testing in place of the endangered American burying beetle (USFWS 2008). Beetles were tested to ensure the new method is no more damaging than current marking systems.

The second objective was to use remote sensing datasets for various environmental conditions and prioritize and weight them appropriately to gain maximum accuracy in predicting ABB occurrence in the Northern Nebraska Prairie ecoregion.

Marking

1. Developed a permanent marking technique which showed the capacity for individual marks.
2. Tested for mortality associated with current permanent and temporary marks against the new method.
3. Tested the legibility of the new mark after prolonged exposure to moist soil.

4. Tested the efficiency of the new marking technique compared to current marking techniques.

Distribution Modeling

1. Generated a large presence/absence trap dataset in the target ecoregion.
2. Developed a preliminary model to test for accuracy based on the presence/absence data set.
3. Tested and improved the model based on the presence/absence data set.
4. Produced a final model including a visual map of ABB likelihood of occurrence for the target ecoregion.

CHAPTER II

ELYTRON-BRANDING AS A PERMANENT MARKING TECHNIQUE FOR *NICROPHORUS* BEETLES (COLEOPTERA: SILPHIDAE)

The American burying beetle, *Nicrophorus americanus* Olivier, (ABB) is a federally endangered species native to North America (USFWS 2008). Historically, the ABB inhabited 35 U.S. states and three Canadian provinces (Lomolino and Creighton 1996; Bedick *et al.* 1999). The current range of the ABB is limited to areas in Arkansas, Kansas, Nebraska, Oklahoma, Rhode Island, and South Dakota (USFWS 2008).

Because the ABB is an endangered species, sites are often surveyed to determine its presence or estimate the population size using mark-recapture methods (Krebs 1999). Population estimates through mark-recapture require some type of a mark to be placed on the beetle. An ideal mark is permanent, does not impede the activity of the beetle in any way, and does not cause any additional risk of mortality or reduced fitness to marked individuals (Kozal *et al.* 1988; Lomolino *et al.* 1995).

Non-permanent marks include queen bee marking tags and enamel paint (Butler *et al.* 2012). These marks may be lost after a few days (Jurzenski *et al.* 2011) and, bee

tags may accumulate moist soil, which can potentially affect survival and reproductive success (Butler *et al.* 2012). Permanent marking methods used on the ABB include burning a hole through an elytron using an electric cauterizer, or the removal of a small triangle from the rear (distal) edge of the elytron using scissors.

These existing permanent marks may impede flight, and elytron clipping can affect stridulatory communication and reduce brood success (Hall *et al.* 2015). Burning a small hole through the elytron has been used as a relatively safe method of creating a permanent mark (Butler *et al.* 2012). The most common injury resulting from using a cauterizer to burn a hole through the elytron is an inability to fold the wing under the elytron on the marked side. This may be a result of damage to the folding mechanism of the wing, or injury to the abdomen. The hole through the elytron likely exposes the wing to damage during burial, and likely affects flight which could impact the population through competition or lost ability to avoid predation. In addition, puncturing the elytron may affect the stridulatory structures of the ABB. Although elytron clipping did not cause direct mortality, Hall *et al.* (2015) found that beetles marked by elytron clipping had a significant difference in stridulation abilities, and substantially lowered brood success.

The purpose of this study is to test a new method of permanent marking, elytron-branding, and compare it to other marking methods currently used on *Nicrophorus* spp.

Materials and Methods

Laboratory testing occurred during the summer of 2014 in Payne County Oklahoma. *Nicrophorus* spp. were collected locally as needed for testing, and beetles

were not separated by sex. Mortality from marking and mark readability were tested with *N. orbicollis* (Say). Because of limited numbers of available *Nicrophorus*, tests of marking speed were performed on a mixture of *N. orbicollis*, *N. marginatus* (F.), *N. tomentosus* (Weber), and *N. pustulatus* (Herschel). After collection from the field, beetles were kept for no longer than a week prior to experimentation in a 37.85 L glass aquaria half filled with moistened soil, kept at room temperature, and on a 12:12 light:dark cycle. Beetles were provided water and ground beef *ad libitum*.

Based on laboratory results, marks applied with brands were used on ABB during summer sampling projects in Nebraska as part of field implementation with the permission of USFWS (B. Harms pers. comm.). These surveys use the standard five trap night protocol and captured ABB were marked with brands starting with the upper right maculation on the first day, then the lower right on the second day, upper left on the third day, and lower right on the fourth day of sampling. Beetles were not marked on the fifth day of sampling. During surveys, between June and August, 1,762 ABB were captured.

Mortality by Marking Technique.

N. orbicollis was chosen for mortality tests and mark readability in branding trials because it is the most closely related species to the ABB (Szalanski *et al.* 2000; Sikes and Venables 2013) and direct testing of mortality on the ABB is prohibited. Mortality associated with marking was assessed following procedures of Butler *et al.* (2012). Twenty individuals, 10 males and 10 females, were used for each of four treatments: elytron brand; elytron hole; bee tag; and control. All beetles were marked on the distal portion of the right elytron (Figure 2) with the exception of the control.

Elytron branding does not penetrate the elytron but instead ablates a maculation. Branding was performed using a medical cauterizer (Bovie High Temperature Model AA01®). We bent the tip to a 45 degree angle in relation to the axis of the handle to reduce the potential for penetrating the elytron and to improve ergonomics for the individual marking the beetles (Fig. 1). The unheated cauterizer tip was applied to the distal maculation of the right elytron while the beetle was held (Fig. 2). No attempt was made to separate the elytron from the abdomen. Once positioned, the cauterizer was then activated long enough to slightly melt the outer epicuticle and blacken the orange maculation, then immediately removed.

To create a hole through the elytron, an unmodified medical cauterizer (Bovie High Temperature Model AA01®) was heated for approximately two seconds until the element was visibly red. The beetle's thorax was lightly squeezed along the outer edges just hard enough to lift the elytra away from the wings and abdomen. A small hole was then burned through the distal portion of the right elytron.

Queen bee marking tags (The Bee Works©, Orillia, Ontario, Canada) were affixed to the distal portion of the right elytron over the lower (distal) maculation using Loctite© Gel Control Super Glue (contains cyanoacrylate). A small droplet of glue was placed on the elytron, then the tag was placed on the glue droplet with a pair of straight point forceps.

Control beetles were held by the pronotum in the same way beetles were held with other marking techniques. The control beetles were then lightly touched with the handle end of a marker on the lower right elytron.

After marking, all beetles were placed inside separate 207 ml condiment cups with snap shut lids along with a moistened cotton ball to provide both water and humidity. Lids of the containers were perforated to allow airflow. Beetles were checked for mortality every 12 h for three days (Butler *et al.* 2012). Beetles were kept on a 12:12 light:dark cycle at room temperature during the test period and no food was provided.

Marking Speed.

Timed trials of elytron-branding, burning a hole, elytron clipping, and application of queen bee marking tags were performed. For elytron-clipping, beetles were lightly squeezed along the beetle's sides to raise the elytra away from the wings and abdomen, and then dissecting scissors were used to cut a 3 mm triangle shaped piece from the distal edge of the left elytron. Other marking techniques were performed as above. Once each beetle was marked, the individual was placed into a holding container before moving to the next beetle. Each of the four techniques were repeated 10 consecutive times without pause and marking times for each beetle from the time it was picked up until it was returned to the container were recorded. Results of marking speed were evaluated using a single factor ANOVA ($\alpha=0.05$) followed by a Tukey HSD test to determine significant differences in marking speed. For the purpose of this test, the null hypothesis was that all techniques would be equal in speed and variation between individuals.

Elytron Branding Retention and Legibility.

Fifty *N. orbicollis* were used for this study. Twenty five beetles were branded in the manner described previously, and 25 were left unmarked. Marking was performed by me. Two containers were prepared using red clay soil from Payne County Oklahoma

which was chosen because it often sticks to beetles and affects legibility of marks in the field. Beetles were placed in their respective containers (marked or unmarked) and left for 48 hours. After 48 hours all beetles were placed into a single container and W. Wyatt Hoback, who had not previously seen the test beetles, attempted to separate marked from unmarked beetles.

Results

Mortality by Marking Technique.

No significant difference in mortality was found using ($\chi^2(3, N=80) = 0.054; p = 0.87$). One beetle died in the queen bee marking tag and elytron-branding methods, and two beetles died in the control group as well as in the group that was marked with a hole burned through the elytron.

Marking Speed.

Significant differences in marking speed were observed among techniques (F Critical = 2.86, F = 45.38, $p < 0.001$, N = 40). Mean time (± 1 S.D.) for branding the elytra (6.6 ± 0.30 s) and burning a hole through the elytra (7.8 ± 0.26 s) were not significantly different from one another (Fig. 3). Marking beetles by clipping the distal edge of the elytra and gluing a bee tag to the elytra took the longest (20.0 ± 1.71 s) (27.4 ± 2.39 s).

Mark Retention and Legibility.

In laboratory trials, branded beetles were distinguished from unmarked beetles with 100% accuracy without the use of magnification. In Nebraska field trials, ABB

were marked with brands in June and August. Of the 1,762 ABB that were marked, 195 were recaptured at least once, 18 ABB were recaptured at least twice, and 16 ABB were recaptured three times. Identifiably-branded individuals, including those marked in June were recaptured during the second activity period in August (Fig. 4B).

Discussion

To estimate population sizes, marking of captured ABB is required. Temporary marks, including paint and bee tags, are often lost as a result of daily periods where beetles dig into the soil. Butler *et al.* (2012) found that queen bee tags were retained by $75.5\% \pm 4.1$ S. E. of marked individuals after 2 weeks. This loss of tags and resulting error causes overestimation of population size. By comparing recaptures of dual marked ABB (queen bee tags and burned holes), the authors found that 19.7% of beetles lost their tags in the field. The resulting population estimate using queen bee tags was 22% higher than the estimate from permanent marks. Elytron modification through burning a hole and clipping are permanent marking techniques that have been shown to cause low mortality in laboratory tests (Butler *et al.* 2012). However, recent research has shown potential reduction in reproduction by clipped individuals as a result of damage to the stridulatory apparatus (Hall *et al.* 2015). Burning holes in the elytra causes low mortality under laboratory conditions. The most common injury resulting from hole burning is the inability to fold the wing under the elytron on the marked side. This may be a result of damage to the folding mechanism of the wing, or be a result of stress as a result of injury to the abdomen. Damage to the elytra exposes the wing to damage, and likely affects flight which could impact the population through competition or lost ability to avoid predation. In addition, puncturing the elytron may affect the stridulatory structures of the

ABB as found with elytron clipping. Hall, et al. (2015) found that beetles marked by elytron clipping had a significant difference in stridulation abilities, and substantially lowered brood success.

Therefore, a new permanent marking technique is needed. In the current study, elytron-branding was tested for mortality compared to attachment of queen bee tags and burning a hole through the elytron of *N. orbicollis*. Mark retention and legibility tests were performed for elytron-branding in the laboratory on *N. orbicollis*. Subsequently, ABB were marked in the field and marks were retained and still legible even after 2 mo. (fig. 4). A test of marking speed was performed for elytron-branding, hole burning, elytron-clipping, and queen bee marking tags on a mixture of *Nicrophorus* species. These species vary in size and biology and all were successfully marked without apparent harm or altered mortality rates.

For a number of population estimates, identification of individuals is required to track movement and multiple recapture events. Individually numbered queen bee tags have been used to track individuals. Queen bee tags are numbered one through 99 and in 5 colors for allowing 495 unique individuals to be followed. Unfortunately, queen bee marking tags are the slowest marking technique and result in additional handling of the beetles that likely leads to increases in stress. In addition, Butler *et al.* (2012) found that queen bee tags tend to cause soil to build up on the tag, which may interfere with flight or other behaviors. For this reason, the USFWS in Nebraska restricts the use of bee tags to August surveys (B. Harms, pers. comm.).

For marking with elytron brands, a possible branding strategy is as follows. Beetles caught once could be marked using elytron-branding on the upper or lower left or right elytra, beetles caught twice could be marked again in one of the three remaining regions and so on until all four possible marks were made on the elytra. If more than four captures of a beetle was expected, the cauterizer tip can be placed on only one half of a maculation resulting in a possible maximum of 8 marks. It would also be possible to use this eight mark technique to create an individualized identity mark. If each of the eight possible marks is evaluated separately there is a possibility of having 40,319 unique marks (8 factorial minus 1 for an unmarked individual).

In conclusion, elytron-branding provides an easy, efficient, economical, and potentially individualized marking system for *Nicrophorus* spp. including the ABB. Elytron-branding offers an advantage over queen bee marking tags or paint because it is permanent, much more efficiently applied, and can yield many more marks. Although not directly tested in this study, elytron-branding should allow permanent marks of any beetle with elytral maculations where permanent marks are needed for population monitoring. Tiger beetles, Scarab beetles, and Longhorn beetles (Family: Carabidae, Scarabaeidae, and Cerambycidae) of larger sizes are likely to be good candidates for this technique. Additional studies to assess the potential alteration of stridulatory abilities and brood success when elytron brands are applied to *Nicrophorus* should be undertaken to further document the effects of this technique.

Figures.

Figure 1. A. Cauterizer with the tip bent at a 45 degree angle. B. Position of the cauterizer on the lower left elytron maculation prior to heating the element to apply a brand.

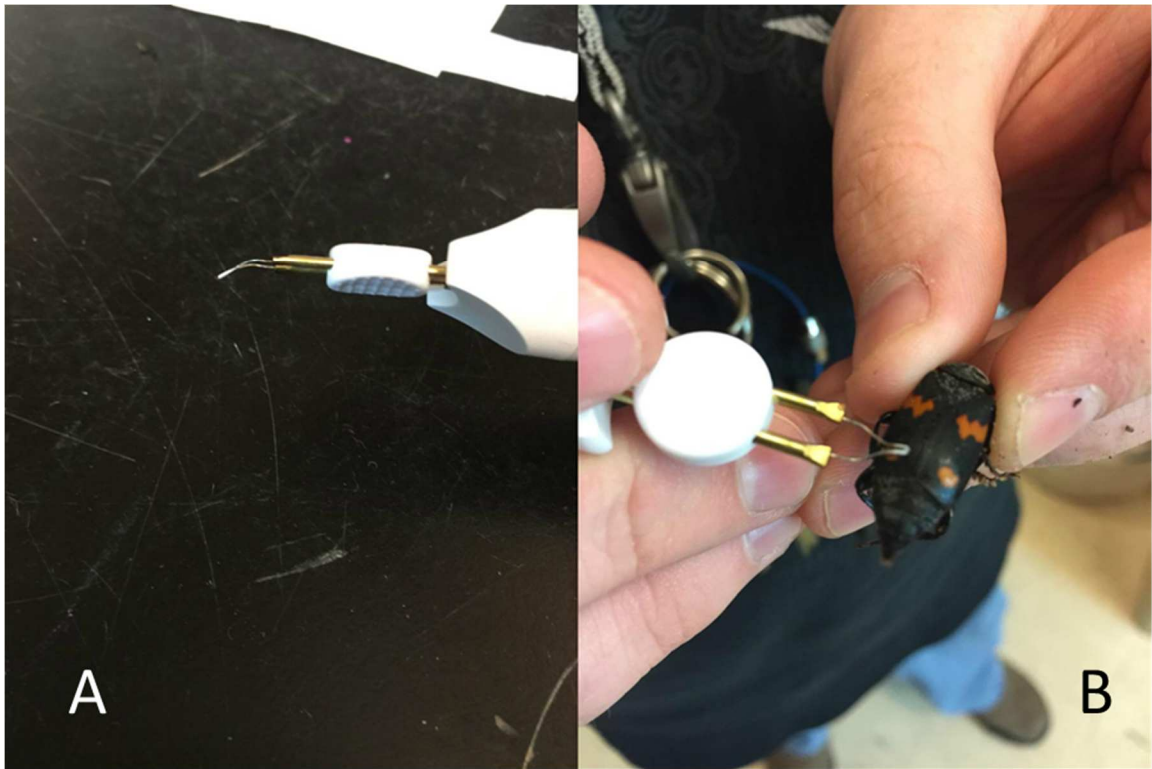


Figure 2. A. Mark created by Elytron-brand. B. Hole burn. C. Queen bee marking tag. D. Elytron-clipping.

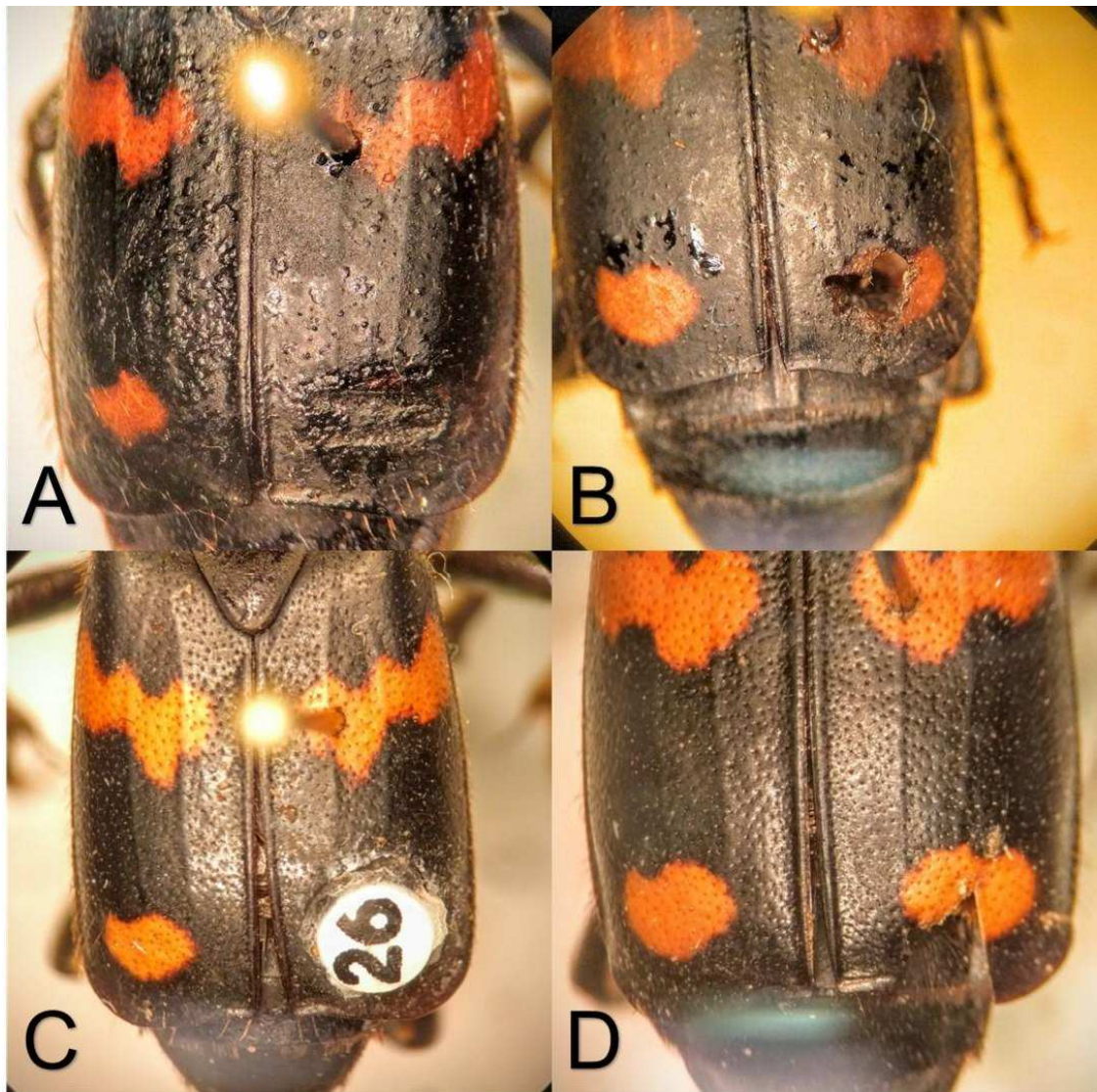


Figure 3. The time required to mark an individual beetle (mean \pm 1 S.D. Like letters denote means which are not significantly different ($\alpha = 0.05$).

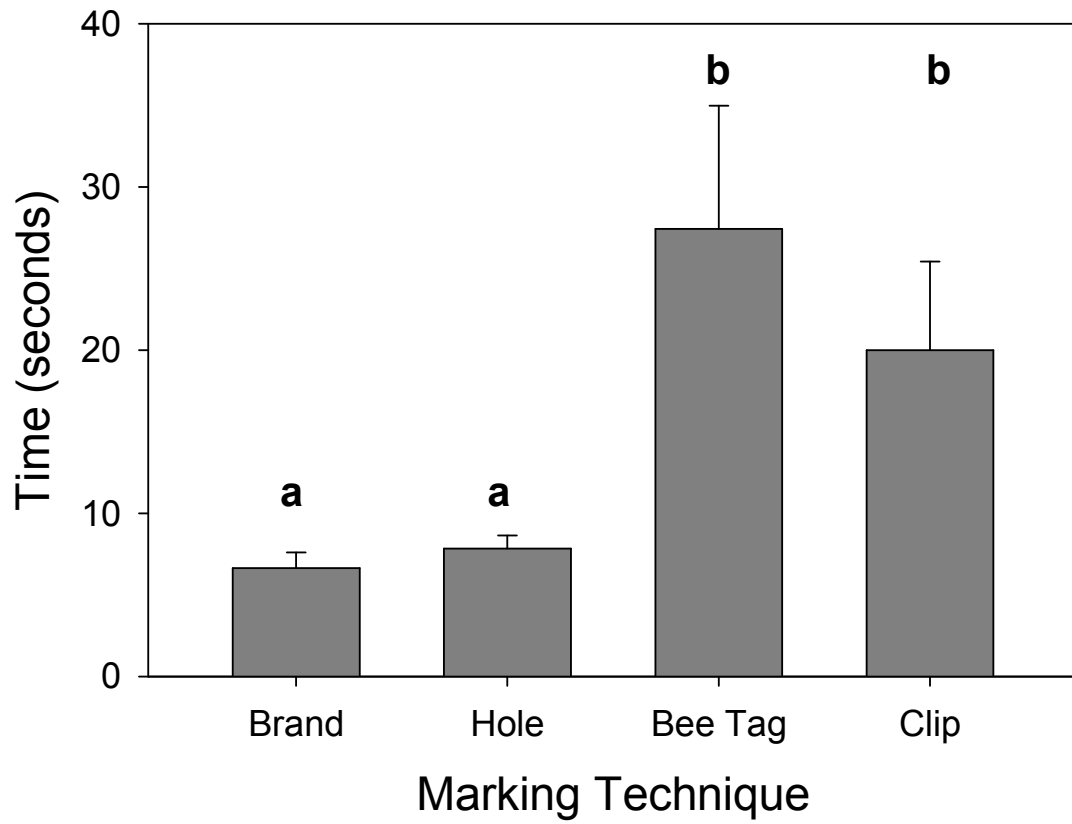
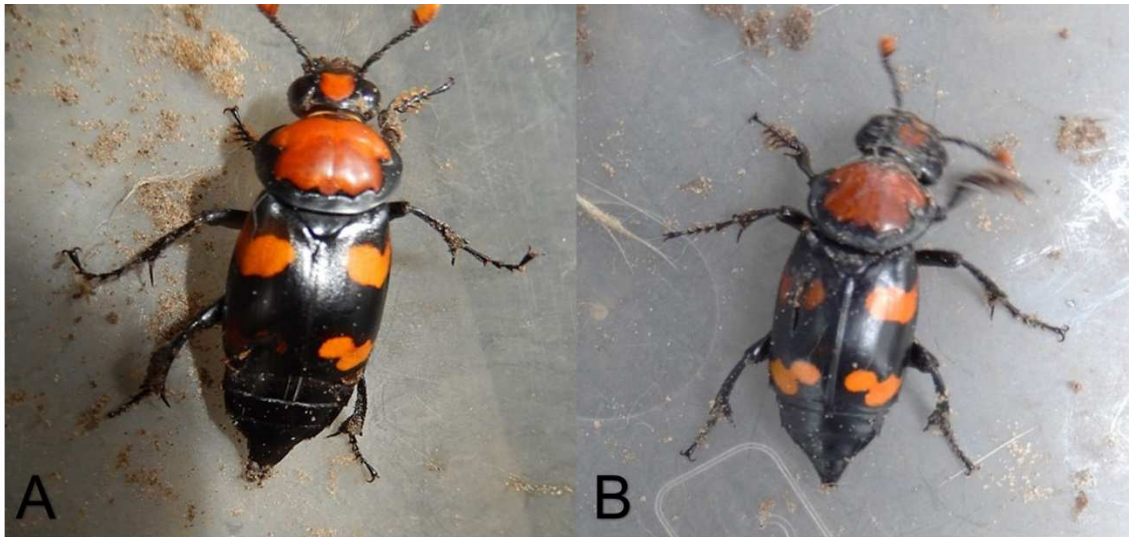


Figure 4. A. A teneral American burying beetle branded on the distal maculation of the left elytron. B. A senescent American burying beetle branded on the basal maculation of the left elytron.



CHAPTER III

DISTRIBUTION OF THE ENDANGERED AMERICAN BURYING BEETLE AT THE NORTHWESTERN LIMIT OF ITS RANGE

Determining occurrence and habitat preference is critical to conserving endangered and threatened species and their habitats (Jurzenski *et al.* 2014). A number of tools have been developed based on conducting surveys to establish presence and absence and then extrapolating likelihood of occurrence based on the model calculations.

The American burying beetle (ABB), *Nicrophorus americanus* (Olivier), is a federally endangered species native to North America (USFWS 2008). The ABB's range historically extended into 35 U.S. states and three Canadian provinces (Lomolino and Creighton 1996; Bedick *et al.* 1999). However, the current range is limited to areas of six states; Arkansas, Kansas, Nebraska, Oklahoma, Rhode Island, and South Dakota (Godwin and Minich 2005; USFWS 2008), representing a more than 90% reduction in range occupied by the ABB (Lomolino *et al.* 1995).

The ABB is characterized as a habitat generalist, and no critical habitat has been designated due to the variability or contradiction found among variables that are strongly linked

with ABB occurrence. Several models of predicted occurrence have been developed for Oklahoma and Nebraska where populations are associated with the Loess Canyons and Sandhills ecoregions (Crawford and Hogland 2010; McPherron *et al.* 2012; Jurzenski *et al.* 2014). Both the Loess Canyons and Sandhills models in Nebraska included validation and produced an AUC of 0.765 and 0.82, respectively (McPherron *et al.* 2012; Jurzenski *et al.* 2014). These models provide information to assist determining priority conservation areas and management of the remaining high quality habitat occupied by the ABB to be maintained. As part of the five year review of ABB (USFWS 2008) future conservation efforts should emphasize the preservation of large unfragmented habitat areas of the ABB and focus less on preservation of small fragmented habitat. One of the key tools for the implementation of such a plan is large scale predictive mapping to allow for identification of areas with high probability of ABB occurrence.

It is not possible to fully sample a large area for ABB presence/absence due access and the costs associated with handling an endangered species, and also placing and checking hundreds or thousands of traps both on public and private land. Because of this limitation, the use of tools that can make predictions outside the gathered datasets. This type of predictive tool uses the habitat conditions present in the area around a known trap site, and uses values of the environmental condition, along with presence or absence datasets, to predict occurrence in areas where sampling is impractical.

Previous studies undertaken in South Dakota overlap the Northwestern Plains ecoregions used in this model, but these studies were simply presence/absence datasets (Backlund and Marrone 1997) or a presence/absence datasets used to produce a population estimate (Backlund *et al.* 2008). These datasets were generated using variable

trap spacing from 0.2-3.22km, a non-standardized 5L bucket style trap, rotted beef kidney bait, and 7-8 trap night survey lengths. These variations of standardized methods inhibit the use of the data in my study due to the inaccuracy that occurs when combining incompatibly gathered datasets. It is possible to use these South Dakota studies to help determine priority areas for possible trap locations using standardized trapping protocols.

In my study, the distribution of the ABB in northern Nebraska and southern South Dakota were sampled and positive and negative trap locations were analyzed with environmental characteristics (Fig. 1) measured within an 800m radius around each site using GIS. We used ecologically relevant characteristics of climate, soils, land cover, and human impacts. Because our study area is at the extreme northwestern corner of the species' range, we hypothesized that decreasing annual precipitation would limit the western edge of the distribution and colder winter temperatures would limit the northern distribution. The predicted geographic distribution of the ABB and its correlations with environmental covariates were compared using two modeling approaches, a machine learning algorithm (random forest) and a generalized linear model (logistic regression).

Methods

Field Methods

Presence or absence of the ABB was determined at 456 sites in northern Nebraska and southern South Dakota (Fig. 5) from 2005 to 2015 (mostly post-2008) using federally approved bucket-style baited pitfall traps. Sites selected specifically for the generation of this model were selected using stratified random sampling methods. Ecoregions in the study area included the Northwestern Great Plains, the Northwestern Glaciated Plains,

and a small fragment of Nebraska Sand Hills (USEPA 2013). Sites were spaced at least 1600 meters apart to maintain independence of samples, based on the assumption that the effective sample radius of baited pitfall traps is about 800 meters. There is some empirical support for this effective trap radius (Leasure *et al.* 2012, Butler *et al.* 2013), but the trap radius is likely to be influenced by environmental conditions including wind and precipitation. Five nights of trapping were conducted at each site to minimize the chance of false negatives. Previous studies have estimated the probability of detecting an ABB population with baited pitfall traps to be about 50% for a night of trapping and between 85.7 (\pm 5.3% S.E.) to 93.7 (\pm 5.1% S.E.) after 5 consecutive trap nights (Leasure *et al.* 2012, Butler *et al.* 2013). This would result in a false negative rate of about 3-10% across five nights of trapping, and we considered this error rate was satisfactory for the purposes of this study (Butler *et al.* 2013; USFWS 2014).

Trap locations were selected by identifying areas in the Northwestern Prairie ecoregions that lacked presence/absence sample data in the last 10 years, were accessible by public roads, and were not directly within 1600m of previously sampled areas. Surveys were conducted using federally compliant 18.9L in ground bucket pitfall traps (USFWS 2014). These traps were dug into the ground with approximately 3cm of the bucket lip above ground to prevent the entrance of water during rain events. Soil was packed against the outside of the bucket lip to create a ramp to ease the entrance of beetles into the trap. Approximately 8cm of moistened soil was added to reduce competition among individuals, and protect against overheating and desiccation. Soil moisture was checked daily and water was added if needed. Each trap was baited with an extra-large previously frozen laboratory rat carcass (RodentPro.com®) which had been

rotted in a dark colored 18.9L bucket in the sun for 2-4 days, depending on temperature. Traps were covered using two, 5x5cm sticks cut into 45cm lengths and a piece of plywood measuring 45x45cm. The sticks were placed on the lip of the bucket in parallel to allow beetles space to enter the trap, and the plywood was then placed on top of the sticks. A large piece of sod was then placed on top of the plywood to prevent removal by scavengers or wind.

Upon capture of an ABB, the individual was aged, sexed, and its pronotum width measured (USFWS 2014). Because open bait allowed the direct contact between the ripened rat carcass and the captured beetle was used, additional handling and feeding time was not required. Beetles were released approximately 100m away from traps where they were caught to reduce the likelihood of artificially high recapture rates. At the release site beetles were released by using a stick or other implement to create an artificial burrow, releasing the beetle oriented into the hole which they readily crawl into, and brushing a small amount of loose soil or vegetation over the opening. This method likely reduces stress, potential predation, heat exhaustion, and desiccation unlike releasing the beetles on the surface or forcing the beetles to fly during daylight may cause. A single capture of ABB resulted in a positive result for the trap site, while no ABB over 5 trap nights resulted in a negative result.

Environmental Covariates

Based on hypothesized ecological relationships, we identified 16 environmental covariates to assess as predictors of ABB occurrence including metrics of climate, soil texture, human impacts, and land cover (Table 1). For comparability, an effort was made

to use similar predictors to those used in a previous study in the Nebraska Sandhills (Jurzenski *et al.* 2014). A combination of automated GIS scripts and manual GIS processing were used to delineate an 800 m sample area around each trap location and to summarize the underlying GIS layers representing our covariates within the circular sample areas surrounding each trap location (ESRI 2013, Python 2012). This process was repeated for grid a of points spaced 500m apart to collect covariate data throughout our study area necessary for mapping the expected distribution of the ABB.

Three climate metrics were selected as environmental covariates: annual precipitation, average minimum winter temperature, and average summer temperature (Table 1). I hypothesized that annual precipitation was related to overall ecosystem productivity and to desiccation risk. Burying beetles are susceptible to desiccation in dry environments leading to increased risk of mortality (Bedick *et al.* 2006). I also hypothesized that average minimum winter temperature was related to overwintering survival (Schnell *et al.* 2008), and that average summer temperature influenced habitat suitability related to temperature-dependent flight activity (Merrick and Smith 2004) during summer months when beetles actively search for reproductive carcasses.

Three soil texture covariates were selected: percent sand, silt and clay in the topsoil horizons, O (organic matter), and A (uppermost mineral based horizon with some organic matter incorporated) (Table 1). Soil texture has been identified as an important habitat characteristic for burying beetles related to suitability of soils for constructing underground brood chambers (Scott 1998). The ABB occurrences appear to be related with sandy loam soils (Lomolino *et al.* 1995, Jurzenski *et al.* 2014).

Five metrics of human influence were selected: road density, highway density, coverage of developed areas, coverage of crops, and coverage of hayfields (Table 1). These metrics could all have indirect effects on habitat suitability because of general habitat degradation and fragmentation that could affect availability of reproductive carcasses across the landscape (USFWS 1991; Jurzenski *et al.* 2014; McPherron *et al.* 2012). In addition to these indirect effects, intensive agriculture, hayfields, and developed areas could have direct negative effects on the ABB from soil disturbance and pesticide applications.

Five land cover metrics were selected: coverage of water, grasslands, wet prairies, wetlands, and forests (Table 1). In dry environments, availability of open water could conceivably benefit burying beetles at risk of desiccation, but in general we would expect open water to be negatively related to burying beetle abundance simply due to decreased availability of terrestrial habitats. Previous studies have indicated that ABBs were associated with grasslands and wet prairies in Nebraska (Kozol *et al.* 1988; Bedick *et al.* 1999, Jurzenski *et al.* 2014).

Analysis

Our response variable was presence or absence of the ABB at each site ($N = 456$). We had sixteen predictor variables, using the GIS-based environmental covariates measured within 800 meters of each site. Covariates were centered and scaled prior to analysis, except percentages which were left unscaled. We compared results from two modeling approaches, a generalized linear model and a random forest model (Breiman 2001).

The generalized linear model (logistic regression) was implemented using the R statistical programming language (R Core Team 2014). To avoid collinearity among predictors in the model, environmental covariates were screened to avoid Spearman correlation coefficients greater than 0.6. Nine of the 16 environmental covariates were selected for logistic regression (Table 1). Predictors were centered and scaled prior to analysis. Regression coefficients and p-values were used to infer the strength and direction of correlations among ABB occurrence and environmental covariates in the model. The influence of each observation on model parameters (leverage) was assessed graphically using the `glm.diag.plots` and `influence.measures` functions from the R package `boot` (Canty and Ripley 2014). We focused on the Cook's D and hat statistics. High leverage observations were removed to avoid a small number of points having a large pull on the predictions produced.

The random forest model was implemented using the R package “randomForest” (Liaw and Wiener 2002). Random forest is a machine learning algorithm that produces ensemble predictions from a large number of classification trees trained on bootstrap samples of the data. We used 10,000 trees in our model. To build each tree, the algorithm first selected four predictors at random and searched for a threshold that could be applied to one of them to best separate our samples into ABB presence versus absence sites. The best predictor and threshold were retained as the first node in the tree, and the algorithm moved to the next node in each branch (i.e. above and below the selected threshold) to randomly select a new set of four predictors to assess. We included all 16 predictors in the random forest model because the algorithm handles correlations among predictors. Model fit was assessed based on the “out-of-bag” classification error rate. Out-of-bag

error rates are produced by the random forest algorithm by making predictions at each site using only those trees in the model that did not include that site in their training data (see Breiman 2001 for more). These error rates are considered conservative estimates that reflect expected error when extrapolating the model to new sites within the study area.

The importance of predictors in the random forest model was assessed based on decreases in the Gini index (a measure of homogeneity in predicted presence and absence bins) resulting from random permutations of each predictor (Breiman 2001, Liaw and Wiener 2002).

To compare fit between the random forest model and the logistic regression, the area under the curve statistic (AUC) was calculated using the R package ROCR (Sing *et al.* 2005). AUC is a measure of how well predicted probabilities of the ABB occurrence fit our presence-absence observations. It is a threshold-independent fit statistic, meaning that we do not have to arbitrarily select a threshold for inferring presence or absence based on predicted probabilities of occurrence. We used AUC to compare model fit between our logistic regression and random forest models. AUC greater than 0.8 is considered a good fit (Franklin 2010). We identified a threshold for each model to convert probabilities of occurrence into binary presence-absence predictions that balanced false positive and false negative rates using R package ROCR (Sing *et al.* 2005).

Results

Of the 456 trap sites, 177 sites were positive, with a total of 1,201 ABB captured. Both models had relatively good fits to the data. The logistic regression model had an

AUC of 0.83 and a non-significant chi-square deviance test ($p = 0.529$). Five false negative trap sites were removed due to high leverage based on Cook's D and hat statistics. This is not surprising based on previous studies which have shown five percent or more beetles present in an area cannot be caught even under ideal conditions after five trap nights (Butler *et al.* 2013). The random forest model had an AUC of 0.82 and an out-of-bag classification error rate of 25.6%. A threshold of 0.4 to convert probabilities of occurrence to discrete presence-absence predictions balanced the false positive and false negative rates for both models. The predicted distributions of ABB from the two models also agreed closely (Fig. 6).

The importance of predictors in each model were noticeably different, except that minimum average winter temperature (twinter) was always a strong predictor (Fig. 7). For the random forest model the most important predictors (Fig. 7) were minimum average winter temperature, average precipitation (which was negatively correlated with minimum average winter temperature), clay, which was correlated with sand and silt, grasslands which were negatively correlated with crops, and roads. In the logistic regression, minimum average winter temperature and percent coverage of wet-grasslands had significant positive relationships with the presence of ABB, while the presence of water and forest had significant negative relationships (Fig. 8).

Discussion

This study represents the first model created specifically for predicting occurrence of ABB in the northwestern limit of its current range (McPherron *et al.* 2012; Jurzenski *et al.* 2014). Our results showed minimum average winter temperature as the strongest

single predictive factor in both the random forest and linear regression models, which was not included in either the Loess Canyons or Sandhills models (McPherron *et al.* 2012; Jurzenski *et al.* 2014). Average minimum winter temperature represents the average from 1950-2000. Correlation with warmer average winter temperatures may suggest that areas with a lower amount of temperature fluctuation during the winter months increase the likelihood of ABB occurrence along with areas with warmer climate. The Northern Plains lacks large bodies of open water, but the Ogallala Aquifer is close to the surface (McMahon *et al.* 2007). The areas of both the random forest model and generalized linear model with high likelihood of ABB occurrence lay in an area close to the surface groundwater from the Ogallala Aquifer (McMahon *et al.* 2007). This would suggest that minimum average winter temperature may be acting as a surrogate for proximity to subsurface water rather than simply minimum average winter temperature alone.

This correlation between ABB occurrence and proximity to subsurface water may partly explain the differences in habitat association between Nebraska and Oklahoma populations of ABB. Lomolino and Creighton (1996) noted that in its southern range, the ABB appeared to be a forest specialist. The results of this study contradict this finding as ABB had negative correlations with forest and open waters (often surrounded by trees in the study region). This may be another product of an association of Nebraska ABBs with the Ogallala Aquifer as a source of soil moisture and temperature stability. ABBs in Oklahoma cannot access aquifer moisture and likely depend on forestation and tree cover to help retain soil moisture (Lomolino and Creighton 1996; McMahon *et al.* 2007; Walker and Hoback 2007). ABBs present in the Loess Canyons were also found to associate with

water features and trees, and in these areas the Ogallala Aquifer is deep underground, and inaccessible to the beetles (McMahon *et al.* 2007; McPherron *et al.* 2012).

Precipitation was a strong predictor in the random forest model. This is in agreement with Jurzenski *et al.* (2014) with precipitation representing the strongest predictive factor in that study. ABB had negative associations with clay in the random forest model and this too would be in agreement with the Sandhills model as ABBs seem to prefer a more sand dominant soil texture, but will avoid areas without trace amounts of silt and clay as the soil is likely not stable enough to maintain a brood chamber (Jurzenski *et al.* 2014). The presence of development represented by crops and roads in the random forest model were negative predictors while areas with high percentages of grassland was a positive predictor of ABB presence. These findings would be consistent with past models and literature that states ABBs avoid areas of developed land such as agriculture (Sikes and Raithel 2002; McPherron *et al.* 2012; Jurzenski *et al.* 2014). Areas in this model designated 70 - 100% probability of presence should be considered for additional conservation measures and preservation as especially suitable ABB habitat.

Future studies contributing to the conservation and recovery of the ABB may include population density distribution over an area to better manage potential disturbances from development projects. The predictive power of this model and other predictive occurrence models is limited to only predict absence or predicted presence on average over a period of years. This long term trend prediction is not translatable into density estimation data as predictive occurrence models are based on long term climate trends and general habitat characteristics. The ABB is a highly mobile animal that is able to routinely travel 1.23km per night, as far as 2.9km in a single night, and up to 10km in

6 nights (Creighton and Schnell 1998). This gives the ABB the ability to move into areas with better real time conditions in terms of climate, food availability, and mate availability. These types of year to year fluctuations would render any density models built upon long term temperature or precipitation trends unacceptably inaccurate.

Future studies should seek to also utilize or generated detailed distribution data of not only the ABB but also common carrion sources. Carrion availability is likely the single most important factor in determining presence of ABBs, but due to the lack of fine scale distribution data across a broad range of possible carrion sources, such modeling efforts remain out of reach at the time of this study. While the possible loss of the passenger pigeon (*Ectopistes migratorius* L.) has been widely implicated as a driving force which contributed to the loss of habitat of ABB due to lack of suitable carrion sources, the wide scale suppression and loss of black tailed prairie dog towns as an ecosystem has not. Populations of black tailed prairie dogs are currently about 2 percent of their historical population size (Summer and Linder 1978). Black tailed prairie dog (*Cynomys ludovicianus* Ord) towns support a rich diversity of vertebrates as potential carrion sources of appropriate size for use by ABBs, but such an association has yet to be properly evaluated at depth (Sikes and Rathel 2002; Whicker and Detling 1988; Lomolino and Smith 2003).

Tables.

Table 1. Predictors used in the logistic regression (GLM) and random forest (RF) models.

GLM	RF	Covariate	Description	Citation
	•	precip	Average annual precipitation 1950-2000	Hijmans <i>et al.</i> 2005
•	•	twinter	Avg. min. winter temperature 1950-2000	Hijmans <i>et al.</i> 2005
•	•	tsummer	Avg. summer temperature 1950-2000	Hijmans <i>et al.</i> 2005
•	•	sand	% sand in top soil horizon	USDA 2006
	•	silt	% silt in top soil horizon	USDA 2006
	•	clay	% clay in top soil horizon	USDA 2006
•	•	road	Road density in 2011 (km / km ²)	USDC 2011
	•	hwy	Highway density in 2011 (km / km ²)	USDC 2011
•	•	develop	% coverage of developed areas	Homer <i>et al.</i> 2015
	•	crop	% coverage of crops	Homer <i>et al.</i> 2015
	•	hay	% coverage of hayfields	Homer <i>et al.</i> 2015
•	•	water	% coverage of open water	Homer <i>et al.</i> 2015
•	•	grass	% coverage of grasslands	Homer <i>et al.</i> 2015
•	•	wetgrass	% coverage of wet prairies	Homer <i>et al.</i> 2015
•	•	wetland	% coverage of wetlands	Homer <i>et al.</i> 2015
•	•	forest	% coverage of forests	Homer <i>et al.</i> 2015

Table 2. Regression coefficients for each predictor in the logistic regression model. Asterisks indicate statistical significance when alpha = 0.05 (*) or alpha = 0.01 (**).

Coefficient	Estimate	SE	p	
(Intercept)	0.812	0.132	< 0.001	**
tsummer	0.084	0.151	0.579	
twinter	1.25	0.146	< 0.001	**
sand	0.07	0.138	0.61	
develop	-0.041	0.145	0.779	
road	-0.169	0.151	0.265	
grass	0.201	0.15	0.179	
wetgrass	0.378	0.146	0.01	**
water	-0.605	0.293	0.039	*
forest	-0.75	0.216	< 0.001	*

Figures.

Figure 5. Study area and *N. americanus* sample sites.

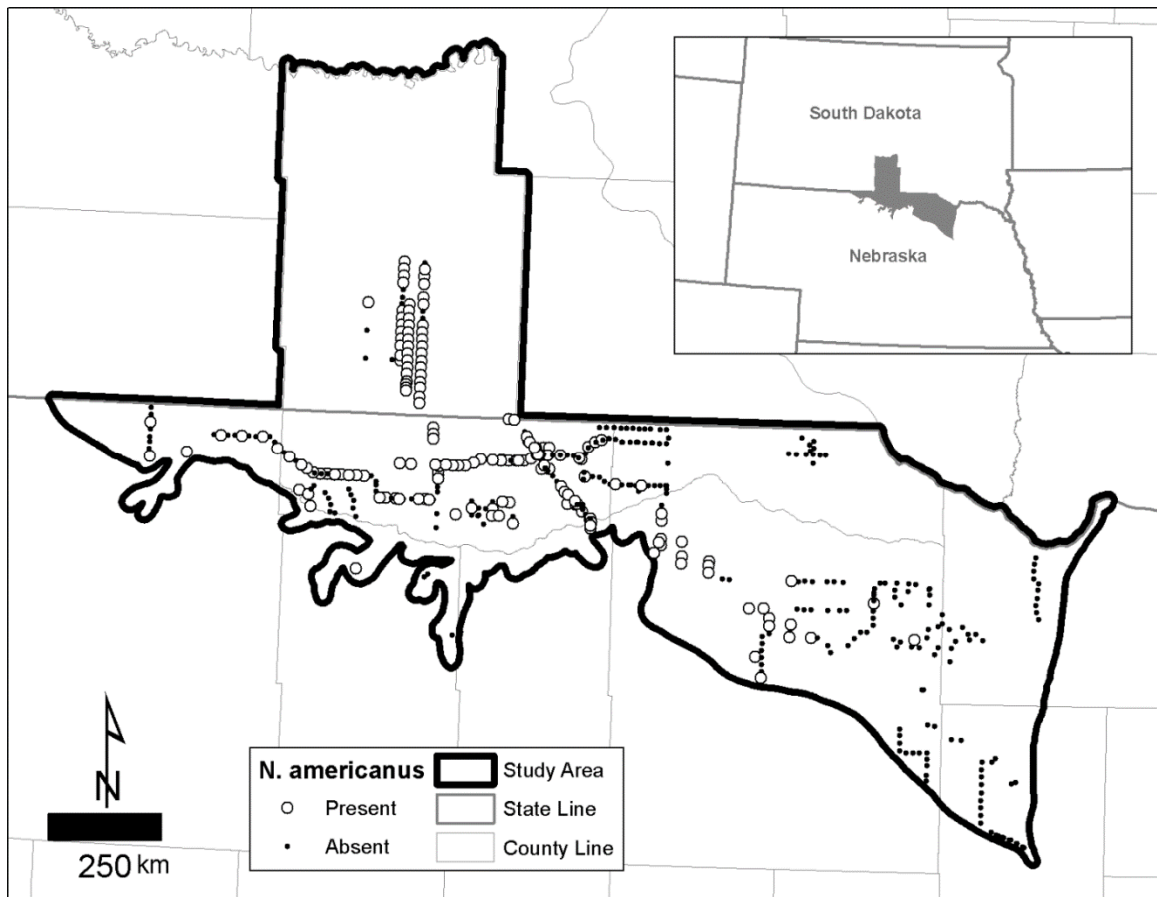


Figure 6. Model predictions throughout study area. Color schemes use a probability of occurrence of 0.4 as the threshold to distinguish a presence versus an absence site because this balances the rates of false positives and false negative in both models.

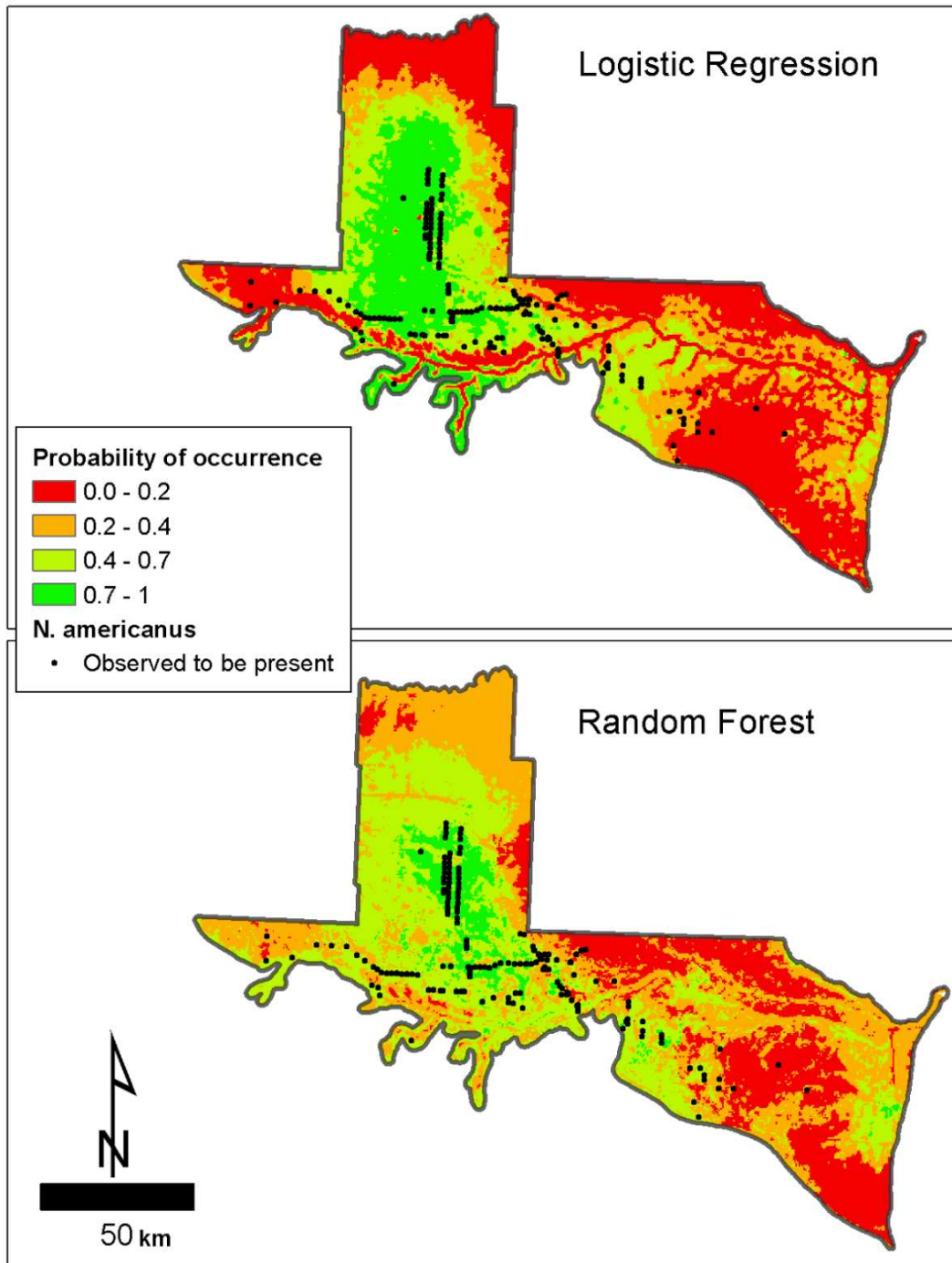


Figure 7. Variable importance of predictors in the random forest model.

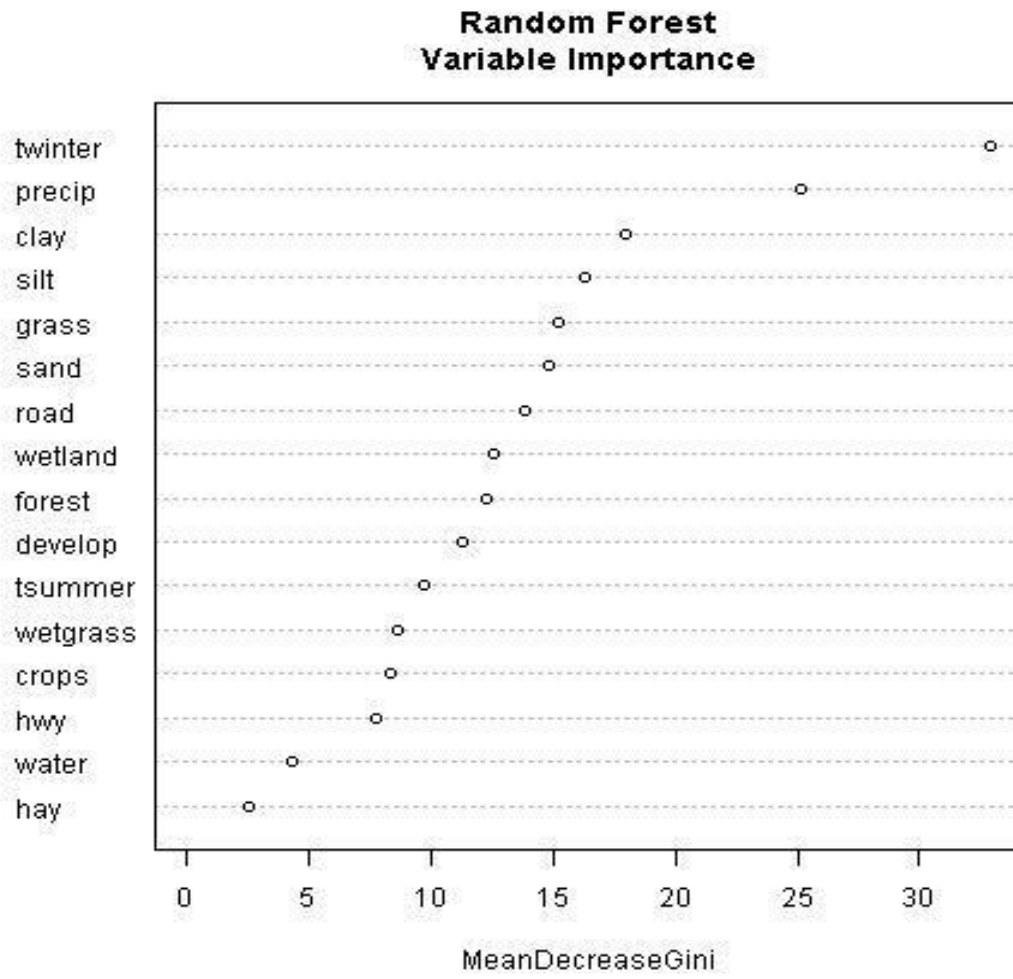
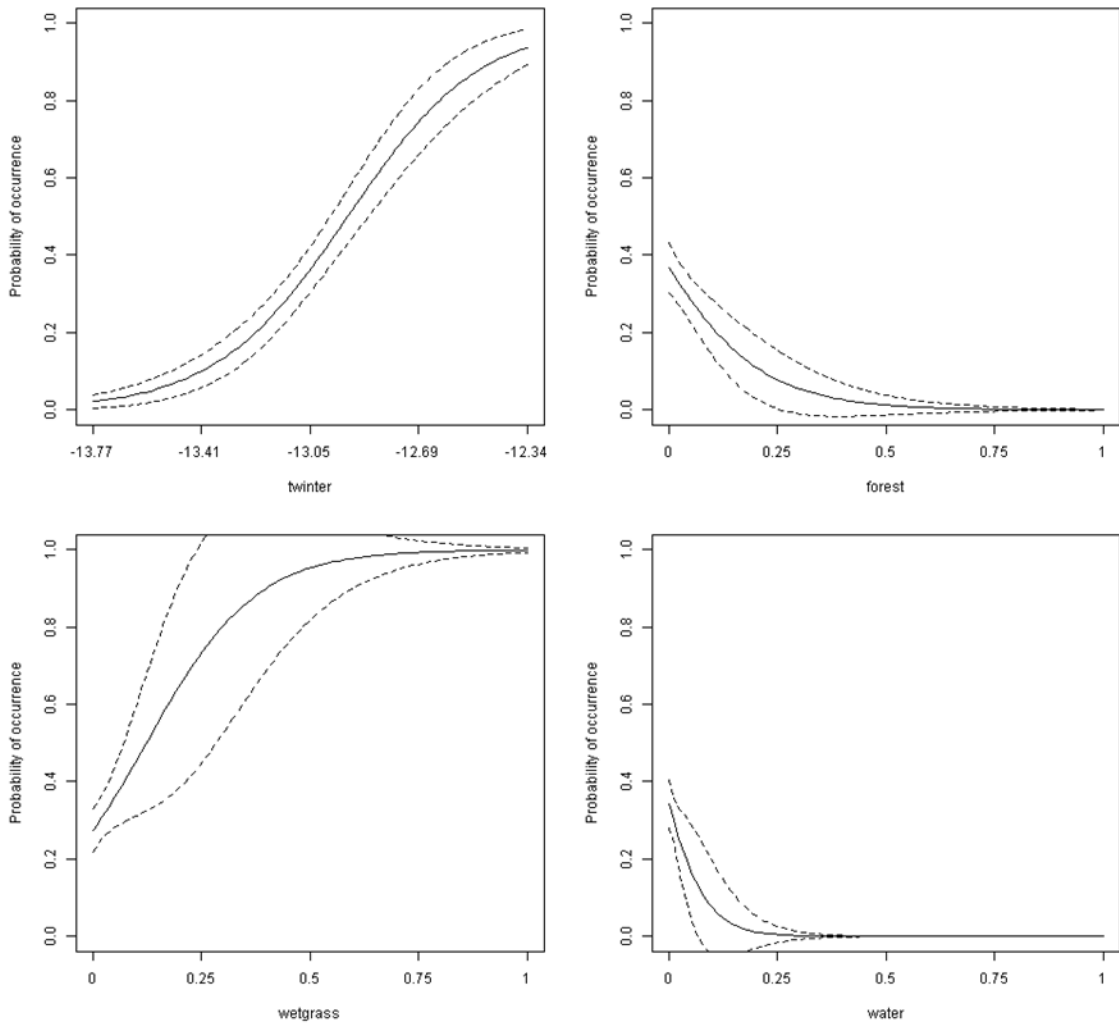


Figure 8. Partial regression plots of significant predictors in the logistic regression model.



CHAPTER IV

MANAGEMENT IMPLICATIONS OF ELYTRON BRANDING AND PREDICTED OCCURRENCE MODELING OF AMERICAN BURYING BEETLE IN THE NORTHERN PLAINS ECOREGIONS OF NEBRASKA

The objectives of this project were to develop a new, safe, and efficient means to permanently mark the federally endangered American burying beetle (ABB). The second objective was to produce an accurate model of predicted occurrence across the North Prairie ecoregions of Nebraska and South Dakota. Marking captured ABBs represents the majority of time spent directly handling beetles by technicians. Marking also represents the activity which may have the highest likelihood of directly causing stress, damage, or mortality to ABBs.

A number of permanent marking techniques have been used for the ABB including elytron clipping, bee tagging, elytron cauterization, tarsal clipping, and others. These techniques can potentially affect the biology of ABB, and a new technique which causes less damage to beetle health or behavior is needed. Elytron branding offers a permanent marking option that does not increase mortality when compared to bee tagging, elytron cauterization, and an unmarked but handled control group. Elytron

branding is also easier, faster, and safer for a technician to apply when compared with either bee tags, or elytron cauterization. This is because the cauterizer is stabilized on the elytron while cool before branding takes place. In contrast, elytron cauterization is similar, but the cauterizer is applied hot and not stabilized increasing the likelihood of causing damage to the beetle or its wings. Elytron cauterization also leaves an open hole through the elytron which could expose the membranous wings to damage from the cauterizing tool, the soil, or other abrasive particles.

Elytron branding was also the fastest of the marking techniques drastically reducing handling time needed to mark beetles when compared with bee tagging, or elytron clipping. This reduction in handling time may reduce beetle stress, and desiccation as the beetle is returned to the soil quickly.

Future work concerning elytron branding should include testing its effects on flight quality of marked beetles to ensure no reduction of mobility is associated with elytron branding. Testing elytron branded beetles in breeding trials would also be beneficial to evaluate if there are any effects on brood rearing by ABBs.

The need to be able to accurately predict occurrence across the range of ecoregions inhabited by the ABB is of high importance to management and recovery efforts for the species, as well as disturbance mitigation from development projects in suitable ABB habitat. Currently the farthest northern distribution of ABB in Nebraska and South Dakota is located in the Northern Prairie ecoregions. Predicted occurrence models were developed previously for all other areas of ABB occurrence across the state of Nebraska including the Loess Prairie and the Nebraska Sandhills. This study

completes the modeling of predicted occurrence of the ABB for Nebraska and the most important area of South Dakota.

Predicted occurrence models are an integral part of ABB research, conservation, and recovery. By prioritizing the conservation and preservation of areas with high habitat suitability and likelihood of occurrences for ABB it is possible to ensure habitat maintenance for ABB into the future. These models also help to make decisions associated with construction management in areas that are under development. If areas have been found to have a low likelihood of ABB occurrences, most projects could proceed with minimal delay or cost associated with surveys and preconstruction modification to conserve ABBs. On the other hand, if development is to take place in an area predicted to have a high likelihood of ABB occurrence because of suitable habitat conditions then mitigation, management, and monitoring plans can be put in place to minimize negative impacts to ABB.

In this study two models, a random forest and a generalized linear model, were produced to predict the occurrences of ABBs across the North Prairie ecoregions of Nebraska and South Dakota. The random forest model has the benefit of using a sophisticated computer learning algorithm to analyze coefficients of correlated covariates together to produce a model that has higher accuracy when compared to other modeling techniques. Random forest models have the benefit of typically higher accuracy where predicted occurrence is concerned. However, the random forest model does not have a mechanism which allows the analysis of each variable to be viewed. This is because of the intermingling of covariates which are unable to be individually analyzed during or after running the model.

This limitation of the random forest model lead to the inclusion of the generalized linear model in this study. Because general linear models use independent variables and produce a high amount of variable separation with operator control the generalized linear model can produce a result which can be interpreted more easily during and after the model is run. In this study the generalized linear model required the removal of five high leverage presence/absence data points. These points were diagnosed with the use of a Cook's D statistical analysis. These points could be the result of ABB being highly mobile and attracted to baited traps placed in generally unsuitable habitats.

The predictions of both models in this study reach the threshold of good fit (AUC 0.82 and 0.83 respectively), and as such are suitable for use as management tools for prediction of ABB occurrence. The random forest model did not require external intervention and also included more input variables which may lead to a higher accuracy in the ecoregions. For this reason it may be preferable to rely heavily on the random forest model's predictions than those produced in the generalized linear model in areas where the two models are in disagreement. Areas predicted to have the highest likelihood of occurrence in the random forest model should be considered for priority protection from development or modification of habitat. A total of 3,074.5 km² were identified as having a 0–20% likelihood of ABB occurrence based on the random forest model. These areas of very low likelihood of ABB occurrence should not require surveys to show absence, and resources may be utilized by surveying for possible presence in other areas shown to have a greater chance of ABB presence.

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APPENDICES

Trap#	UTMEast	UTMNorth	UTMzone	GPSCordinateSys	Year	County	Start#1	End#1	Start#2	End#2	TotABB
1	456215	4760730	14 North	Nad 83	2012	Keya Paha	8/3/2012	8/7/2012			0
2	454829	4760773	14 North	Nad 83	2012	Keya Paha	8/3/2012	8/7/2012			1
3	461294	4754280	14 North	Nad 83	2012	Keya Paha	8/3/2012	8/7/2012			0
4	463066	4754310	14 North	Nad 83	2012	Keya Paha	8/3/2012	8/7/2012			3
5	464431	4754334	14 North	Nad 83	2012	Keya Paha	8/3/2012	8/7/2012			1
6	462124	4752720	14 North	Nad 83	2012	Keya Paha	8/3/2012	8/7/2012			1
7	464071	4752725	14 North	Nad 83	2012	Keya Paha	8/3/2012	8/7/2012			3
8	464557	4749499	14 North	Nad 83	2012	Keya Paha	8/3/2012	8/7/2012			6
9	462718	4749469	14 North	Nad 83	2012	Keya Paha	8/3/2012	8/7/2012			3
10	469080	4741516	14 North	Nad 83	2012	Keya Paha	8/3/2012	8/7/2012			2
11	470906	4741520	14 North	Nad 83	2012	Keya Paha	8/3/2012	8/7/2012			2
12	472565	4741501	14 North	Nad 83	2012	Keya Paha	8/3/2012	8/7/2012			0
13	491112	4738398	14 North	Nad 83	2012	Holt	8/3/2012	8/7/2012			3
14	491073	4739941	14 North	Nad 83	2012	Holt	8/3/2012	8/7/2012			14
15	491059	4734287	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			8
16	491056	4732726	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			15
17	495927	4733718	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			1
18	495925	4730417	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			3
19	495924	4728792	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			2
20	502295	4729436	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			4
21	502295	4728489	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			3
22	502296	4726835	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			3
23	505537	4725474	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			0
24	507150	4725482	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			0
25	512011	4719076	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			3
26	513714	4707969	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			2
27	515410	4719082	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			1
28	516798	4716849	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			2
29	516809	4715159	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			2
30	516820	4713392	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			0
31	521648	4712723	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			1
32	521641	4714327	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			0
33	521719	4715580	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			3
34	526516	4712737	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			2
35	528069	4712728	14 North	Nad 83	2012	Holt	8/8/2012	8/13/2012			0
36	575233	4681285	14 North	Nad 83	2012	Antelope	8/7/2012	8/12/2012			0
37	574321	4680974	14 North	Nad 83	2012	Antelope	8/7/2012	8/12/2012			0
38	569584	4685743	14 North	Nad 83	2012	Antelope	8/7/2012	8/12/2012			0
39	570373	4686596	14 North	Nad 83	2012	Antelope	8/7/2012	8/12/2012			0
40	562229	4690502	14 North	Nad 83	2012	Antelope	8/7/2012	8/12/2012			0
41	559946	4690495	14 North	Nad 83	2012	Antelope	8/7/2012	8/12/2012			0
42	555983	4693488	14 North	Nad 83	2012	Holt	8/7/2012	8/12/2012			0
43	554299	4693675	14 North	Nad 83	2012	Holt	8/7/2012	8/12/2012			0

44	531261	4709546	14 North	Nad 83	2012	Holt	8/7/2012	8/12/2012			0
45	530516	4711145	14 North	Nad 83	2012	Holt	8/7/2012	8/12/2012			0
46	473030	4754264	14 North	Nad 83	2012	Keya Paha	8/12/2012	8/17/2012			0
47	474446	4755701	14 North	Nad 83	2012	Keya Paha	8/12/2012	8/17/2012			0
48	485423	4745016	14 North	Nad 83	2012	Boyd	8/12/2012	8/17/2012			0
49	483718	4746314	14 North	Nad 83	2012	Boyd	8/12/2012	8/17/2012			0
50	481521	4746314	14 North	Nad 83	2012	Boyd	8/12/2012	8/17/2012			0
51	491879	4746387	14 North	Nad 83	2012	Boyd	8/12/2012	8/17/2012			0
52	491977	4744627	14 North	Nad 83	2012	Boyd	8/12/2012	8/17/2012			0
53	490936	4741968	14 North	Nad 83	2012	Boyd	8/12/2012	8/17/2012			0
54	534609	4719225	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			0
55	533023	4719202	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			0
56	530302	4719175	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			0
57	526353	4719161	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			0
58	524755	4719148	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			0
59	523158	4719141	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			0
60	533710	4725660	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			0
61	531501	4725649	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			0
62	529923	4725612	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			0
63	526476	4725608	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			0
64	524714	4725596	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			0
65	523281	4725467	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			0
66	521573	4725550	14 North	Nad 83	2014	Holt	6/21/2014	6/26/2014			1
67	525838	4757712	14 North	Nad 83	2014	Boyd	6/21/2014	6/26/2014			0
68	525846	4756104	14 North	Nad 83	2014	Boyd	6/21/2014	6/26/2014			0
69	525865	4752586	14 North	Nad 83	2014	Boyd	6/21/2014	6/26/2014			0
70	525033	4755446	14 North	Nad 83	2014	Boyd	6/21/2014	6/26/2014			0
71	524887	4756981	14 North	Nad 83	2014	Boyd	6/21/2014	6/26/2014			0
72	523082	4758522	14 North	Nad 83	2014	Boyd	6/21/2014	6/26/2014			0
73	528677	4754695	14 North	Nad 83	2014	Boyd	6/21/2014	6/26/2014			0
74	527293	4754686	14 North	Nad 83	2014	Boyd	6/21/2014	6/26/2014			0
75	525657	4754674	14 North	Nad 83	2014	Boyd	6/21/2014	6/26/2014			0
76	524030	4754667	14 North	Nad 83	2014	Boyd	6/21/2014	6/26/2014			0
77	521780	4754658	14 North	Nad 83	2014	Boyd	6/21/2014	6/26/2014			0
78	520043	4754643	14 North	Nad 83	2014	Boyd	6/21/2014	6/26/2014			0
79	472910	4747947	14 North	Nad 83	2014	Keya Paha	6/27/2014	7/2/2014			1
80	474686	4747933	14 North	Nad 83	2014	Keya Paha	6/27/2014	7/2/2014			0
81	476249	4747929	14 North	Nad 83	2014	Keya Paha	6/27/2014	7/2/2014			0
82	477401	4747018	14 North	Nad 83	2014	Keya Paha	6/27/2014	7/2/2014			0
83	478727	4746326	14 North	Nad 83	2014	Keya Paha	6/27/2014	7/2/2014			0
84	480155	4746322	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			1
85	481589	4746327	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
86	483098	4746333	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
87	484707	4746283	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
88	486129	4746283	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			1
89	487708	4746365	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0

90	489084	4746373	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
91	490488	4746376	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
92	491792	4746388	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
93	475925	4759445	14 North	Nad 83	2014	Keya Paha	6/27/2014	7/2/2014			0
94	477477	4759472	14 North	Nad 83	2014	Keya Paha	6/27/2014	7/2/2014			0
95	479331	4759469	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
96	480870	4759461	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
97	482267	4759462	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
98	483759	4759462	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
99	485151	4759467	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
100	486400	4759456	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
101	487774	4759456	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
102	490002	4759450	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
103	491545	4759452	14 North	Nad 83	2014	Boyd	6/27/2014	7/2/2014			0
104	578289	4732670	14 North	Nad 83	2014	Knox	7/30/2014	8/4/2014			0
105	577955	4731214	14 North	Nad 83	2014	Knox	7/30/2014	8/4/2014			0
106	577879	4729584	14 North	Nad 83	2014	Knox	7/30/2014	8/4/2014			0
107	577851	4727902	14 North	Nad 83	2014	Knox	7/30/2014	8/4/2014			0
108	579020	4726773	14 North	Nad 83	2014	Knox	7/30/2014	8/4/2014			0
109	578881	4725278	14 North	Nad 83	2014	Knox	7/30/2014	8/4/2014			0
110	578782	4723454	14 North	Nad 83	2014	Knox	7/30/2014	8/4/2014			0
111	578602	4721695	14 North	Nad 83	2014	Knox	7/30/2014	8/4/2014			0
112	578803	4720117	14 North	Nad 83	2014	Knox	7/30/2014	8/4/2014			0
113	579340	4718673	14 North	Nad 83	2014	Knox	7/30/2014	8/4/2014			0
114	547964	4692525	14 North	Nad 83	2014	Holt	7/30/2014	8/4/2014			0
115	547975	4691072	14 North	Nad 83	2014	Holt	7/30/2014	8/4/2014			0
116	547985	4689412	14 North	Nad 83	2014	Holt	7/30/2014	8/4/2014			0
117	548014	4687576	14 North	Nad 83	2014	Holt	7/30/2014	8/4/2014			0
118	549470	4687195	14 North	Nad 83	2014	Holt	7/30/2014	8/4/2014			0
119	551088	4687206	14 North	Nad 83	2014	Holt	7/30/2014	8/4/2014			0
120	552539	4687216	14 North	Nad 83	2014	Holt	7/30/2014	8/4/2014			0
121	554372	4687230	14 North	Nad 83	2014	Holt	7/30/2014	8/4/2014			0
122	554406	4685830	14 North	Nad 83	2014	Holt	7/30/2014	8/4/2014			0
123	554414	4684425	14 North	Nad 83	2014	Holt	7/30/2014	8/4/2014			0
124	554438	4682217	14 North	Nad 83	2014	Holt	7/30/2014	8/4/2014			0
125	554447	4680854	14 North	Nad 83	2014	Holt	7/30/2014	8/4/2014			0
126	567207	4685702	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
127	567234	4683900	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
128	567257	4682271	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
129	567271	4680583	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
130	567282	4678579	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
131	567297	4676785	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
132	567311	4675214	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
133	567325	4673466	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
134	567336	4671775	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
135	577619	4666395	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0

136	575917	4667383	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
137	574267	4668083	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
138	572535	4668640	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
139	571100	4669188	14 North	Nad 83	2014	Antilope	8/5/2014	8/10/2014			0
140	535256	4711200	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
141	535256	4711200	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			1
142	538043	4714443	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
143	538043	4714443	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
144	539582	4714445	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
145	539582	4714445	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
146	540881	4719345	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
147	540881	4719345	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
148	540867	4720997	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			1
149	540867	4720997	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
150	540955	4724332	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
151	540955	4724332	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
152	543334	4725792	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
153	543334	4725792	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
154	546075	4725825	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
155	546075	4725792	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
156	545670	4724197	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
157	545670	4724197	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
158	545678	4722593	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
159	545678	4722593	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
160	547319	4721012	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
161	547319	4721012	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
162	548869	4723936	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
163	548869	4723936	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
164	553737	4721183	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
165	553737	4721183	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
166	556967	4718964	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
167	556967	4718964	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
168	556978	4716869	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
169	556978	4716869	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
170	555960	4716219	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
171	555960	4716219	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
172	553572	4713636	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
173	553572	4713636	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
174	550552	4712964	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			1
175	550552	4712964	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
176	547383	4712468	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
177	547383	4712468	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
178	551814	4711362	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
179	551814	4711362	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
180	555422	4709747	14 North	Nad 83	2014	Holt	6/21/2014	6/25/2014			0
181	555422	4709747	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0

182	437876	4741594	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			0
183	436106	4741610	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			1
184	434441	4741602	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			2
185	432769	4741587	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			0
186	430848	4741583	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			0
187	429128	4741617	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			1
188	426881	4741663	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			1
189	425277	4741739	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			1
190	424417	4743124	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			0
191	424433	4745299	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			0
192	423259	4746542	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			0
193	421555	4746566	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			1
194	419860	4746588	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			2
195	418120	4746622	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			5
196	416280	4746661	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			1
197	453474	4751146	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			1
198	455132	4751143	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			3
199	456900	4751127	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			7
200	458730	4751114	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			2
201	460259	4751734	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			4
202	461548	4752720	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			2
203	463235	4752717	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			0
204	465270	4752753	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			0
205	466967	4752770	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			1
206	468636	4752844	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			0
207	470301	4752612	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			0
208	471865	4751980	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			1
209	472870	4753226	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			0
210	473492	4754840	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			1
211	475013	4756059	14 North	Nad 83	2015	Keya Paha	6/16/2015	6/21/2015			1
212	414606	4746706	14 North	Nad 83	2015	Keya Paha	6/23/2015	6/28/2015			1
213	412969	4746801	14 North	Nad 83	2015	Keya Paha	6/23/2015	6/28/2015			2
214	411350	4746750	14 North	Nad 83	2015	Keya Paha	6/23/2015	6/28/2015			1
215	409732	4746946	14 North	Nad 83	2015	Keya Paha	6/23/2015	6/28/2015			2
216	408327	4747841	14 North	Nad 83	2015	Keya Paha	6/23/2015	6/28/2015			3
217	406954	4748716	14 North	Nad 83	2015	Keya Paha	6/23/2015	6/28/2015			2
218	405590	4749587	14 North	Nad 83	2015	Keya Paha	6/23/2015	6/28/2015			0
219	404153	4750502	14 North	Nad 83	2015	Keya Paha	6/23/2015	6/28/2015			3
220	402782	4751376	14 North	Nad 83	2015	Keya Paha	6/23/2015	6/28/2015			0
221	401328	4752304	14 North	Nad 83	2015	Cherry	6/23/2015	6/28/2015			1
222	400651	4753783	14 North	Nad 83	2015	Cherry	6/23/2015	6/28/2015			0
223	399513	4755000	14 North	Nad 83	2015	Cherry	6/23/2015	6/28/2015			0
224	397852	4755008	14 North	Nad 83	2015	Cherry	6/23/2015	6/28/2015			1
225	396213	4755016	14 North	Nad 83	2015	Cherry	6/23/2015	6/28/2015			0
226	394603	4755024	14 North	Nad 83	2015	Cherry	6/23/2015	6/28/2015			0
227	392956	4755033	14 North	Nad 83	2015	Cherry	6/23/2015	6/28/2015			1

228	391341	4755046	14 North	Nad 83	2015	Cherry	6/23/2015	6/28/2015			0
229	389691	4755056	14 North	Nad 83	2015	Cherry	6/23/2015	6/28/2015			0
230	388079	4755091	14 North	Nad 83	2015	Cherry	6/23/2015	6/28/2015			2
231	386445	4755134	14 North	Nad 83	2015	Cherry	6/23/2015	6/28/2015			0
232	476565	4756472	14 North	Nad 83	2015	Keya Paha	6/23/2015	6/28/2015			1
233	478133	4756888	14 North	Nad 83	2015	Keya Paha	6/23/2015	6/28/2015			0
234	479822	4756743	14 North	Nad 83	2015	Boyd	6/23/2015	6/28/2015			0
235	481371	4756250	14 North	Nad 83	2015	Boyd	6/23/2015	6/28/2015			0
236	483111	4756233	14 North	Nad 83	2015	Boyd	6/23/2015	6/28/2015			0
237	484992	4756250	14 North	Nad 83	2015	Boyd	6/23/2015	6/26/2015			0
238	486604	4756253	14 North	Nad 83	2015	Boyd	6/23/2015	6/28/2015			0
239	488233	4756249	14 North	Nad 83	2015	Boyd	6/23/2015	6/28/2015			0
240	489946	4756247	14 North	Nad 83	2015	Boyd	6/23/2015	6/28/2015			0
241	491579	4756235	14 North	Nad 83	2015	Boyd	6/23/2015	6/28/2015			0
242	553702	4724647	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
243	536610	4712811	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
244	540845	4723030	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
245	556219	4711401	14 North	Nad 83	2015	Holt	6/8/2015	6/14/2015			0
246	492090	4751654	14 North	Nad 83	2005	Boyd	N/A	N/A			0
247	491873	4745610	14 North	Nad 83	2005	Boyd	N/A	N/A			0
248	490484	4733467	14 North	Nad 83	2005	Holt	N/A	N/A			9
249	489504	4731029	14 North	Nad 83	2005	Holt	N/A	N/A			11
250	437309	4756826	14 North	Nad 83	2005	Keya Paha	N/A	N/A			17
251	438770	4746403	14 North	Nad 83	2005	Keya Paha	N/A	N/A			6
252	436997	4724531	14 North	Nad 83	2006	Brown	6/20/2006	6/25/2006	8/6/2006	8/10/2006	0
253	436246	4723965	14 North	Nad 83	2006	Brown	6/20/2006	6/25/2006			0
254	427337	4773552	14 North	Nad 83	2006	Tripp, SD	7/21/2006	7/30/2006			0
255	429229	4773486	14 North	Nad 83	2006	Tripp, SD	8/5/2006	8/11/2006			2
256	421169	4773658	14 North	Nad 83	2006	Tripp, SD	7/21/2006	7/30/2006	8/5/2006	8/11/2006	0
257	421265	4780073	14 North	Nad 83	2006	Tripp, SD	7/21/2006	7/30/2006	8/5/2006	8/11/2006	0
258	421330	4786494	14 North	Nad 83	2006	Tripp, SD	7/21/2006	7/30/2006	8/5/2006	8/11/2006	1
259	438826	4743341	14 North	Nad 83	2007	Keya Paha	6/16/2007	6/25/2007			0
260	438790	4744484	14 North	Nad 83	2007	Keya Paha	6/16/2007	6/25/2007			0
261	438781	4746420	14 North	Nad 83	2007	Keya Paha	6/16/2007	6/25/2007			3
262	438781	4748049	14 North	Nad 83	2007	Keya Paha	6/16/2007	6/25/2007			2
263	438787	4747221	14 North	Nad 83	2007	Keya Paha	6/16/2007	6/25/2007			1
264	438829	4749370	14 North	Nad 83	2007	Keya Paha	6/16/2007	6/25/2007			2
265	553091	4701724	14 North	Nad 83	2008	Holt	8/17/2008	8/19/2008			0
266	552775	4701726	14 North	Nad 83	2008	Holt	8/17/2008	8/19/2008			0
267	443137	4710800	14 North	Nad 83	2009	Brown	8/9/2009	8/13/2009			0
268	569956	4669713	14 North	Nad 83	2010	Antelope	8/10/2010	8/14/2010			0
269	571510	4669014	14 North	Nad 83	2010	Antelope	8/10/2010	8/14/2010			0
270	572929	4668473	14 North	Nad 83	2010	Antelope	8/10/2010	8/14/2010			0
271	574500	4667998	14 North	Nad 83	2010	Antelope	8/10/2010	8/14/2010			0
272	575962	4667339	14 North	Nad 83	2010	Antelope	8/10/2010	8/14/2010			0
273	577280	4666579	14 North	Nad 83	2010	Antelope	8/10/2010	8/14/2010			0

274	515216	4712626	14 North	Nad 83	2010	Holt	6/9/2010	6/9/2010			0
275	515218	4711036	14 North	Nad 83	2010	Holt	6/9/2010	6/9/2010			0
276	515218	4707823	14 North	Nad 83	2010	Holt	6/9/2010	6/10/2010	6/18/2010	6/20/2010	0
277	515220	4709409	14 North	Nad 83	2010	Holt	6/9/2010	6/10/2010	6/19/2010	6/21/2010	0
278	515252	4706203	14 North	Nad 83	2010	Holt	6/9/2010	6/10/2010	6/18/2010	6/20/2010	0
279	515261	4704593	14 North	Nad 83	2010	Holt	6/9/2010	6/10/2010	6/18/2010	6/20/2010	0
280	515276	4703222	14 North	Nad 83	2010	Holt	6/9/2010	6/10/2010	6/18/2010	6/20/2010	5
281	438669	4735231	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/5/2010			0
282	443093	4738355	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/6/2010			2
283	446813	4738309	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/5/2010			0
284	448379	4738238	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/5/2010			0
285	449632	4736341	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/5/2010			0
286	436936	4741595	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/6/2010			1
287	429955	4741589	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/5/2010			1
288	428179	4741637	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/5/2010			0
289	424439	4744498	14 North	Nad 83	2010	Keya Paha	8/2/2010	8/6/2010			0
290	420252	4725260	14 North	Nad 83	2010	Keya Paha	8/2/2010	8/7/2010			1
291	437346	4758312	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/10/2010			28
292	418011	4746625	14 North	Nad 83	2010	Keya Paha	8/2/2010	8/7/2010			1
293	414874	4746713	14 North	Nad 83	2010	Keya Paha	8/2/2010	8/7/2010			2
294	413271	4746807	14 North	Nad 83	2010	Keya Paha	8/2/2010	8/6/2010			0
295	411696	4746781	14 North	Nad 83	2010	Keya Paha	8/2/2010	8/6/2010			0
296	410096	4746774	14 North	Nad 83	2010	Keya Paha	8/2/2010	8/6/2010			0
297	437313	4756825	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/10/2010			43
298	437328	4755732	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/10/2010			39
299	432366	4749622	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/10/2010			10
300	429806	4749651	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/10/2010			9
301	438749	4738356	14 North	Nad 83	2010	Keya Paha	8/1/2010	8/5/2010			0
302	450165	4751205	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/24/2010			0
303	448586	4751216	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/26/2010			9
304	446935	4750415	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/27/2010			8
305	445071	4749611	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/24/2010			6
306	443469	4749623	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/25/2010			3
307	442076	4749612	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/25/2010			3
308	440521	4749614	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/25/2010			6
309	438064	4749645	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/25/2010			3
310	438839	4746414	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/25/2010			5
311	471410	4752165	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/24/2010			2
312	470176	4752641	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/24/2010			0
313	468694	4752865	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/24/2010			0
314	467103	4752784	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/24/2010			0
315	457359	4751110	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/24/2010			1
316	455611	4751144	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/24/2010			0
317	453314	4751117	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/24/2010			3
318	451364	4751177	14 North	Nad 83	2010	Keya Paha	6/20/2010	6/26/2010			6
319	474347	4738181	14 North	Nad 83	2010	Keya Paha	8/11/2010	8/15/2010			5

320	472515	4739853	14 North	Nad 83	2010	Keya Paha	8/11/2010	8/15/2010			2
321	471279	4741683	14 North	Nad 83	2010	Keya Paha	8/11/2010	8/15/2010			1
322	463802	4749502	14 North	Nad 83	2010	Keya Paha	8/11/2010	8/15/2010			0
323	462196	4752733	14 North	Nad 83	2010	Keya Paha	8/11/2010	8/15/2010			4
324	461326	4754282	14 North	Nad 83	2010	Keya Paha	8/11/2010	8/15/2010			12
325	459443	4755903	14 North	Nad 83	2010	Keya Paha	8/11/2010	8/15/2010			1
326	456159	4760622	14 North	Nad 83	2010	Keya Paha	8/11/2010	8/15/2010			1
327	492108	4757461	14 North	Nad 83	2011	Boyd	8/4/2011	8/8/2011			0
328	481264	4756266	14 North	Nad 83	2011	Boyd	8/4/2011	8/8/2011			0
329	484579	4756258	14 North	Nad 83	2011	Boyd	8/4/2011	8/8/2011			0
330	488273	4756263	14 North	Nad 83	2011	Boyd	8/5/2011	8/9/2011			0
331	380212	4750933	14 North	Nad 83	2011	Cherry	N/A	N/A			1
332	371731	4760954	14 North	Nad 83	2011	Cherry	6/12/2011	6/17/2011			0
333	371706	4759330	14 North	Nad 83	2011	Cherry	6/12/2011	6/17/2011			0
334	371671	4757532	14 North	Nad 83	2011	Cherry	6/12/2011	6/17/2011			1
335	371637	4755935	14 North	Nad 83	2011	Cherry	6/12/2011	6/17/2011			0
336	371591	4754148	14 North	Nad 83	2011	Cherry	6/12/2011	6/17/2011			0
337	371545	4753116	14 North	Nad 83	2011	Cherry	6/12/2011	6/17/2011			0
338	371531	4751441	14 North	Nad 83	2011	Cherry	6/12/2011	6/17/2011			0
339	371489	4750541	14 North	Nad 83	2011	Cherry	6/12/2011	6/19/2011			0
340	371751	4749703	14 North	Nad 83	2011	Cherry	6/12/2011	6/19/2011			1
341	458540	4757943	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			0
342	466404	4746623	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			0
343	467465	4745553	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			1
344	468298	4744316	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/7/2011			1
345	470203	4742605	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			0
346	469061	4743608	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/7/2011			1
347	470743	4742293	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/9/2011			1
348	471880	4741458	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			0
349	472570	4740389	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			0
350	473377	4739227	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			0
351	474424	4738339	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/7/2011			4
352	474658	4737516	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			3
353	459172	4756874	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/12/2011			8
354	459775	4755619	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/10/2011			3
355	461045	4754305	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/10/2011			3
356	461045	4754295	14 North	Nad 83	2011	Keya Paha	6/21/2011	6/25/2011			3
357	461745	4753026	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			0
358	462523	4751656	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			0
359	460559	4754340	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			0
360	463329	4750278	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/15/2011			10
361	464183	4748944	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			0
362	465334	4747801	14 North	Nad 83	2011	Keya Paha	8/2/2011	8/6/2011			0
363	474415	4738350	14 North	Nad 83	2011	Keya Paha	6/21/2011	6/25/2011			0
364	472569	4740360	14 North	Nad 83	2011	Keya Paha	6/21/2011	6/25/2011			0
365	471258	4741486	14 North	Nad 83	2011	Keya Paha	6/21/2011	6/25/2011			0

366	463678	4749515	14 North	Nad 83	2011	Keya Paha	6/21/2011	6/21/2011			0
367	461913	4752756	14 North	Nad 83	2011	Keya Paha	6/21/2011	6/25/2011			2
368	459774	4755629	14 North	Nad 83	2011	Keya Paha	6/21/2011	6/25/2011			0
369	463637	4749584	14 North	Nad 83	2011	Keya Paha	6/22/2011	6/25/2011			0
370	446849	4741539	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			0
371	455579	4741507	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			6
372	453957	4741525	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			4
373	451674	4741479	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			0
374	451666	4739871	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			4
375	446826	4739897	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			2
376	448436	4738399	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			0
377	448440	4739931	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			0
378	450061	4739899	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			0
379	451647	4738312	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			1
380	453189	4738287	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			3
381	456452	4736701	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			1
382	456468	4738312	14 North	Nad 83	2011	Keya Paha	5/26/2011	8/28/2011			0
383	471239	4752239	14 North	Nad 83	2011	Keya Paha	8/4/2011	8/8/2011			0
384	475205	4756120	14 North	Nad 83	2011	Keya Paha	8/4/2011	8/8/2011			0
385	473395	4754754	14 North	Nad 83	2011	Keya Paha	8/4/2011	8/8/2011			0
386	472847	4753235	14 North	Nad 83	2011	Keya Paha	8/4/2011	8/8/2011			0
387	472601	4748970	14 North	Nad 83	2011	Keya Paha	8/4/2011	8/8/2011			0
388	472595	4747293	14 North	Nad 83	2011	Keya Paha	8/4/2011	8/8/2011			0
389	478265	4756963	14 North	Nad 83	2011	Keya Paha	8/4/2011	8/8/2011			0
390	476872	4756545	14 North	Nad 83	2011	Keya Paha	8/4/2011	8/8/2011			0
391	474547	4736183	14 North	Nad 83	2011	Rock	8/2/2011	8/10/2011			5
392	474559	4736701	14 North	Nad 83	2011	Rock	6/21/2011	6/25/2011			4
393	550022	4711341	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
394	551835	4711354	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
395	546582	4711342	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
396	545813	4713003	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
397	540884	4716087	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
398	540875	4717725	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
399	540865	4719363	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
400	540851	4721672	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
401	540841	4723471	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
402	542179	4725762	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
403	548834	4725805	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
404	550459	4725656	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
405	553752	4724875	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
406	553764	4723343	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
407	553751	4715478	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
408	566564	4713319	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
409	564806	4714712	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
410	563397	4713491	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
411	563142	4714697	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0

412	561749	4716296	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
413	558588	4715704	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
414	560153	4713058	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
415	559356	4711439	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
416	559004	4708265	14 North	Nad 83	2012	Holt	6/12/2012	6/17/2012			0
417	415100	4737873	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			0
418	413895	4738170	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			0
419	414106	4739687	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			0
420	413470	4741131	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			0
421	412635	4742371	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			0
422	409360	4739329	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			2
423	406685	4742812	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			1
424	408772	4741975	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			3
425	409322	4743191	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			0
426	410036	4743981	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			0
427	418131	4743353	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			0
428	418639	4742080	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			0
429	419361	4740798	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			0
430	419322	4739182	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			0
431	419926	4737244	14 North	Nad 83	2012	Keya Paha	8/18/2012	8/22/2012			0
432	391077	4875807	14 North	Nad 83	2012	Lincoln	8/8/2012	8/17/2012			0
433	429468	4796047	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			1
434	429442	4794445	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			2
435	429453	4792837	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			1
436	429361	4791231	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			2
437	429389	4789623	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			0
438	429350	4787924	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			0
439	429235	4786410	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			0
440	429328	4784836	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			2
441	429313	4783189	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			1
442	429297	4781582	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			6
443	430946	4786355	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			1
444	430930	4784793	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			6
445	430918	4783153	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			5
446	430908	4781543	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			1
447	429247	4779969	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			15
448	429210	4778355	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			19
449	429213	4776755	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			20
450	429242	4774529	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			11
451	430888	4779938	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			5
452	430874	4778291	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			4
453	430975	4776730	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			13
454	430850	4775118	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			8
455	430838	4773526	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			3
456	430844	4771906	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			11
457	430811	4770418	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			8

458	430804	4768695	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			4
459	430776	4767959	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			6
460	430786	4767163	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			23
461	430768	4766601	14 North	Nad 83	2014	Tripp, SD	5/26/2014	5/30/2014			11
462	434259	4795905	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			0
463	434259	4794391	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			4
464	434259	4794391	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			17
465	434219	4792749	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			1
466	434203	4791005	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			4
467	434185	4788052	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			2
468	434166	4786346	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			13
469	434141	4784743	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			0
470	434134	4783138	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			0
471	434123	4781533	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			12
472	434114	4779910	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			11
473	434092	4778312	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			15
474	434078	4776712	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			22
475	434091	4775087	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			2
476	434047	4773449	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			7
477	434036	4771884	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			10
478	434020	4770244	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			2
479	434001	4768639	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			6
480	433993	4767360	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			8
481	433928	4765198	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			3
482	433953	4763821	14 North	Nad 83	2014	Tripp, SD	5/29/2014	6/2/2014			2

VITA

Tanner M. Jenkins

Candidate for the Degree of

Master of Science

Thesis: ELYTRON BRANDING AND PREDICTED OCCURRENCE MODELING
OF AMERICAN BURYING BEETLE IN THE NORTHERN PLAINS
ECOREGIONS OF NEBRASKA AND SOUTH DAKOTA

Major Field: Entomology and Plant Pathology

Biographical:

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Completed the requirements for the Master of Science in Entomology and Plant Pathology at Oklahoma State University, Stillwater, Oklahoma in May, 2016.

Completed the requirements for the Bachelor of Science in Environmental Resource Management at Chadron State College, Chadron, Nebraska in 2014.

Experience:

Research and teaching assistant in the Department of Entomology and Plant Pathology.

Conducted field research involving the federally endangered American burying beetle, and managed a field team to survey in remote areas.

Professional Memberships:

Member of the Entomological Society of America