A novel method to detect spotted knapweed (*Centaurea biebersteinii* DC.) using specially trained canines (grant awarded 2004)

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ABSTRACT

Invasive weeds continue to invade natural communities and permanently damage ecosystems at an alarming rate. Rapid weed spread results from small populations with high spread rates that go undetected. Although early control programs can slow weed spread, their efficacy relies on successful detection of incipient invasions. Complete ground inventories performed at adequate frequency remain the best method to detect new weeds, but such inventories are difficult and unlikely across large areas. Complete ground inventories may become practical and extensively implemented when augmented with the use of specially trained, point source detector dogs. Scientifically valid studies support the usefulness of canines as an effective detection technology for a variety of targets based on advantages in sampling efficiency, sensitivity, target noise discrimination, and gradient detection. Domestic dogs may be effective in sampling plant communities to detect early invasions of spotted knapweed (*Centaurea biebersteinii* DC.) based on these advantages and their ability to cover more area over manual, or human, searches. This study has successfully established proof of concept, demonstrating the ability of a specially trained canine to reliably detect spotted knapweed with high accuracy. The efficacy of the canine to discriminate spotted knapweed (n = 41) in a controlled environment was calculated as 96.5 percent. The mean accuracy of the canine to detect naturally occurring spotted knapweed (n = 91) in field trials across two testing sets was calculated as 90.1 percent (SE 3.4 percent). We will evaluate the feasibility of this novel spotted knapweed detection method by comparing the efficacy between specially trained canines and human surveyors to locate spotted knapweed incursions in field trials. If successful, this new method may improve early weed detection efficacy and slow invasive weed spread.

1. INTRODUCTION

Invasive weeds are considered the single greatest threat to rangeland ecosystem health and stability (Liebhold et al. 1995, USDI 1996) and one of the most serious problems facing western region land managers (Hobbs and Humphries 1995). Most invasive weeds have a competitive advantage (CAST 2002) over native species through resource preemption and greater net reproductive capacity (LeJeune and Seastedt 2001), resulting in native plant and animal displacement (Rejmanek and Richardson 1996). This alters wildlife habitat suitability (Trammell and Butler 1995), threatens native biodiversity (Pimm and Gilpin 1989, Randall 1996, Wilcove 1998, Kolar and Lodge 2001) and permanently damages (Coblentz 1990, Hobbs and Humphries 1995) the structure and function of biological communities and ecosystems (Vitousek 1986, Schofield 1989, Cronk and Fuller 1995, Kolar and Lodge 2001). Invasive weeds can have devastating economic impacts. Weeds in rangeland reduce forage yield and quality and increase the costs of producing livestock. This causes an estimated loss of $2 billion annually in the United States (Quimby et al. 1991).

Invasive weeds continue to invade natural communities (Asher and Spurrier 1998, Pimental et al. 2000, Buhler 2002) up to 14 percent annually (Whitson 1998, FICMNEW 1998, USGAO 2001). The rapid spread of invasive weeds occurs in spite of management efforts (USDI 1996) and current trends indicate weed spread will only continue to increase (Forcella and Harvey 1983). Rapid weed spread results from spatially distributed populations with high spread rates (Auld and Coote 1980, Auld et al. 1983, Mack 1985, Moody and Mack 1988, Simberloff 2003) that go undetected (Asher and Spurrier 1998). Radosevich et al. (2003) states small populations are often undetected during the introduction phase and small metapopulations, or groups of spatially separated populations of the same species which interact at some level, may remain undetected for extended time periods, setting the stage for further invasion (With 2002).

Rapid management response to small populations is crucial to preclude spread (Mack 1981, Navaratnam and Catley 1986, Moody and Mack 1988, Cousens and Mortimer 1995, Stohlgren et al. 1998, Radosevich et al. 2003) by significantly reducing the probability of colonization. Rapid response efforts, however, have been significantly hindered by the lack of effective early detection systems (USGAO 2001). Remote sensing misses local scales that are paramount in the detection of incipient invasions and modeling of weed invasions has had varying degrees of success (Radosevich et al. 2003). Ground inventories remain the best sampling method to detect new invasions (Radosevich et al. 2003). They are improved when periodically repeated at the highest level of detection confidence practicable (Dewey and Andersen 2005). Ground inventories are also improved when based on uncertainty where sampling is expanded to geographic borders or habitat limits, anticipating an invasion is larger than expected (Zamora et al. 1989). Such geographically extensive inventories are desperately needed (Keating et al. 1998), but may not be a reasonable goal based on the challenge (Zamora et al. 1989) and expense (Shafii et al. 2003) in finding new populations when density is very low (NRC 2002). Furthermore, Dewey et al. (1991) state it is unlikely we will ever have enough trained personnel needed to perform adequate inventories across large areas. Effective landscape-scale inventories are performed where one person can survey 20 to 40 ha per day with a detection confidence of at least 90 percent for patches 40 m^2 in size and larger (Dewey and Andersen 2005). This method, however, is not intended to detect isolated plants prior to reproduction or newly established patches prior to seedbank development. These early strategies are needed to maximize the chances of eradication success (DiTomaso 2000) and prevent rapid spread (Zamora et al. 1989). Eradication strategies must eliminate every plant from an infestation (Zamora et al. 1989) and unless eradication is total, the remnant populations become the foci for reinvasion (Mack 1985, Bazzaz 1986). Eradication treatments must be highly effective (Zamora et al. 1989), located strategically, and cover sufficient area to stop spread (Simberloff 2003). Complete ground inventories performed strategically and with adequate frequency and improved efficacy will improve the detection of new weeds to increase the chances of eradication success and stop spread. Such inventories may become practical when augmented with the use of specially trained, point source detector dogs.

Canine detection has been recognized as an effective method to cover more area than by manual personnel searches (Campbell and Rodda 1998, Lorenzo et al. 2003) and it seems domestic dogs (*Canis familiaris* L.) may be effective at locating invasive weeds in low density because of their high sensitivity to a target (Waggoner et al. 1998). Experts state dogs are the most effective and broadly usable detection technology currently available (OTA 1992, Joynt 2003). In spite of considerable investment and rapid advances in other types of sensor technologies, detector dogs remain the most widely used, broadly sensitive, accurate, fast, mobile, flexible, and durable system available for detecting a variety of targets (OTA 1991), lending a high-level of satisfaction to end-users (Myers 1992). The U.S. Department of Defense uses benchmarks set by detection dogs against which to measure their electronic vapor detectors (Joynt 2003), as the detection ability of dogs is better than instruments by many orders of magnitude (Göth et al. 2003, Phelan and Webb 2003). Experts have provided formal support for the usefulness of canines as an effective detection technology (Williams and Johnston 2002) based on advantages in sampling efficiency, sensitivity, target noise discrimination, and gradient detection (Waggoner et al. 1998). Furton and Myers (2001) state there is sufficient scientifically valid data to demonstrate dogs can be, and are, trained to reliably detect a variety of targets.

Dogs have an excellent ability to discriminate specific odors and are capable of smelling some odors in the tens of parts per billion to 500 parts per trillion (Johnston 1999). Odor discrimination studies have shown dogs are able to separate the target odor under conditions where the target odor was degraded by extremely high concentrations of a non-target odor, even when the non-target odor was orders of magnitude higher in concentration and overwhelming to humans (Johnston 1999). That is, dogs are able to discriminate extremely faint signals against a very noisy background. The ability of a dog to detect very low odor concentrations results from a very large number of olfactory receptor cells, together with the specialized behavior of sniffing (Gazit et al. 2003). Airflow allows odors to make contact with the olfactory mucosa and build concentration for odor sampling via odor-binding proteins that act as carrier molecules to the olfactory receptor molecules. Odor and receptor molecules then interact to allow for discrimination of separate odors followed by transduction, or transportation of the stimulus to the nervous system, and neural coding, or the process of coding these signals by neurons (Myers 1990). Modifying airflow pattern through sniffing enhances odor perception (Laing 1983) by increasing humidity in front of the nose (Fjellanger 2003). This works to increase the availability of odor molecules in the vapor drawn back into the nose to make contact with the remote areas of the olfactory mucosa (Neuhaus 1981, Youngentoub et al. 1987, Steen et al. 1996).

The sense of smell in domestic dogs has been successfully used for specific detection purposes for many years. The most obvious and widespread use of dogs in detection purposes is for hunting (Myers 1990). Dogs have also been trained to detect criminals, disaster victims, and missing persons (OTA 1991); human remains (Rebmann et al. 2000); marijuana, heroin, hashish, opium, and other drugs (Pearsall and Verbruggen 1982); scats of the endangered San Joaquin kit fox (*Vulpes macrotis mutica* M.) (Smith et al. 2003) and other specific animals (USDA 2003) demonstrating certain species are present in an area under study; endangered desert tortoises (*Gopherus agassizii* C.) (Cablk and Heaton 2005); smuggled foxes (*Vulpes* spp.) at airports (Barbeliuk 2001); invasive animals such as brown tree snakes (*Boiga irregularis* C.) (Engeman et al. 1998 and 2002) and gypsy moth egg masses (*Lymantria dispar* L.) (Wallner and Ellis 1976); harmful species such as screwworm (*Cochliomyia hominivorax* C.) (Welch 1990) and habu (*Trimeresurus flavoviridis* Gray) (Shiroma and Ukuta 1999), an extremely venomous snake in Japan; microbial growth in buildings (Kauhanen et al. 2002); prohibited fruits, plants, and meat at international airports and select mail facilities (USDA 1997); off-flavor compounds (Shelby 2004); leaks in natural gas pipelines (Johnson 1977); hazardous waste (Skovronek et al. 1987); accelerant and explosives, including landmines and tripwires (Hayter 2003, McLean 2003); and other substances.

Canines have increased inspection and survey efficiency for various targets with economic savings. Handwerk (2002) states U.S. Customs Service dogs can examine a vehicle for illegal narcotics in about five minutes, whereas even a cursory search by an inspector would take at least 20 minutes. Killam (1990) and Komar (1999) state the use of dogs to locate human remains is effective because they are accurate, relatively inexpensive, quick, thorough, and can cover large areas while functioning day or night. Canines offer a relatively low-tech and flexible option in difficult post-war situations for demining purposes where they can verify suspected land faster than manual (i.e., human) demining teams (Göth et al. 2003). Cablk and Heaton (2005) found canines were able to detect more and smaller desert tortoises than humans, which they state will improve efficacy of tortoise surveys. They report dogs found tortoises as small as 30 mm, whereas the smallest tortoise located by human survey teams was 110 mm. Detector dogs may be effective in locating spotted knapweed as isolated individuals and newly established patches across large areas. This study will evaluate the viability of using specially trained canines to sample plant communities for spotted knapweed incursions. If successful, this novel detection method may address the need for more early detection systems (USGAO 2001). The implications of this research for early invasive weed management are important in that detector dogs may be used to effectively and reliably sample plant communities for early spotted knapweed invasions either as a stand-alone method or in conjunction with other sampling methods. The use of invasive weed detector dogs may work to improve detection efficacy, increase the chances of eradication success, prevent reinvasion, and stop spread.

2. Objectives

The purpose of this project is to evaluate the potential of canine detection as a novel sampling method to locate new invasions of spotted knapweed (*Centaurea biebersteinii* DC.). A series of experimental trials were conducted with a single, specially trained canine to demonstrate proof of concept (Objectives 1 and 2). Objective 1 measured the effectiveness of the canine to correctly discriminate spotted knapweed odor with a minimum number of mistakes in a controlled environment. Discrimination was the process by which the canine selectively responded to the target odor among a number of other odors. Objective 2 measured the accuracy of the canine to detect naturally occurring spotted knapweed in an uncontrolled field setting. Accuracy was measured as the number of targets detected out of the number of targets available for detection. Detection was the process by which the canine discovered and alerted to the presence of the target odor in a field setting. A series of experimental field trials will be conducted in September 2005 to statistically quantify and compare the efficacy between canines and human surveyors to detect spotted knapweed incursions (Objective 3). Efficacy will be measured as the ability to successfully detect spotted knapweed targets with high accuracy, maximum detection distance (i.e., the distance from the target to the canine or surveyor upon detection), and minimum search duration. Canine efficacy will be a measure of the usefulness of detector dogs as an early detection survey tool, which can then be compared with preliminary measures of human abilities to locate spotted knapweed incursions. The primary purpose of Objective 3 is to quantify the efficacy of canines to detect naturally occurring spotted knapweed. Secondary is to quantify the efficacy of human surveyors across the same trials for initial comparison to canines. This will support exploratory data gathering and method development for purposes related to future investigation outside of thesis work.

Objective 1: Quantify the number of correct and incorrect discriminations (i.e., effectiveness) of spotted knapweed by a specially trained canine through a series of odor discrimination trials in a controlled environment.

Hypothesis 1_0: A specially trained canine is not able to discriminate spotted knapweed with high effectiveness in a controlled environment.

Hypothesis 1_a: A specially trained canine can discriminate spotted knapweed with high efficiency in a controlled environment.

Objective 2: Quantify the accuracy of a specially trained canine to detect naturally occurring spotted knapweed through a series of field trials.

Hypothesis 2_0: A specially trained canine is not able to detect naturally occurring spotted knapweed with high accuracy in field trials.

Hypothesis 2_a: A specially trained canine can detect naturally occurring spotted knapweed with high accuracy in field trials.

Objective 3: Quantify and compare the accuracy, detection distance, and search duration of canines and surveyors to detect spotted knapweed incursions through a series of field trials.

Hypothesis 3_0: Canines and surveyors can detect spotted knapweed incursions with equal accuracy, detection distances, and search durations in field trials.

Hypothesis 3_a: Canines can detect spotted knapweed incursions with lower accuracy, greater detection distances, and more rapid search durations compared to surveyors in field trials.

3. Methods

3.1 Controlled Trials - Objective 1

Canine

One 2.5 year old, female, Rocky Mountain shepherd was used in this study. She was trained in basic obedience and selected for the study based on physical and behavioral health and not assessed for scenting ability to ensure an average canine was selected. A local handler trained the canine. The canine is the property of Montana State University, Department of Land Resources and Environmental Sciences. The housing and care of the canine was supervised by Montana State University, Animal Resource Center.

Target weed and odor source material

Spotted knapweed is a state-listed noxious weed (MDA 2003), infesting over 1.6 million ha in Montana (Duncan 2001). Spotted knapweed is declared noxious in 14 other states (USDA 2004). This weed has been associated with increased soil erosion (Lacey et al. 1989) and changes in wildlife habitat suitability (Hakim 1979). Spotted knapweed impacts the livestock industry by lowering yield and quality of forage, interfering with grazing, increasing costs of managing and producing livestock, and reducing land value (DiTomaso 2000). Displacement of native plants by spotted knapweed impacts Montana cattle producers over $42 million annually (Hirsch and Leitch 1996). Spotted knapweed was chosen as the target weed based on its ability to invade and dominate a variety of healthy and relatively undisturbed plant communities (Rutledge and McLendon 1998), increasing the probability it will continue to spread (Zamora et al. 1989). Localized spread occurs through peripheral enlargement of existing stands (Watson and Renney 1974). Long distance spread occurs as seed transport in contaminated crop seed and livestock forage, and through wildlife and livestock dispersal and human activity (Sheley et al. 1999). Seeds are also transported along waterways and roadsides whereby spread occurs into adjacent grasslands (Tyser and Key 1988). Based on estimates of selected climate and physiographic parameters, Rice et al. (1997) predicts the potential range of spotted knapweed in Montana is the majority of the state. Effective early detection of spotted knapweed is crucial to preclude spread and further invasion throughout Montana and the western region.

A variety of spotted knapweed odor source materials were used to train the canine. Logan (1976) states using a variety of stimuli (i.e., spotted knapweed odor) improves training by facilitating persistence in the learning of a response, such as scratching or sitting as a response to an odor. Previously frozen stem and leaf material was used to establish a scent association with the canine during fall / winter 2003. This material was also used as a target odor source during exploratory odor discrimination trials conducted during March 2004. Previously frozen stem and leaf material was originally clipped from naturally occurring plants, sealed in airtight plastic bags, immediately frozen during fall 2003, and thawed as needed. Spotted knapweed field transplants were used for formalized odor discrimination trials during July 2004. Transplants were maintained in a greenhouse at Montana State University, Plant Growth Center. For the purpose of this study, the dominant vapor constituents of these odor source materials were collected and analyzed with a volatile collection system and gas chromatography coupled with a mass spectrometry detector (instrument specifications forthcoming). This work was conducted by Montana State University, Department of Entomology. Vapor was collected and analyzed from field transplants ($n = 6$), transplants severed from the root crown to represent clipped material ($n = 6$), and previously frozen material (mean weight 14.3 g; SE 2.7 g). Methods describing volatile collections and analyses will be included at a later date. Table 1 presents summary results of the volatile analyses, completed during December 2004. Vapor analyses demonstrate the odor chemicals produced by fresh clipped (< 24 hrs old) and previously frozen spotted knapweed material (>24 hrs old) is in poor agreement to the odor chemicals produced by living spotted knapweed. However, the canine did not seem to display any difficulty in recognizing the active odor chemicals produced among the various odor source materials as target substances. It seems the canine was able to generalize among the odor chemicals, likely using a bouquet of vapor constituent information to detect the various target substances that were presented. Determining which odor chemicals the canine used for detection is important for understanding the basic science of canine detection and is also useful in improving performance and training aids for developing reliable canine detection methods (Göth et al. 2003, Lorenzo 2003).

Training procedure

Training a detector dog involves two lessons. First, the canine must learn an association between the target odor and a reward, and second, the canine must learn to give a specific response in order to gain access to the reward. The association between the target odor and the reward induces the animal to search for the target odor, while the response provides an "indication" that alerts the handler to the location of the target (Hilliard 2003). Training the canine these lessons was based on the process of instrumental conditioning where the dog learned to associate a voluntary behavior, or the instrumental response, with its result, or the consequence. Positive reinforcement was a consequence that encouraged prior behavior, such as play and praise to reinforce an alert response to the target, while negative reinforcement was a consequence that discouraged prior behavior, such as a verbal correction and task repetition to reinforce an alert response to a non-target.

The canine was trained to discriminate living spotted knapweed plants with standard narcotics detection training protocol (Robicheaux and Jons 1996) involving a line of cement cinder blocks, or "scent line," where one block contains the target scent among other identical blocks not containing the target scent. Procedures outlined in Hilliard (2003) to train canines to detect the odor of a landmine from suspect land were also incorporated. Air from suspect land is vacuumed through a filter and transferred to a canine at a controlled testing station for area reduction of full clearance landmine operations. Additional information was found useful and incorporated to better understand dog behavior, learning and motivation (Logan 1976), and odor perception (Engen 1982).

Initial training sessions for the canine began in October 2003 with daily obedience training and ended with scent association training at the end of each daily session. A regime based on retrieval (USCS 1979) was used to establish the scent association. Previously frozen spotted knapweed material was wrapped in a small towel and secured with duct tape as a retriever-training dummy. The dummy was repeatedly thrown by the handler for the canine to retrieve. The canine was praised and reinforced to an enthusiastic game of tug-of-war upon retrieval. Once the canine was consistently retrieving the scented training dummy, we performed a series of controlled search drills. This involved training the canine to search for the target scent by randomly hiding the training dummy in one of ten widely spaced cement cinder blocks in a field setting. The remaining cinder blocks contained blank training dummies, or training dummies without the target odor. On the command "find-it," the dog would search for the odor by sampling each position. The canine was trained to remove the target dummy from the target sampling position as the alert response.

Once the canine was consistently providing alert responses to the target odor, we performed a series of exploratory discrimination trials. The purpose of these trials was to develop methodology for formalized controlled odor discrimination trials (Objective 1). Exploratory trials also worked to improve training of the canine in preparation for formalized odor discrimination trials. This was accomplished by challenging the dog to ignore novel, non-target scents and focus on discriminating for the target odor through instrumental conditioning. Exploratory trials were conducted during March 2004 in two metal buildings with dirt floors located in Gallatin County, Montana. Ten training sessions were conducted over a period of 14 days. Each session was comprised of nine trials. Each discrimination trial consisted of ten sampling positions comprised of cement cinder blocks and arranged in a parallel array. Each sampling position contained plastic scent tubes (Figure 1) where one scent tube contained previously frozen spotted knapweed material, two scent tubes were empty, and seven scent tubes each contained a non-target odor. These odors included wood, sugar, tea, tobacco, soil, superfatted soap, bouillon cubes, celery flakes, dill seed, etc. Non-target scents were chosen based on novelty to the dog and to challenge the canine to ignore new odors (Matre 2003). Training to ignore novel, non-target odors augments the capability of odor discrimination (Johnston 1999) and decreases false negative alerts (Williams and Johnston 2002). Each scent tube randomly changed for each new trial. Clean target scent tubes were used for each new trial to avoid odor cues from saliva and human handling during reward sessions. Alert response to the target odor was a removal of the plastic tube containing the target odor. Alert responses to the target were positively reinforced with praise and play with the target plastic tube. Correctly ignoring non-target odors were not reinforced. Missing a target resulted in repetition of the task until the dog alerted on the odor. Alert responses to non-target dummies were negatively reinforced with a verbal correction and repetition of the task. Data collected during correction procedures were not used for analysis. Ninety trials were performed. Two blank trials were conducted during each session where there was no target to detect. Average temperature and humidity across sessions was calculated as 9 degrees C and 46 percent, respectively. Seventy spotted knapweed (n = 70) targets were available for detection across the set. The canine sampled 587 positions represented by target, blank, and non-target odors. The canine missed two targets and gave three false alerts (i.e., alert response when no target). Discrimination accuracy was calculated as 97.1 percent. Discrimination for the target odor as previously frozen spotted knapweed material was demonstrated and the alert response was established.

Experimental setting

Formalized odor discrimination trials were conducted during July 2004 in a 12 x 8 m metal building with a concrete floor located at Rocky Mountain Command Dogs training facility in Gallatin County, Montana. Seven testing sessions were conducted during a seven-day period. Each session was comprised of seven trials. Each trial consisted of eight sampling positions arranged in a linear array. Each position was approximately 1 m apart. The blank position contained moist soil and the target position contained living spotted knapweed to emphasize a natural odor signature and concentration to the canine (Johnston 1999). The remaining six positions contained native, non-target plants. The target position randomly changed for each new trial. The other positions randomly changed for each new session. Forty-nine trials were performed. One to two blank trials were conducted during each session where there was no target to detect. Forty-one spotted knapweed (n = 41) sampling positions were available for the canine to detect across the testing set. Average temperature and humidity across sessions was calculated as 33 degrees C and 23 percent, respectively.

Non-target plants and blank soil worked as controls to ensure the canine was responding to the target odor. Non-target plants were chosen based on availability and nativity to Montana. These included prairie coneflower [*Ratibida columnifera* (Nutt.) Woot. & Standl.], littleflower penstemon (*Penstemon procerus* Dougl. ex Graham), western pearly everlasting [*Anaphalis margaritacea* (L.) Benth.], white prairie clover (*Dalea candida* Michx. ex Willd.), wholeleaf Indian paintbrush (*Castilleja integra* Gray), and Sandberg bluegrass (*Poa secunda* J. Presl). Native forbs were purchased as greenhouse-grown plugs and transplanted into six-inch diameter pots filled with a pasteurized soil mixture consisting of 1/3 silt loam, 1/3 sand, and 1/3 peat. Spotted knapweed and Sandberg bluegrass were transplanted from a field site located in Gallatin County, Montana into six-inch diameter pots with the same soil mixture. Blank positions contained equal amounts of pasteurized soil mixture and soil from the field site. Plants were maintained in a greenhouse at Montana State University, Plant Growth Center for one week and transplanted into cement cinder blocks that rested on a plywood base (Figure 2). A fine wire mesh was placed over each block to prevent visual discrimination opportunities to the canine. A second cinder block was placed on top of the first (Figure 3) and identical, empty plastic tubes were placed on top of the wire mesh in each position. Alert response to the target was a removal of the plastic tube. Praise and play with the plastic tube was used as the reinforcer throughout the trials. Correctly ignoring non-target or blank sampling positions were not reinforced. Missing a target resulted in repetition of the task until the dog alerted on the odor. Alert responses to non-target odors were negatively reinforced with a verbal correction and repetition of the task. Data collected during correction procedures were not used for analysis. Clean tubes were placed in the target position before each new trial to avoid odor cues from saliva and human handling during reward sessions. Clean tubes were placed across all sampling positions before each new session.

Positions were placed outside to maintain plant health between sessions. Plants were replaced when they appeared stressed or unhealthy.

Testing procedure

Single-blind trials were performed where the scorer knew the details of each trial but the handler and canine did not. The handler and canine would enter the building at the beginning of each trial and on the command "find-it," the canine would independently sample each position off-leash while the handler remained stationary. When the dog alerted, the handler queried the scorer by asking, "hit?" If the dog responded to a non-target or blank, the scorer replied "no," a verbal correction was given, and the task was repeated until the dog ignored the odor. If the position contained the target, the scorer would reply, "hit," the handler would reward the dog, and the trial would end. If the dog missed the target, the scorer informed the handler of the miss after all positions were sampled and the task was repeated until the dog indicated. Data collected during correction procedures were not used for analysis. Each trial returned any of the following possible responses by the canine, which were recorded by the scorer: (1) alerted to the target (hit), (2) correctly ignored non-targets or blank (correct rejection), (3) alerted to non-target or blank (false positive), and (4) missed the target (miss).

3.2 Field Trials - Objective 2

Canine

One 2.5 year old, female, Rocky Mountain shepherd was used in this study. She was trained in basic obedience and spotted knapweed scent discrimination. A local handler trained the canine. The canine is the property of Montana State University, Department of Land Resources and

Environmental Sciences. The housing and care of the canine was supervised by Montana State University, Animal Resource Center.

Target weed and odor source material

Spotted knapweed was chosen as the target weed based on its widespread distribution in Montana (Duncan 2001) and its ability to invade and dominate a variety of healthy and relatively undisturbed plant communities (Rutledge and McLendon 1998) throughout the western region. Spotted knapweed may increase soil erosion (Lacey et al. 1989) and change habitat suitability for wildlife (Hakim 1979). Spotted knapweed impacts the livestock industry by lowering yield and quality of forage and increasing costs of producing livestock (DiTomaso 2000). Displacement of native plants by spotted knapweed impacts Montana cattle producers over $42 million annually (Hirsch and Leitch 1996). Spotted knapweed continues to spread through peripheral enlargement of existing stands (Watson and Renney 1974), contaminated crop seed and livestock forage, wildlife and livestock movement, and human activity (Sheley et al. 1999). If allowed to continue to spread, Rice et al. (1997) predicts the potential range of spotted knapweed in Montana could represent the majority of the state. Effective early detection of spotted knapweed is crucial to preclude spread and further invasion throughout Montana and the western region.

Spotted knapweed plants were transplanted from the field into cement cinder blocks for scent training and discrimination testing. This odor source material was also used to train the canine to scratch at the target odor as an alert response. Once the canine was consistently providing this alert response to the target odor, we used fresh clipped material from naturally occurring spotted knapweed plants as training aids for search drills in preparation for field trials. Clipped material that was older than one hour was discarded and not used as a training aid. Naturally occurring spotted knapweed plants were used as the odor source material for field trials. To establish which odor chemicals were available to the canine for detection, the dominant vapor constituents of spotted knapweed transplants and clipped material (< 24 hrs old) were collected and analyzed with a volatile collection system and gas chromatography coupled with a mass spectrometry detector. This work was conducted by Montana State University, Department of Entomology. Table 1 presents summary results of the volatile analyses. We attempted to collect and analyze vapor from six naturally occurring spotted knapweed plants ($n = 6$) during June and August 2004, but we were unsuccessful in collecting enough vapor to conduct an adequate analysis. Although the clipped material analysis indicates a poor chemical fit to the transplants and likewise to naturally occurring plants, the canine did not seem to display any difficulty in transitioning among the odor source materials. Previous studies have stated dogs learn to recognize a target using only one or two of its most abundant vapor constituents (Williams et al. 1997). We believe, however, that the canine was likely using a bouquet of vapor constituent information produced among the materials to detect the target. This is suggested as the canine was originally trained on previously frozen material, of which the dominant acetate was entirely absent (Table 1). Using a variety of odor source materials during training may have been beneficial to facilitate persistence in the learning of an alert response (Logan 1976) and odor generalization to variants. Johnston (1999) states the more varieties of a substance dogs are trained on, the more likely they are to alert to non-trained variants, such as other knapweeds.

Training procedure

The canine was trained to discriminate the natural odor signature and concentration (Johnston 1999) of spotted knapweed with a series of controlled odor discrimination trials coupled with instrumental conditioning. We conditioned the canine to associate voluntary behavior with positive or negative reinforcement during odor discrimination trials. An alert response to the target odor was positively reinforced with play and praise and an alert response to a non-target was negatively reinforced with verbal correction and task repetition. Controlled odor discrimination trials were based on standard narcotics detection training protocol (Robicheaux and Jons 1996) using a line of double stacked cement cinder blocks containing target and non-target odors, or “scent line,” and procedures outlined in Hilliard (2003) to train canines to detect the odor of a landmine from suspect land at a controlled testing station. The canine was trained to remove an empty plastic tube from the target cinder block as an alert response to the target. The dog was then trained to scratch at the target odor as a suitable alert response to naturally occurring spotted knapweed targets in preparation for field trials. This self-rewarding (Livingood 2004) indication response keeps the dog highly motivated (Robicheaux and Jons 1996) and is a natural and spontaneous alert response by most detector dogs (Welch 1990, Bulanda 1994, Shiroma and Ukuta 1999, Kauhanen et al. 2002). Training the canine to scratch at the target odor as an alert response was accomplished by positioning the second cinder block on top of the first in a diagonal manner. This positioning allowed the canine to see, but not easily remove, the plastic tubes. This encouraged the canine to scratch at the target position in an attempt to obtain the reward. Alert scratching responses to the target were reinforced with play and praise.

After the canine was competent at scratching as an alert response to the target, we subjected the dog to numerous search drills in a variety of field settings (Syrotuck 1972, Bryson 1976) with naturally occurring spotted knapweed plants and training aids comprised of clipped material (< 1 hour old) from naturally occurring spotted knapweed plants. Training the canine to search for the scent was based on procedures used to train "point source oriented" detector dogs where the single element source of the target odor is located in an area. Point source detector dogs independently work an area off-leash and cast back and forth until either the target odor is located or it is established the target odor is not present. Once the canine is aware the odor is present, it follows increasing levels of odor intensity and finds the target point source of strongest concentration. Point source detector dogs are very versatile in application as once the dogs have learned the basic lesson to "go find the source," they can easily search out any given area and determine if the target odor source is present at the site (Syrotuck 1972). Training techniques included, but were not limited to, those used to train dogs to detect narcotics (Robicheaux and Jons 1996), human remains (Rebmann et al. 2000), and to scent track (Syrotuck 1972, Bryson 1976, Johnson 1977, Pearsall and Verbruggen 1982, Davis 1987, and Bulanda 1994). Additional information was found useful in understanding dog behavior, learning and motivation (Logan 1976), and odor perception (Engen 1982).

Study areas

Field trials were conducted in a plant community grazed by cattle and dominated by cheatgrass (*Bromus tectorum* L.) and introduced and native forbs. Thirteen small ($9 m^2$) and seven large (9 x 18 m) search areas were developed in an area of high spotted knapweed density at the edge of an infestation located in Gallatin County, Montana. Two $9 m^2$ search areas were blank. Although the ultimate purpose of this project is to evaluate the potential of canine detection as a novel sampling method to locate spotted knapweed in low density, a high density site was chosen to maximize target sample size. Our primary focus was to determine the accuracy of a specially trained canine to detect naturally occurring target plants in a community of other naturally occurring, non-target plants. Search areas were delineated with flagging and based on the lowest spotted knapweed density present without modifying natural occurrence. Forty-six spotted knapweed plants (n = 46) occurred across sites as juvenile and adult plants. Mean size of targets in height and width was calculated as 27.1 x 39.4 cm (SE 12.5 x 17.5 cm). Mean density of targets was 2.56 plants per site (SE 1.22). Targets predominately occurred as isolated individuals across each site. Three sites contained patches comprised of three to four individuals. A patch was defined as more than one individual target within a 1 m diameter area. Each patch was recorded as one target location. Detection of an individual within the patch was considered detection of the target. A general area map presenting search sites (Figure 4) and reference map of specific search sites and target locations (Figure 5) was produced with a Trimble GeoExplorer XT GPS receiver and ArcView software. Manual search site maps with specific site information were used for reference during field trials.

Testing procedure

Each site searched was considered a field trial. Forty field trials were performed during 17 August to 14 September 2004 across twelve testing sessions. An average of three field trials comprised each testing session. Testing was conducted across two sets where each site was randomly searched once across each testing set. An average of 11 days elapsed before each site was searched again by the canine. Average temperature and humidity across trials was 28 degrees C and 27 percent, respectively. Forty-six spotted knapweed targets (n = 46) were available for detection across the first testing set. The canine detected and killed a juvenile target.

Forty-five targets (n = 45) were therefore available for detection across the second testing set. At the beginning of each field trial, the handler and canine would enter the search site at a random corner unless a prevailing breeze directed a search strategy conducive to the direction of the wind. On the command "find-it," the dog would begin to sample the search site off-leash for the target odor. The handler remained stationary unless the canine required additional direction to completely sample the site. The handler was not aware of target locations. When the dog alerted, the handler queried the scorer similar to procedures described in Objective 1. Each trial returned any of the following responses by the canine, which were recorded by the scorer: (1) alerted to the target (hit), (2) alerted when no target (false positive), and (3) missed the target (miss). Detection distance, or the distance from the target to the dog, was measured by target. This distance was measured from the point where the dog detected the scent, indicated by noticeable difference in search behavior, to the target plant. The scorer collected and recorded canine responses and detection distance data.

3.3 Field Trials - Objective 3

A canine / handler and human surveyor deployment design across field trials is forthcoming, which is dependent on final approval of sites appropriate to this study. As of 1 August 2005, five sites have been located, but we anticipate locating more and / or improved sites prior to 26 August 2005. Field trials are scheduled to begin on 9 September 2005.

Canines and handlers

Canines will be chosen for this study based on associated handler availability and capability of the handlers to adequately train and prepare their canines to participate in field trials. Four dogs are planned to be used in this study. Dog 1 is a 2.5 year old, female Rocky Mountain shepherd. This canine is the property of Montana State University, Department of Land Resources and Environmental Sciences and is trained in spotted knapweed scent discrimination and detection. A local handler trains this canine. Dogs 2 and 3 are a male German shepherd and male Australian shepherd, respectively. These canines are the property of, and will be trained by, a handler located in Stanislaus County, California. Dog 2 is eight years old and has six years of carnivore scat detection training. Dog 3 is three years old and has one year of carnivore scat detection training. Dog 4 is a six year old, female Labrador retriever. This canine is the property of, and will be trained by, a training specialist with the United States Department of Agriculture – Animal and Plant Health Inspection Service – Plant Protection and Quarantine – National Detector Dog Training Center (USDA - APHIS – PPQ – NDDTC) located in Orange County, Florida. Dog 4 is trained in basic obedience. Montana State University, Animal Resource Center will supervise the housing and care of the dogs during the investigation.

Human surveyors

An equal number of human surveyors to canines will be selected to participate in this study. Four human surveyors will be randomly chosen from a population of individuals that will be generated based on availability to participate in the study, and experience in identifying and detecting spotted knapweed and using a Trimble GeoXT GPS receiver. These individuals will be recruited from Montana State University, College of Agriculture and Gallatin and Park County Weed Districts.

Target weed and odor source material

Spotted knapweed was chosen as the target weed based on its widespread distribution in Montana (Duncan 2001) and its ability to invade and dominate a variety of healthy and relatively undisturbed plant communities (Rutledge and McLendon 1998) throughout the western region. Watson and Renney (1974) found spotted knapweed infestations decreased bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) A. Löve] yield by 88 percent. This displacement lowers yield and quality of livestock forage and increases costs of producing livestock, impacting Montana cattle producers over $42 million annually (Hirsch and Leitch 1996). Spotted knapweed may also increase soil erosion (Lacey et al. 1989) and impact wildlife habitat suitability for wildlife. Hakim (1979) found elk use was reduced 98 percent on spotted knapweed-dominated range compared to bunchgrass-dominated sites. Spotted knapweed continues to spread from existing stands (Watson and Renney 1974), contaminated crop seed and livestock forage, wildlife and livestock movement, and human activity (Sheley et al. 1999). Rice et al. (1997) predicts the potential range of spotted knapweed in Montana may include the majority of the state. Effective early detection of spotted knapweed is crucial to preclude spread and further invasion throughout Montana and the western region.

Spotted knapweed will be transplanted into six-inch diameter pots from sites located in Gallatin County, Montana during summer 2005. These plants will be maintained in a greenhouse at Montana State University, Plant Growth Center or an outside environment. Plants will be shipped via airmail to the California and Florida handlers every two to three weeks. Shipments to the California handler will be sent under an approved State of California, Department of Food and Agriculture permit to ship live noxious weeds. One shipment of six plants to the California handler occurred on 25 July 2005. Three shipments of six plants to the Florida handler occurred on 7 June, 29 June, and 1 August 2005. The handlers will use fresh clipped (< 1 hour old) stem and leaf material from these transplants as training aids to establish a scent association with Dogs 2, 3, and 4. Fresh clipped spotted knapweed material produces a chemical odor in poor agreement to the odor produced by living spotted knapweed transplants, as demonstrated by the volatile analysis summarized in Table 1. Volatile collection and analysis of clipped material and living spotted knapweed transplants were conducted by Montana State University, Department of Entomology. This analysis indicates the same dominant vapor constituents are present in both the clipped and living material. The clipped material vapor constituents, however, are present at much higher mean amounts, averaging 22.9-fold (SE 30.8-fold), over the living material. Small amounts of clipped material will be used to establish a scent association and as training aids to partially compensate for increased volatile production that results from clipping. Spotted knapweed transplants will be planted into cement cinder blocks and used for odor discrimination training across Dogs 2, 3, and 4 during August 2005 with "scent line" procedures described below. Training with living targets in a controlled setting will teach the canines a natural odor signature and concentration (Johnston 1999) in preparation for field trials. Dogs 2 and 3 will be trained to search for the target scent during August 2005 with fresh clipped spotted knapweed material and naturally occurring spotted knapweed plants located in Stanislaus County, California. Dog 4 will be trained to search for the target scent during August 2005 with fresh clipped spotted knapweed material. Naturally occurring spotted knapweed plants in Gallatin County, and possibly Park County, Montana will be used as the odor source material across field trials.

Canine training procedures

Dog 1 has been trained in spotted knapweed scent discrimination and detection. To prepare for field trials, the canine will be trained to search for extended periods in areas with low target availability in preparation for field trials. This will be accomplished by increasing canine duty cycle and training the canine to expect targets only occasionally by increasing the number of searches where there is no target to detect. Gazit and Terkel (2003) found bomb-sniffing dogs in Israel became less effective if they were given fewer samples to locate than they had been trained to find. Additionally, Fjellanger (2003) considers blank searches to be a background strategy to ensure variation in reinforcement conditions. This strategy is beneficial to training as variability in reinforcement facilitates persistence in the learning of correct responses (Logan 1976). The canine will be trained to work for extended periods of time by increasing her duty cycle. This will be accomplished by encouraging the dog to sample plant communities for incrementally longer periods of time during each training session. We will motivate the canine to continue sampling for longer periods of time with a detection event planned for the canine at the end of random sessions. These detection events will reinforce continued sampling effort and will involve fresh clipped material as a training aid or naturally occurring targets that are strategic to the search. "Duty cycle" refers to the amount of time a canine will work without observed deterioration in its performance. Garner et al. (2000) states an effective detector dog duty cycle under moderate environmental conditions is 90 - 120 minutes of continuous searching. Training to continuously search for extended periods of time and ignore distractions and diversion odors will prepare the canine to maintain search focus and improve target detection in preparation for field trials.

Dogs 2, 3, and 4 will be trained to discriminate spotted knapweed with instrumental conditioning coupled with standard narcotics training protocol (Robicheaux and Jons 1996), similar to "scent line" procedures described in Objective 1 and detailed in Smith et al. (2003). While training dogs to detect San Joaquin kit fox scat, the researchers placed the target odor source in a block with a 5 cm hole. This block was placed among five to ten other identical blocks containing non-target odors. They led each dog down the line of blocks, tapping each hole to lead the dog's nose to the hole. When the dog reached the hole of the block containing the target, they immediately rewarded the dog. This conditioned the dog to associate the target scent with their reward. The dogs were then conditioned to indicate they found the target by sitting as an alert response. Dogs 2 and 3 will be trained to look at the handler and "freeze" as the alert response to the target, as a pointer would do in the presence of birds. Alert responses to the target will be reinforced with play and praise. Dog 4 will be trained to scratch at the target odor as the alert response and reinforced with food rewards. Correctly ignoring non-target or blank sampling positions will not be reinforced. Missing a target will result in repetition of the task until the canine alerts on the odor. Alert responses to non-target positions will be negatively reinforced with verbal correction and repetition of the task. Target scent discrimination and alert response training will progress at the discretion of the handlers. After the canines are consistently alerting to the presence of the target odor, they will be trained to search areas for the scent in a variety of field settings (Syrotuck 1972, Bryson 1976). Dogs 2 and 3 will be trained to search areas for the scent using fresh clipped material as training aids and naturally occurring spotted knapweed plants in Stanislaus County, California. Dog 4 will be trained to search an area for the target scent in Orange County, Florida using fresh clipped material. This trainer expressed interest in planting spotted knapweed transplants and control plants across a field setting for scent search training.

We discouraged the handler from performing this technique as we attempted this same strategy during spring 2004 with negative results to our training program. The canine learned to detect the target by sampling for disturbed soil, which we believe may have had a stronger odor than the target. Disturbed soil continues to be of sampling interest to the dog. All canines will be trained to search for the target scent with a variety of techniques frequently used in tracking (Syrotuck 1972, Johnson 1977, Pearsall and Verbruggen 1982, Davis 1987, Koehler 1987, Bulanda 1994).

Study areas

Search efforts to locate appropriate field trial sites will be narrowed to livestock-grazed dryland pastures that are adjacent to weed pathways in Gallatin County, and possibly Park County, Montana. Proximity to weed pathways, such as waterways and roads, may increase invasion incidence (Timmins and Williams 1991, Pyšek and Prach 1994, Thompson 1999) through repeated propagule introductions (Bazzaz 1986). Grasslands have frequent breaks in plant cover (Baker 1986, Tyser and Key 1988), which may increase the probability that weed propagules will encounter safe sites (Green 1983) with conditions sufficient for germination and growth. Grazed grasslands with reduced canopy cover may also be more vulnerable to airborne entry of weeds (NRC 2002) and high rates of seed dispersal distance and establishment (Almasi 2000) as many isolated small populations (Bazzaz 1986) compared to plant communities with a closed canopy (Simberloff 2003). These plant community characteristics and environmental conditions may guide efficient location of field trial sites with spatially separated spotted knapweed targets.

Field trials will be conducted during September 2005. A minimum of six, 1 ha sites will be located. Each site will be considered a field trial. Sites will contain no more than six naturally occurring spotted knapweed targets. Targets will occur as isolated individuals or patches. A patch will be defined as more than one individual within a 1 m diameter area. Each patch will be recorded as one target location. Detection of a spotted knapweed individual within the patch will be considered detection of the entire target. We anticipate sites will contain targets as juvenile and adult plants, as continuous spotted knapweed seedling emergence facilitates development of a hierarchy of age classes within a population (Sheley and Larson 1996). Grazing pressure may prevent adult plants from reaching full growth height. One to two field trials will be blank, where targets will not be available for detection. Sites will be similar in elevation and across grassland habitat types. Grassland community types will be determined following Mueggler and Stewart (1980). Sites will occur on flat land with a zero or gentle percent slope (< 20 degrees).

Potential sites will be initially assessed with one surveyor walking parallel transects. Target occurrences will be marked with survey flags. Sites may be rejected if more than six targets occur within a 1 ha area. Chosen sites will be delineated and targets will be inventoried with a Trimble GeoExplorer XT GPS receiver. This receiver can achieve submeter accuracy with post-processed differential correction. The search area will be delineated with survey flags and captured as a survey feature. Transects will be 20 m wide and 100 m in length, marked with survey flags, and captured as a survey feature. Transect widths are based on the lateral distance anticipated to be maintained by the canines from the handlers. Each site will contain ten transects in a crisscrossed grid pattern, but only five transects in the same cardinal direction (i.e., east to west, north to south) will be used during each trial. Targets occurring as isolated individuals or patches will be captured as point features by logging at least 10 GPS positions. Standing in the center of a patch will capture the patch location. Attribute data will be collected and linked to the geographic weed features with a data dictionary. The data dictionary will incorporate the life stage and size dimension (height, width, and root crown diameter) of each isolated individual, and length and width of each patch, number of targets comprising each patch, and estimated life stage of the majority of the population. Ground truth of each site will be determined at the end of the testing set by overlaying search results from the initial scorer, canines, and surveyors. Each site will have been searched nine times following the completion of the testing set.

Canine / handler team testing procedure

Two early morning field trials will comprise daily canine testing sessions. Scenting ability is affected by variations in target odor concentrations, which vary in complex ways with climatic variables (Phelan and Barnett 2001, Fjellanger 2003). Field trials will be conducted, therefore, during early morning when the atmosphere is cool, moist, and when wind velocities are low. These conditions may provide the optimum conditions for canine vapor sensing (Phelan and Webb 2003). Canines traveling from California and Florida will be acclimatized to Montana field conditions for three days prior to field trial deployment.

Canines will search each site by sampling vapor from the plant community while casting back and forth in front of the handler. The role of the handler is to interpret scenting behavior, remain on transect centerline, and ensure the canine completely samples each site. Handlers will perform an open grid search by walking transect centerlines so the area is covered evenly (Rebmann et al. 2000). Each site will contain ten transects in a crisscrossed grid pattern, but only five transects in the same cardinal direction (i.e., east to west, north to south) will be used during each trial. Transect direction of each field trial will be chosen based on the optimal search pattern to work the canine either perpendicular or into the prevailing wind direction. In the presence of wind, a maximum of six random field trial entry points will be available where deployment may start at transect one or five at any three sides of the field trial site. In the absence of wind, there will be eight available field trial entry points. That is, deployment may start at transect one or five at any four sides of the field trial site. Available entry points will be determined immediately prior to each field trial. A random entry point will then be generated.

The order sites are searched and the order canines are deployed will be randomized across the testing set. A canine will complete the entire testing set when all sites have been searched. Each site will be searched one time by canines each day to ensure at least 24 hours elapse among canine deployments to allow dispersion of any human or canine scent that might lead to the targets. Every effort will be made to lengthen the period of time between canine deployments across the same sites. The canine / handler team will be allowed a maximum of one hour to conduct the field trial. This time limit is based on cadaver search proficiency test guidelines for a 1 ha area with moderate vegetation (Rebmann et al. 2000).

At the beginning of each trial, the canine / handler team and the scorer will enter the search site at a random entry position dependent on prevailing wind. On the command “find-it,” the trial will start and search time will begin. The handler will not be aware of target locations. The handler will be instructed to avoid surveying the plant community for targets to prevent unintended messages to the canine. Handlers will be informed some sites contain targets and others do not (i.e., blank field trials). This way, handlers will not expect targets to occur across each field trial. This will work as a quality control measure to ensure handlers do not over compensate canine search efforts, or make excessive corrections, for fear of their canine missing a target they know is present.

The handler will carry weighted flags or survey flags to mark alert responses and detection distances by the canine. The handler will mark the detection distance, or the noticeable change in canine behavior, with a blue flag. This is the point where the canine detects the target scent. When the canine alerts, the handler will mark the location with a red flag and query the scorer by asking, “hit?” If the canine alerts to a target, the scorer will reply, “hit.” The handler will reward the dog while the scorer captures both the detection distance and hit location as point features and makes note on a manual map. If the canine false alerts to a target, the scorer will reply, “no.” The scorer will capture the false alert as a point feature and make note on the manual map. False alerts may occur if the canine responds to spotted knapweed scent pools remote from the source. Scent pools form when wind-borne scent molecules pool at airflow barrier locations (Rebmann et al. 2000), such as rocks or tufts of grass. The handler will reward the canine only when the dog alerts at the scent source. The handler will continue the field trial until the site has been completely searched. End search time will be recorded. The handler will not be informed of any missed targets.

Human surveyor testing procedure

The primary purpose of this study is to statistically quantify the efficacy of canines to locate isolated spotted knapweed targets. Secondary is to quantify the efficacy of human surveyors for comparison to canines for purposes of gathering exploratory data and formulating method development. Human surveyor testing protocol, therefore, will adhere to canine deployment and testing protocol as practically as possible and to ensure testing procedures between canines and surveyors are consistent. We will essentially treat each surveyor as another canine. The order sites are searched and the order surveyors are deployed will be randomized across the testing set. Each site will be searched one time by surveyors each day across daily testing sessions. A surveyor will complete the testing set when all sites have been searched. Two early morning field trials will comprise daily surveyor testing sessions. Each site will contain ten transects in a crisscrossed grid pattern, but only five transects in the same cardinal direction will be used across each trial. The process of determining the cardinal direction of transects and associated random field trial entry points will follow prevailing wind direction protocol described in canine / handler testing protocol. Surveyors will be informed some sites contain targets and others do not (i.e., blank field trials). This way, surveyors will not expect targets to occur across each field trial. This will work as a quality control measure to ensure surveyors do not over compensate their search efforts, or make excessive corrections, for fear of missing a target they know is present. Surveyors will enter the search site and search time will begin. A scorer will record search duration and accompany the surveyor to manually record target detections and measure and record detection distance by target. Each surveyor will be allowed a maximum of one hour to conduct the field trial (Rebmann et al. 2000). The surveyor will search for targets using an open grid search across each site by walking in a zigzag pattern across each transect width. The surveyor will immediately stop upon target detection and inform the scorer of the detection. The scorer will measure and record this distance, or the distance between the point of visual detection and the target. Target locations will be captured as point features by the surveyor and detection distance will be manually noted and recorded as an attribute linked to the target point feature. The surveyor will continue searching each transect until the site has been thoroughly searched.

This will conclude the field trial. End search time will be recorded. The surveyor will finish the entire testing set after all sites have been searched. That is, after all field trials have been completed.

Canine / handler team and surveyor data collection and management

Search duration will be measured and recorded. Weather data, such as temperature, humidity, and wind direction and velocity, will be recorded at the beginning and end of each canine / handler team and surveyor field trial. Canine responses, such as hits, misses, and false alerts, will be captured as point features and manually recorded on site maps by the scorer. Target detections by surveyors will be captured as point features by the surveyor and manually recorded on site maps by the scorer. Canine detection distances will be marked by the handler, and measured and recorded by the scorer as GPS attributes linked to the target. Surveyor detection distance will be measured and recorded by the scorer across each field trial. Canine field trials will be videotaped. Global positioning system receiver data and manually recorded data will be identified to each canine and surveyor. Management of GPS data will be similar to procedures described by Wood (2003). The raw, unedited and uncorrected GPS data will be transferred to a computer and saved at the end of each daily testing session. A copy of the original GPS file will be differentially corrected using the closest functioning base station. The percent correction achieved will be recorded. The corrected GPS file will be exported to ArcView shapefile format using the UTM coordinate system and the North American Datum of 1983.

4.0 Analyses

All statistical analyses will be carried out using SAS Version 9.1 (SAS Institute, Cary NC, USA). A significant finding will be indicated as a p-value less than or equal to 0.05.

4.1 Controlled Trials - Objective 1

We calculated the effectiveness of a single canine to discriminate living spotted knapweed using the Predictive Value Model (Galen 1979, Mass and Galen 1981). The parameters that interact in this model are sensitivity, specificity, and positive and negative predictive values. Sensitivity and specificity values are properties of the test and the predictive values are properties of both the test and the test results. Sensitivity of the method is calculated by the following formula: (true positives / true positives + false negatives) x 100. Specificity of the method is calculated by the following formula: (true negatives / false positives + true negatives) x 100. Positive predictive value of the method is calculated by the following formula: (true positives / true positives + false positives) x 100. Negative predictive value of the method is calculated by the following formula: (true negatives / true negatives + false negatives) x 100. Taking these ratios one step further, effectiveness of a method can be calculated by the following formula: (true positives + true negatives / true positives + false positives + false negatives + true negatives) x 100.

4.2 Field Trials - Objective 2

We calculated the accuracy of a single canine to detect naturally occurring spotted knapweed targets using the percentage of hits. Accuracy calculations were used to describe detection performance, or the dog’s sensitivity to the stimulus (Waggoner et al. 1998). Accuracy was calculated as the number of targets located divided by the total number of targets available for detection. A high proportion of hits would indicate accurate detection performance. Decreased proportion of hits would indicate decreased detection performance. Increased frequency of false alerts may not affect detection performance for the target, but could decrease effectiveness and credibility of the method if the proportion of false alerts could be quantified. Mean and standard deviation of detection distances and search duration (start time – stop time) were calculated. Overall accuracies were calculated for both testing sets with a mean percentage and associated standard error.

4.3 Field Trials - Objective 3

We will calculate the accuracy of each canine and surveyor as the number of targets located divided by the total number of targets available for detection across each trial. We will calculate the search duration (start time – stop time) of each canine and surveyor across each trial. We will calculate target detection distances for each canine and surveyor by target. A range of accuracies and search durations will be presented for each canine and surveyor across the testing set with median and mean accuracies and search durations. A small sample size may require that we combine data across sites, resulting in a single canine data set and a single surveyor data set for analysis. A range of detection distances by target will be presented across each dog team and surveyor with median and mean distances. Frequency histograms will be produced to evaluate data for normality to determine whether parametric or nonparametric statistical tests will be run. A one-way ANOVA or the nonparametric Kruskall-Wallis one-way ANOVA will be used to test for differences in accuracy rates, search duration, and detection distances between dog teams and surveys.

5.0 Results

5.1 Controlled Trials - Objective 1

Dog 1 sampled 227 sampling positions and made eight mistakes, represented by four misses and four false alerts. The sensitivity of the method to detect spotted knapweed was calculated as 90.2 percent [(37 / 37 + 4) x 100]. Specificity of the method to correctly ignore non-targets and blanks was calculated as 98.0 percent [(182 / 4 + 182) x 100]. The positive predictive value of the method was 90.2 percent [(37 / 37 + 4) x 100], while the negative predictive value was 98.0 percent [(182 / 182 + 4) x 100]. The efficiency of the canine in discriminating spotted knapweed was calculated as 96.5 percent [(37 + 182 / 37 + 4 + 4 + 182) x 100]. The values in parentheses are derived from summarized results in Table 2. Our data analysis demonstrates the use of a specially trained canine to discriminate spotted knapweed has high effectiveness.

5.2 Field Trials - Objective 2

Dog 1 detected 43 of 46 targets across the first testing set. The accuracy of the canine to detect naturally occurring spotted knapweed targets was calculated as a percentage of hits, or 93.5 percent [(43 / 46) x 100]. Three misses were recorded when the canine did not respond to the target. Three false alerts were recorded when the canine responded to spotted knapweed scent pools, each within 1 m of a target plant. The canine’s alert response destroyed one juvenile target. This resulted in a reduced number of targets available for detection across the second testing set. The canine detected 39 of 45 targets across the second testing set. Canine accuracy was calculated as a percentage of hits, or 86.7 percent [(39 / 45) x 100]. The canine’s alert response destroyed two additional juvenile targets during the second testing set. Juvenile targets seemed to be susceptible to uprooting, possibly the result of an underdeveloped taproot. Six misses and two false alerts were recorded. Mean accuracy was calculated across testing sets as

90.1 percent (SE 3.4 percent). Mean detection distance was calculated across testing sets as 0.66 m (SE 0.24 m). The greatest detection distance was 1.5 m from the target plant, observed in three separate trials. High target density may have influenced detection distance of the canine. We believe the target odor was disseminated throughout the search sites, which may have posed a difficult situation for the canine to follow increasing levels of odor intensity to locate individual targets. Sensitivity studies have shown initial odor samples prompt the dog to move in directions that lead to higher concentrations (Johnston 1999). We anticipate isolated targets occurring in low density will facilitate greater detection distances by the canine. Our data analysis demonstrates the use of a specially trained canine to detect naturally occurring spotted knapweed has high accuracy.

5.3 Field Trials - Objective 3

Field trials will be conducted and data will be collected during September 2005. We expect canines will have slightly lower detection accuracies, greater detection distances, and more rapid search durations compared to human surveyors. Canines are expected to have slightly lower detection accuracies compared to surveyors, because we believe the surveyors may have the sampling advantage when targets are in the flowering stage. This growth stage is expected to occur during September across field trial sites. Canines, however, may counteract this by having the sampling advantage in locating juvenile or non-flowering targets. Possibly more significant is the assumption that the surveyors may have a sampling advantage related to training and pre-trial search experience. The surveyors will already be fully trained and experienced in detecting spotted knapweed. The experienced surveyors may increase probability of target detection by focusing search efforts to likely sites based on invasion susceptibility, such as disturbed areas and sites in proximity to fence lines. Detector dogs will also learn over time where to sample to increase probability of detection, but canines will be only moderately experienced to inexperienced in spotted knapweed detection prior to field trials. Myers (1990) states the function of detector dogs is largely dependent on the level and amount of training where they become more effective in their task over time. We anticipate a learning curve in how well the canines perform, where they may perform better and find more and smaller targets as the trials progress. The function of detector dogs also is dependent on handler skills. We anticipate misses may be related to handler error and more specifically, to search strategy. If the handler does not cover the entire search area, the canine may not have an opportunity to find each target. And if the handler does not pay close attention to wind direction, the canine may miss upwind targets. Canines are expected to have greater detection distances compared to surveyors, as canines have a demonstrated sensitivity to targets they have been trained to find (Waggoner et al. 1998). Dog 1 has demonstrated this ability by detecting an isolated target from a distance of 18 m during a training session on 22 July 2005. Dogs trained to sit at the first whiff of a mine indicated they could detect mines at distances up to 20 m (Joynt 2003). Cablk and Heaton (2005) found trained dogs could detect desert tortoises at distances up to 60 m. Canines are expected to have more rapid search durations compared to surveyors given their demonstrated ability to cover more area faster over manual (i.e., human) searches (Campbell and Rodda 1998, Lorenzo et al. 2003).

6.0 Future direction

This project will evaluate the potential of canine detection as a novel sampling method to locate new spotted knapweed invasions. We anticipate this project will demonstrate the use of detector dogs holds strong promise as an effective method to detect spotted knapweed early. Future direction of this project, outside of thesis work, is to train more canines and conduct additional trials with surveyors and experienced dog teams under a range of seasonal conditions, target growth stages, plant communities, and land use. This direction is intended under a proposed five-year plan to be developed by Montana State University. This plan is at the request of USDA – APHIS – PPQ to work in partnership with Montana State University to evaluate the efficacy of using invasive weed detector dogs to protect domestic agriculture in the western region.

Skinner et al. (2000) states the genus *Centaurea* is the most abundant noxious weed in the western United States. The inclusion of odor generalization among the knapweeds is another anticipated future direction of this project. The ability of canines to generalize from spotted knapweed to variants, such as yellow starthistle (*Centaurea solstitialis* L.), squarrose (*C. triumfettii* All.), white (*C. diffusa* Lam.), and Russian [*Acroptilon repens* (L.) DC.] knapweed, should be considered and investigated. Dog 1 has demonstrated odor generalization to variants by alerting to two spatially separated white knapweed plants, approximately 90 m apart, during a training session on 14 June 2005. These alert responses to a non-trained variant may indicate white knapweed has an active odor signature similar to spotted knapweed. The ability of the canine to generalize to a target variant, without being trained to do so, may have been unintentionally facilitated by using a variety of odor source materials throughout training. These included previously frozen and fresh clipped spotted knapweed material, field transplants, and naturally occurring plants. Johnston (1999) states the more varieties of a substance dogs are trained on, the more likely they are to alert to non-trained variants. We intend to collect and analyze odor chemicals from white and spotted knapweed field transplants. (Vapor analysis of spotted knapweed transplants presented in Table 1 will not be used). Prior to any conclusions generated from this analysis, however, we aim to better understand the influence of belowground odor chemicals on canine detection of spotted knapweed.

Dog 1 alerted to two dead spotted knapweed plants at least 12 m apart during a training session on 20 May 2005. Detection distance of the canine to both plants was measured as 5 m and 2 m. The plants were less than 20 cm in height and may have been dead prior to 2004, as they were desiccated, brittle upon touch, and bleached in appearance. The canine was trained on previously frozen and fresh clipped spotted knapweed material. This material was also "dead," but it was green, pliable, and actively releasing volatiles (Table 1), with a detectable odor to humans. The dead plants lacked pliable roots, and any sign of potential living material above or below ground. We believe the canine used belowground odor chemicals produced by previous root activity, which likely persisted in the soil, to detect these dead plants. The behavior of the canine suggests belowground chemicals were used for detection, as the canine was intent on capturing and sampling more odors by digging deeper into the soil. (A video clip in .mpg format is available for computer viewing). Long-term observations also indicate the canine may be using belowground odors, at least in part, to detect spotted knapweed. For example, prior to an alert response, the canine confirms the target by habitually sampling the soil surface at the base of the plant in lieu of sampling the aboveground target foliage. Landmine detection dogs sample the soil surface with similar behavior. A series of controlled odor discrimination trials with soil samples, including aged soil based on length of time the target has been removed from the soil, may confirm the canine is using chemicals produced by root activity. If canines are able to also detect spotted and white knapweed via belowground chemicals, which may persist across growth stages, there may be potential for canines to detect knapweed across seasons. Present survey efforts to locate invasive weeds are typically implemented during a two- or three-month flowering period to improve sampling effort (Keating et al. 1998). The potential to effectively detect knapweed incursions during a nine-month (March – November) time period, or longer, may be significant towards improving early detection efforts and slowing knapweed spread. Published reports are available, and shall be reviewed, that describe the ability of canines to locate landmines and the fundamental soil-chemical interactions that influence the availability of chemicals for trace chemical detection (Göth et al. 2003, Phelan and Webb 2003).

We aim to better understand which odor chemicals are used for canine detection of spotted, and possibly white, knapweed. This will work to improve the training process and performance of additional canines through the development of better training aids. Pseudochemical blends are extensively used as detection training aids and will be critical to the future direction of this project. The development of a spotted knapweed pseudochemical will be an improved training aid over clipped material, as the formulated ratios of the vapor constituents will be consistent with the living plant odor signature. A pseudochemical blend will also alleviate effort to transplant and maintain living spotted knapweed plants for odor material needs. In addition, we will avoid shipping whole plants at high costs and high risk to remote trainers and western region states. Montana State University, Department of Entomology is able to formulate a three-component pseudochemical blend comprised of cis-3-hexenyl acetate, beta-cis-ocimene, and alpha-pinene. They are also able to prepare a five-component blend with the addition of beta-myrcene and beta-cuvebene. We anticipate developing the five-component blend and investigating its effectiveness as a training aid. We could also consider developing an improved spotted knapweed pseudoochemical in the future, which may incorporate identified belowground volatiles, or a generalized pseudochemical, which may include the dominant vapor constituents produced across the knapweeds for detector dogs trained to locate all *Centaurea* species.

We anticipate future investigation of this project will demonstrate how the use of invasive weed detector dogs may improve ground inventories and fill broad survey needs, such as to cover large areas, increase sampling accuracy and thoroughness, and decrease search time and expense. A significant potential exists where detector dogs may also have the ability to locate early age class and early season targets that humans typically cannot locate, nor typically survey, to maximize the chances of eradication success. Dog 1 has demonstrated the ability to locate early age class targets by detecting, and killing, as a result of the alert response, three juvenile targets during Objective 2 investigation. This canine also demonstrated the ability to locate early season targets during a training session on 4 March 2005. The canine detected three isolated, early season spotted knapweed plants regrowing from root crowns (Figure 6). The root crowns were not measured, but aboveground biomass was clipped, dried, and weighed. We originally believed the canine used the vapor produced by the minimal living material to detect the targets. We now suggest the canine used belowground odor chemicals, in large part, to detect the targets. We believe the small amount of aboveground living material [0.43 g (SE 0.09)] produced minimal vapor, while the belowground chemicals were made more available to the canine for detection purposes from the prevailing moist soil conditions. Phelan and Webb (2003) states soil acts as a temporary storage reservoir for landmine signature chemicals, releasing them when dew or rain falls, and collecting more as soil water evaporates.

We also anticipate future investigation may demonstrate how the use of invasive weed detector dogs could support predictive modeling and hyperspectral imaging. These technologies could identify hot spots that would then be targeted for a more focused detector dog search. Future direction may investigate invasive weed detector dog teams, trained to indicate on multiple weed signatures. Williams and Johnston (2002) found dogs could easily master at least 10 odor discriminations and learn additional odors with increasing ease. The limits to the number of odors canines can learn have not been explored (Göth et al. 2003). Scenting ability varies more among individual dogs (Johnston 1999) than breeds of dogs. Issel-Tarver and Rine (1997) found scenting ability among breeds of dogs, based on the number of olfactory receptor genes per subfamily, has remained stable in spite of many years of differential selection on the basis of olfactory acuity in scent hounds, sight hounds, and toy breeds. The problem of varied scenting ability among individuals is solved statistically using combined performances of several dogs. Three dogs were required in a team to achieve the 99.6 percent requirement of the United Nations contracts in landmine detection in South Africa (Joynt 2003). The strategic use of highly effective detector dog teams may work similarly to stop spread at population fronts and increase the chances of eradication success. Simberloff (2003) states expanded eradication efforts can potentially affect enormous ecological and economic savings. Expanded eradication efforts would require highly effective strategies where every plant is located and eliminated (Zamora et al. 1989) over a sufficient area (Simberloff 2003). Invasive weed detector dogs may have potential to meet these needs and ultimately function as working advertisements. Detector dogs may promote the urgency of early weed control (Dewey 1995, Hobbs and Humphries 1995, USDI 1996, Allendorf and Lundquist 2003), which is not widely understood (USDI 1996), while at the same time working to curb spread and restrict ecologic and economic impacts (Mack et al.

2000). This project has high public appeal, as it seems the public cannot resist the sight of a working dog assisting in the pursuance of man's duty (Gregory 1999). High public appeal is working to promote this project and the urgency of early weed control through widespread media coverage (CNN 2003, Boston Herald 2003, ESPN 2003, NPR 2003, The Seattle Times 2003, Cal-IPC 2004, Durand 2004, Kirkwood 2004, USDI 2004, Scholastic Library Publishing 2005).

Table 1. Spotted knapweed volatile analysis. Poor odor chemical agreement is demonstrated among field transplants, clipped material (i.e., severed transplants), and previously frozen material (i.e., clipped, fresh frozen, then thawed). The dominant vapor constituent produced by living plants, cis-3-hexenyl acetate, is released from clipped material and exhausted over time.

Vapor constituent	**Field transplants**		**Clipped (< 24 hrs old)**		**Previously frozen (> 24 hrs old)**	
	n = 6		*n* = 6		M 14.3 g, σM 2.7 g	
	Mean	SE	Mean	SE	Mean	SE
cis-3-hexenyl acetate	39.17	21.04	86.10	27.78	0.00	0.00
alpha-pinene	7.98	2.22	19.07	4.64	71.60	8.25
Beta-cis-ocimene	7.22	2.89	14.02	4.63	0.00	0.00
beta-cuvebene	3.82	1.56	404.60	89.36	372.11	64.06
beta-myrcene	3.00	1.54	12.78	7.46	51.75	7.11
caryophyllene	1.98	0.92	188.94	37.56	121.58	22.71
nonanal	1.14	0.10	1.68	0.25	12.96	2.28
cis-3-hexenol	0.71	0.22	7.54	0.98	243.02	53.34
(M)(+)-Epi-bicyclosesquiphellandrene	0.58	0.25	56.84	12.70	87.05	14.16
benzeneacetaldehyde	0.42	0.10	3.47	0.57	129.84	47.61
trans-2-hexenal	0.00	0.00	7.83	2.30	455.01	103.82
1-hexanol	0.00	0.00	2.85	0.43	89.21	21.80
benzaldehyde	0.00	0.00	0.71	0.14	165.50	29.12
benzyl alcohol	0.00	0.00	1.42	0.28	65.64	11.46
phenylethyl alcohol	0.00	0.00	0.49	0.13	51.34	10.93
	Nanograms / plant / hr				Nanograms / hr	

Table 2. Summary of correct and incorrect spotted knapweed discriminations (*n*=227) by Dog 1

	Correct discrimination	Incorrect discrimination	Total
Positions sampled with target	37 (hits)	4 (misses)	41
Positions sampled without target (*i.e.*, soil or non-target)	182 (correct rejection)	4 (false alerts)	186
Total	219	8	227

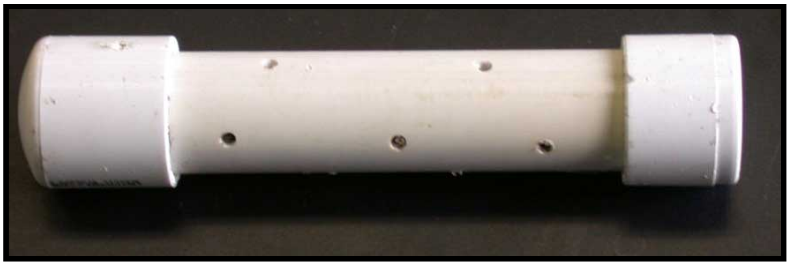

Figure 1. Example of a plastic tube used in the study.

Figure 2. Sampling positions were comprised of cement cinder blocks containing target and non-target odor material beneath a fine wire mesh. From left to right: non-target plant, spotted knapweed target, and moist soil.

Figure 3. Sampling positions were double stacked. Empty, identical plastic tubes (Figure 1) were placed in each sampling position.

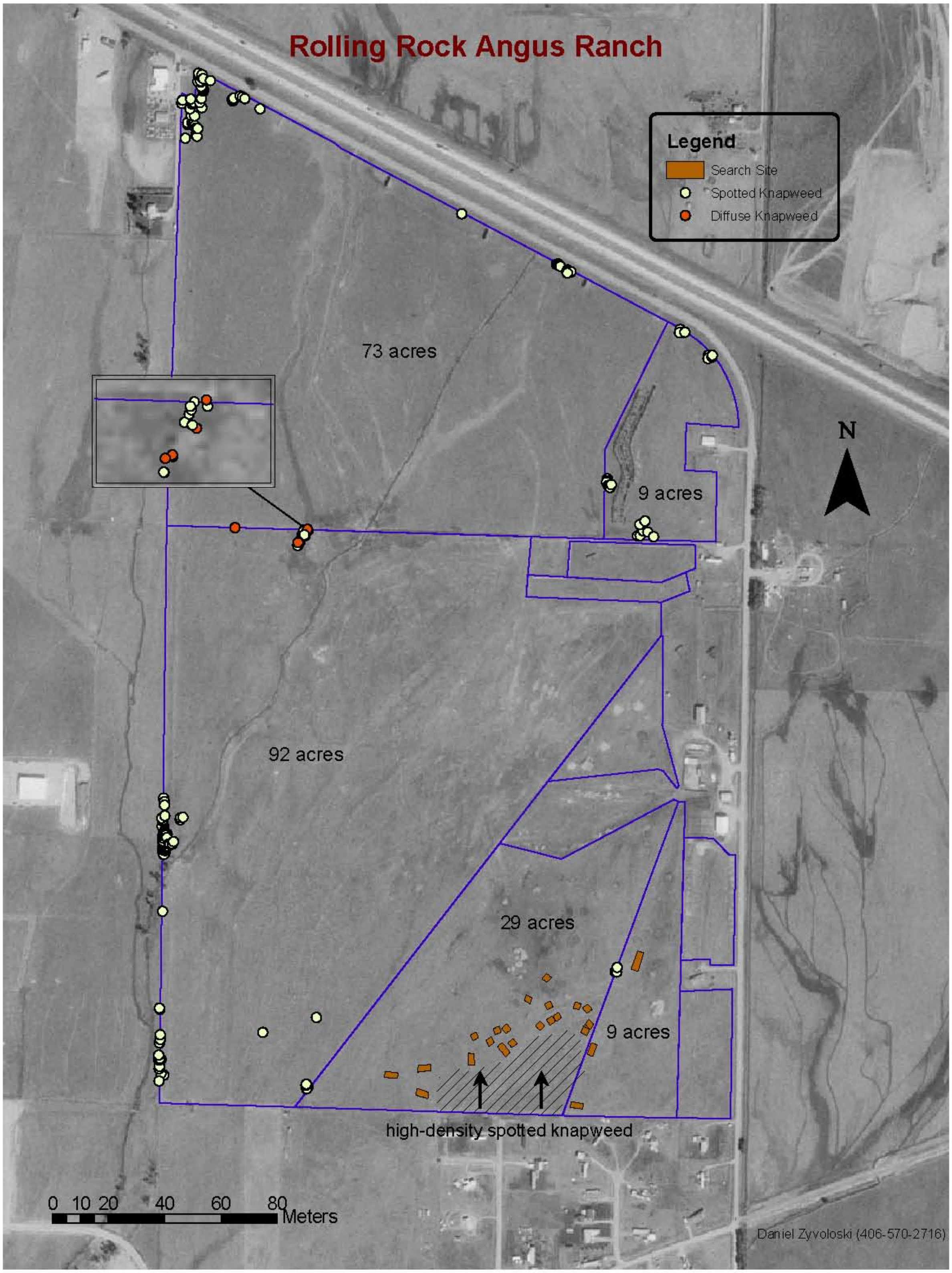

Figure 4. General area map demonstrating location of search sites.

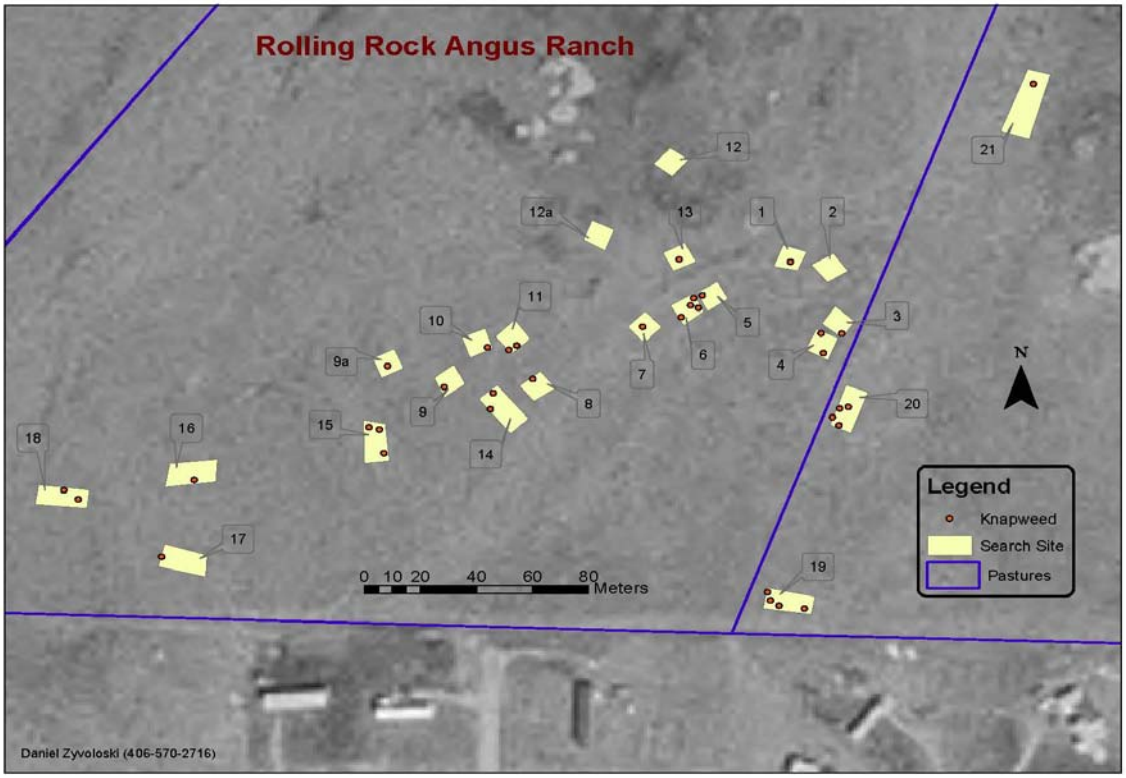

Figure 5. Reference map of search sites and target locations. Spotted knapweed points may represent more than one individual plant. Sites 7 and 21 were not used in the study.

Figure 6. One of three early season spotted knapweed targets detected on 4 March 2005 by Dog 1. Minimal aboveground living material is observed. The canine may have used belowground volatiles to detect early season targets.

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