

USING CANINES TO DETECT SPOTTED KNAPWEED: FIELD SURVEYS AND
CHARACTERIZATION OF PLANT VOLATILES

by

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ABSTRACT

Invasive plants threaten biodiversity as well as the function of ecosystems and cause loss for land users and managers in western North America. Eradication of invasive plant populations is desirable to prevent these impacts. But eradication is difficult to attain due to imprecise detection rates for low density and inconspicuous plants, such as juveniles and small adult plants. In an effort to improve the accuracy of plant detection, we investigated the ability of domestic dogs (*Canis familiaris* L.) to search for and detect rare occurrences of spotted knapweed (*Centaurea stoebe* L.). The objectives of this study were to (1) compare the performance of trained dogs to human surveyors in detecting new invasions of knapweed, (2) evaluate the ability of the dogs to detect below ground odor produced by knapweed roots, and (3) characterize the above and below ground volatile profiles of spotted knapweed. The accuracy of three dogs was compared to human surveyors on a dryland pasture near Belgrade, Montana in 2005 and 2006. Dog accuracy for large-size knapweed infestations (0.52m³) was similar to human accuracy and better than humans (94 vs. 78%) for medium-size infestations (0.13 m³). Dog accuracy (67%) was greater (>81% probability) than humans (34%) for small knapweed targets (plants; 0.02 m³). In September 2006, controlled odor response experiments were conducted in a livestock barn in Bozeman, Montana to evaluate the ability of the dogs to identify spotted knapweed root material without being previously trained to do so. Mean success rate of the dogs to recognize bare and intact roots were 97% and 87% respectively. In 2005 and 2006, volatile collections were conducted. The main compounds of the foliage were found to be (*Z*)-3-hexenyl acetate, β -cis-ocimene, and β -cubebene, which were not present in the roots. The main constituents of the roots were junipene, β -caryophyllene, and thujopsene, which were not present in the foliage (exception: β -caryophyllene), indicating dissimilar volatile profiles between the foliage and the roots. Collectively, these results indicate detection dogs might improve the efficiency and accuracy of plant detection by increasing the likelihood small and inconspicuous plants, including belowground plant targets, will be detected.

INTRODUCTION

Background

Invasive plants are non-native plant species that are introduced into an ecosystem and then spread and become abundant over an extensive area (Richardson et al. 2000). Most invasive plants have a competitive advantage over native plants and can replace native species and plant communities (Louda et al. 1990, Dukes and Mooney 2004). The traits of invasive plants and the mechanisms associated with the elimination of native plants include persistent seed banks, short juvenile periods and early reproductive capacity (Rejmánek and Richardson 1996), resource preemption (LeJeune and Seastedt 2001, Hill et al. 2006), and natural enemy release (Williamson 1996, Mitchell and Power 2003, Torchin et al. 2003) or competitive release from native species by generalist enemies (Keane and Crawley 2002). The displacement of native plants by invasive species can threaten biological diversity and the function of biological communities and ecosystems (Braithwaite et al. 1989, Musil 1993, Wilcove et al. 1998) and cause serious losses for land users and managers in western North America (Hobbs and Humphries 1995). Weeds in rangelands cause an estimated loss of \$6 billion [U.S.] annually in the United States due to control costs and forage reductions (Pimentel et al. 2000).

Early intervention against new invasions can slow weed spread (Navaratnam and Catley 1986, Moody and Mack 1988, Cousens and Mortimer 1995), prevent future weed problems (Hobbs and Humphries 1995), and reduce ultimate management costs (Higgins

et al. 2000, Myers et al. 2000, Panetta 2007). Targeting new invasive plant populations is the most effective management approach (Moody and Mack 1988) with eradication as the ultimate goal. Eradication is the elimination of all reproducing individuals of a species (Myers et al. 1998) and is declared once the failure to detect individuals is equal to the longevity of the seed bank (Woldendorp and Bomford 2004). The process of eradication can last decades under field conditions (Baskin and Baskin 1998, Panetta and Timmins 2004). Eradication relies on a high probability of detection to remove new recruits before reproduction to eliminate seed production and exhaust seed banks (Mack 1985, Bazzaz 1986, Zamora et al. 1989, Myers et al. 2000, Panetta and Timmins 2004), or eliminate vegetative growth in rhizomatous perennial plants. Therefore, effective search protocols are needed (DiTomaso 2000, Radosevich et al. 2003, Cacho et al. 2006) that cover adequate areas (Simberloff 2003).

The detection of rare individuals, or those occurring in low abundance in an area of occupancy (Gaston 1997), is important for eradication and also for accurate and unbiased occupancy estimates of rare species (MacKenzie et al. 2002). Detection rates for plants occurring infrequently in an area are imperfect (Regan et al. 2006) and finding juvenile and small adult plants is particularly problematic (Tomley and Panetta 2002, Panetta and Timmins 2004). In addition, detection becomes increasingly difficult and imprecise as management reduces weed density over time (Regan et al. 2006, Panetta 2007), but eradication requires complete control for many years (Simberloff 2003, Panetta and Timmins 2004, Cacho et al. 2006). Due to declining densities, monitoring usually is reduced before eradication is attained (Whittenberg and Cock 2001, Mack and

Lonsdale 2002). As progress is made toward eradication, increased sampling is needed (Simberloff 2003) to maintain near perfect control. Exhaustive monitoring over a sufficient area using highly accurate detection methods is needed to improve the likelihood of eradication.

Canine Detection Technology

Domestic dogs (*Canis familiaris* L.) trained to search for the presence of specific odors and referred to as detection dogs can be effective in field settings because they have the ability to discriminate and localize odor sources with gradient detection (Waggoner et al. 1998). They can also cover large areas quickly and thoroughly (Killam 1990, Komar 1999, Göth et al. 2003). These capabilities are enhanced by their natural tendency to please the trainer and search for territorial boundaries (Bach and Phelan 2003). Intrinsic rewards (Göth et al. 2003), especially food and praise from the pack leader, allow dogs to be trained to search for and locate targets. Search dogs typically work an area by sinuous forward movement while continuously sampling the air. Detection of a trace of the target odor initiates movement through the odor gradient to its source. The successful dog then gives a trained signal or 'alert' to obtain a reward.

Detection dogs are commonly used for hunting (Myers 1990) where they innately search for odors arising from prey or closely related species (Göth et al. 2003). Dogs are also trained to reliably locate extrinsic odors as an accurate, low input, and versatile detection method (Göth et al. 2003). For instance, the utilization of detection dogs to locate hidden explosives and narcotics is widely known (Furton and Myers 2001, Lorenzo et al. 2003). Dogs have been trained to detect odors signaling the presence of

cancerous tissue in humans (Pickel et al. 2004, Willis et al. 2004, McCulloch et al. 2006). Dogs can readily seek odors arising from desert tortoises, *Gopherus agassizii* C. (Cablak and Heaton 2006), carnivore scat (Smith et al. 2003, Wasser et al. 2004, Harrison 2006, Long et al. 2007a, 2007b), and the presence of black-footed ferrets, *Mustela nigripes* Audubon and Bachman (Reindl-Thompson et al. 2006). Additionally, dogs have been trained to find more than 30 different substances, as listed by Lorenzo et al. (2003) and Browne et al. (2006).

Detection dogs represent an effective and reliable detection technology (U.S. Congress, Office of Technology Assessment 1992) because of their ability to accurately discriminate odor molecules at concentrations of approximately one part per billion (Waggoner et al. 1998). The capacity of dogs to detect minute odor concentrations results from over 200 million olfactory receptor cells (Roppel and Wilson 2000) and is enhanced by the specialized behavior of sniffing (Syrotuck 1972). Sniffing increases odor concentration by repeatedly drawing volumes of air back through the nostrils which make contact with the remote areas of the olfactory mucosa (Neuhaus 1981, Youngentoub et al. 1987). Sniffing also increases odor availability by disturbing particles in the vicinity of a scent source and volatilizing latent chemical traces by the warmth of the expired air (Settles 2005). Mammalian odor detection involves the binding of receptors to signal molecules in the epithelium of the nasal cavity (Engen 1982). This sensory information is processed in the olfactory bulb in the brain and then further refined by central processing to generate a response that allows for odor discrimination (Buck and Axel 1991,

Shepherd 1994). As an example, a thorough discussion of the sense of smell in the canine detection of explosives is given in Furton and Myers (2001).

Odor Signatures

The odors associated with explosives and narcotics in canine detection have been studied (Johnston et al. 1998, Williams et al. 1998, Lorenzo et al. 2003, Harper et al. 2005, Joshi et al. 2009) to improve dog training, deployment, and integration with other technologies (Furton and Myers 2001). Studies using analytical chemistry and certified detection dogs suggest the primary odorant signature chemicals (i.e., those that function as detection cues to trained dogs; Williams et al. 1997) are the volatile compounds associated with the substance rather than the target compound itself (Williams et al. 1998). For example, drug detection dogs alert when methyl benzoate is perceived because it is a volatile decomposition product of the effectively non-volatile cocaine, or benzoylecgonine, molecule (Furton et al. 1997). Canine detectors also alert to piperonal, 3,4-methylenedioxybenzaldehyde, which is more volatile than the target compound 3,4-methylenedioxy-*N*-methylamphetamine, known as MDMA or Ecstasy (Lorenzo et al. 2003). In the case of explosives, most dogs do not respond directly to 2,4,6-Trinitrotoluene or cyclotrimethylenetrinitramine (RDX), the explosive components in TNT and C-4, respectively, as they have low vapor pressures. Canines detect the volatile component 2,4-Dinitrotoluene, an impurity in TNT, or the plasticizer 2-ethyl-1-hexanol in C-4 explosive (Johnston et al. 1998, Williams et al. 1998, Lorenzo et al. 2003, Harper et al. 2005). In addition to volatiles in explosives, dogs might also cue on

substances associated with landmines such as solvents, oils, and plastic housings that might contain polyvinylchlorines and polystyrenes (Göth et al. 2003).

Although dogs are likely to locate substances from the most volatile (Lorenzo et al. 2003) or abundant constituents (Harper et al. 2005), isolating and identifying these important compounds can be difficult (Furton and Myers 2001). Often the constituents themselves or the active concentrations that dogs actually detect are unknown (Göth et al. 2003). Typically, dogs might respond to only a few key compounds as target odorant signatures sometimes as isolated compounds (Harper et al. 2005) and sometimes as blends (Williams et al. 1997). These chemicals might be less abundant but more volatile (Rouhi 1997, Furton and Myers 2001, Bach and Phelan 2003, Harper et al. 2005, Settles 2005) or less volatile but in greater abundance for short-range discrimination or target confirmation. Although great differences in odorant signatures and detection thresholds are unlikely because of similarities in canine olfactory receptor physiology (Johnston et al. 1997, Williams et al. 1997), the variability in odorant signatures used among dogs might be due to previous training with dissimilar odor sources (Williams et al. 1997, Furton and Myers 2001). And perceptual biases in terms of vapor sensing thresholds, physiological sensitivities, or odor pattern recognition of individual dogs (Williams et al. 1997, Laurent 1999, Furton and Myers 2001, Bach and Phelan 2003).

Responses to plant odors have also been intensively studied with most work emphasizing the role of volatile signals in insect herbivory and pollination (Karban and Baldwin 1997, Agrawal et al. 1999, Dicke and Baldwin 2010, Pichersky and Gershenzon 2002). In contrast to the odor profiles of explosives, which show simple emissions

containing only a few abundant compounds (Williams et al. 1997), the volatiles released by plants are in complex mixtures of many different components (Dudareva et al. 2004). The emission of plant volatiles occur passively as a consequence of physiological processes (Peñuelas and Llusía 2004) and mechanical damage (e.g., wind or cutting or insect feeding) due to increased permeability or breached integrity of the tissue cells (Agelopoulos et al. 1999). Plant volatiles are also actively released in response to insect herbivore damage which mediates large changes in volatile quantity and composition (Karban and Baldwin 1997, Agrawal et al. 1999). Herbivore-induced volatiles may serve plant defensive roles via systemic signaling (Arimura et al. 2001, Farmer 2001, Ruther and Kleier 2005) and deterrence of oviposition (Kessler and Baldwin 2001, Engelberth et al. 2004) or by recruiting natural enemies of herbivores (Dicke and Sabelis 1988, Turlings et al. 1990, Rasmann et al. 2005, Pareja et al. 2007). The host-finding and specificity behaviors of insects are also influenced by the volatiles released by undamaged plants (Elzen et al. 1983, Ma et al. 1992, Cortesero et al. 1993, Ngi-Song et al. 1996, Benrey et al. 1997, Takabayashi et al. 1998). For this reason the composition of volatiles emitted by invasive plant species is often studied to evaluate host plant specificity of prospective agents for use in classical biological control, which includes the introduction and release of exotic herbivorous insects that attack and control only the target weed (USDA, APHIS 1988).

Centaurea Species

The invasiveness of *Centaurea* (L.) species (primarily knapweeds) and the ecological impacts to grassland diversity and productivity (Roché and Roché 1991), and

the economic consequences to land managers (Bais et al. 2003) have made control of this genus a priority in the western United States (DiTomaso 2000, Skinner et al. 2000). The volatiles released by *Centaurea* spp. have been investigated to determine the chemical cues that might attract or repel potential biocontrol agents. For example, the volatile composition of *Centaurea solstitialis* L. (Buttery et al. 1986, Binder et al. 1990a) has been compared to *C. calcitrapa* L. (Binder et al. 1990b) to determine the nature of the constituents that might mediate the behavior of insect biocontrol agents. More recently, volatiles from rosette leaves of *C. cyanus* L., *C. solstitialis*, and *C. cineraria* L. have been compared to determine the chemical cues for the weevil *Ceratopion basicorne* Illiger in the herbivory of *C. solstitialis* (Beck et al. 2008). Other chemical studies have investigated the release and identification of phytotoxic compounds by *Centaurea* spp. and the potential for allelopathy. More specifically, compounds in the roots of *C. diffusa* Lam. (Vivanco et al. 2004, Norton et al. 2008, Quintana et al. 2009, Tharayil et al. 2009), and the roots and shoots of *C. stoebe* L. (Kelsey and Locken 1987, Locken and Kelsey 1987, Bais et al. 2002 and 2003, Weir et al. 2003, Perry et al. 2005, Tharayil and Triebwasser 2010) have been studied to address phytotoxicity caused by these plants. The root exudate 8-hydroxyquinoline secreted by *C. diffusa* (diffuse knapweed), was reported as a phytotoxin and found to be abundant in soil extracts at field sites invaded by this plant (Vivanco et al. 2004). But other researchers have had difficulty detecting quinoline in experimental or field collected soils from infested sites (Norton et al. 2008) and from root exudates and root extracts of this plant (Quintana et al. 2009). Thus, the role of quinoline in diffuse knapweed allelopathy has been questioned. Quintana et al. (2009)

reported the phytotoxic activity of this plant was attributable to caryophyllene oxide and linoleic acid. In the case of *C. stoebe* (spotted knapweed), the aerial exudate cnicin was isolated (Kelsey and Locken 1987) but later found not likely to function allelopathically in field settings (Locken and Kelsey 1987). The root exudate catechin has also been reported as an allelochemical in spotted knapweed that contributes to the invasiveness of this species (Ridenour and Callaway 2001, Bais et al. 2002 and 2003, Weir et al. 2003). Other investigators, however, have dismissed the role of catechin in spotted knapweed allelopathy due to an inability to accurately detect even minute amounts of this compound (Blair et al. 2005, 2006, 2009; Tharayil et al. 2008, Duke et al. 2009).

A number of investigations have focused on *Centaurea* ssp. volatile-mediated tritrophic interactions. For example, Pareja et al. (2007) investigated the changes in the volatile chemistry of *C. nigra* L. following aphid, *Uroleucon jaceae* L., attack and that led to attraction of the parasitoid *Aphidius funebris* Mackauer. To explore convergent patterns in floral scent composition, Andersson et al. (2002) compared the volatiles emitted by the flowers of *C. scabiosa* L. to other plant species that also rely on butterflies for pollination. Also, in antimicrobial screening studies, the chemical compositions of the floral essential oils have been reported for *C. cineraria* L. and *C. napifolia* L. (Senatore et al. 2003) and for the aerial tissue of Russian knapweed, *Acroptilon repens* (L.) DC. [syn. *C. repens* L., *C. picris* Pall. ex. Willd.] (Norouzi-Arasi et al. 2006, Tunalier et al. 2006).

Spotted Knapweed

Spotted knapweed, *Centaurea stoebe* L., (Asteraceae) is a biennial to perennial forb with a deep taproot that is native to Eurasia. It was introduced to the Pacific

Northwest in the early 1900s with alfalfa seeds from Turkestan (Groh 1940). By 1988, spotted knapweed dominated over 2.8 million ha in eight western states and one Canadian province (Lacey 1989). It is considered the most common weed in Montana, occupying about 1.5 million ha (Duncan 2005). Spotted knapweed is listed as a noxious weed in 11 western states (USDA, NRCS 2010) and spreads to new sites at an annual rate of 16% (Duncan 2005). It frequently establishes along roads and invades adjacent grasslands (Tyser and Key 1988, Roché and Roché 1991). Impure seed and forage, wildlife and livestock movement, and water pathways represent other avenues of spread (Sheley et al. 1998).

Spotted knapweed is highly invasive in semiarid grasslands (DiTomaso 2000, LeJeune and Seastedt. 2001) and displaces native plants to nearly complete exclusion (Sheley and Larson 1996, Callaway et al. 1999, Ridenour and Callaway 2001, Callaway and Vivanco 2007). The replacement of native plants by knapweed may increase surface water runoff and soil erosion compared to bunchgrass-dominated sites (Lacey et al. 1989) and reduce forage on rangelands (Watson and Renny 1974). This alters habitat suitability for wild ungulates (Thompson 1996) and increases the costs of land management and cattle production (DiTomaso 2000). The invasion success of knapweed into native plant communities has been attributed to prolific seed production (Schirman 1981), seed bank persistence (Davis et al. 1993), and successful competition for limiting resources such as nutrients and water (Velagala et al. 1997, Jacobs and Sheley 1999, LeJeune and Seastedt 2001, Hill et al. 2006).

Research Objectives

Ongoing interest in locating rare weed targets, building on the knowledge that previous investigators have utilized to train dogs to perform these tasks, led to this project. All plant species release gaseous vapors that can serve as a target odorant signature to a canine. The history of using canines to detect concealed plant material such as prohibited fruit imports (USDA, APHIS 1996) and plant derivatives from the opium poppy (heroin), coca leaf (cocaine), and hemp (marijuana, hashish) (Bird 1996) in non-natural settings is a testament to this fact. However, to my knowledge the utilization of canines to locate rare plant species within the overall plant community in natural settings has not been investigated prior to this study.

This thesis study was divided into two parts or chapters. In the first chapter, the ability of dogs to search for and detect rare occurrences of spotted knapweed was compared to the performance of humans at the same sites. The objectives were to test the hypotheses that dogs (1) detect rare spotted knapweed with equal or better accuracy than human surveyors which is the standard detection method, and (2) detect rare spotted knapweed at greater distances than humans. The impetus for the second study was based on our observations from the first study that dogs responded to the below ground odors produced by spotted knapweed plants even though they had not been trained with root material. The objectives were to (1) test the hypothesis that root volatiles contribute to spotted knapweed detection by trained dogs, and (2) characterize the volatile composition of spotted knapweed foliage and root material to evaluate the above and below ground odor profiles.

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Contribution of Authors and Co-Authors

Manuscript in Chapter 1

Chapter 1:

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Contributions: conceived the study and designed and performed experiments, collected and analyzed output data, and wrote the manuscript. J. Wilson wrote code for the permutation and discretization algorithms.

Co-author: Dr. Rick E. Engel

Contributions: discussed the results and implications and commented on the manuscript at all stages. The co-author also generated Figure 2 with data analyzed by K.M.G.

Co-author: Dr. David K. Weaver

Contributions: discussed the results and implications and commented on the manuscript at all stages.

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 Research

Trained Dogs Outperform Human Surveyors in the Detection of Rare Spotted Knapweed (*Centaurea stoebe*)

Kim M. Goodwin, Rick E. Engel, and David K. Weaver*

Invasive plants have devastating effects on ecosystems and biodiversity that early intervention can prevent. Eradication or containment of new invasions is difficult to achieve because of constraints posed by the low density and detectability of individuals. Domestic dogs trained to cue on distinctive scents might provide an effective method to detect spotted knapweed. The objective of this study was to compare the accuracy and detection distances of dogs to humans in locating new spotted knapweed (*Centaurea stoebe*) invasions. Three dogs, trained to detect knapweed using scent discrimination and tracking techniques, were compared with human surveyors. Seven sampling units (0.5 ha [1.2 ac]) were delineated in a grazed dryland pasture. Dogs, with their handlers, and human surveyors performed line-transect surveys in fall 2005 and spring, summer, and fall 2006. Dog accuracy for large-size knapweed targets (infestations 0.52 m³ [18.4 ft³]) was similar to human accuracy and better than humans (94 vs. 78%) for medium-size targets (infestations 0.13m³). Dog accuracy (67%) was greater (> 81% probability) than humans (34%) for small targets (plants; 0.02 m³). Overall dog accuracy (81%) and *F*-measure scores (86%) were better than human scores, 59% and 74%, respectively. Human precision was greater (100%) than dogs at 94%. Dogs detected a larger percentage of small targets (80%) at distances greater than 7.9 m (26 ft) compared with humans at only 20%. Our results indicate dogs are more accurate than humans are, especially at critical detection of small spotted knapweed plants, and from greater distances. Invasive plant monitoring using detection dogs can provide greater overall accuracy of plant detection.

Nomenclature: Domestic dogs, *Canis familiaris* L.; spotted knapweed, *Centaurea stoebe* L.

Key words: Weeds, rangeland, eradication, rare plant monitoring, vapor detection, search dog.

Invasive plants can replace native species (Dukes and Mooney 2004), alter ecosystem function and threaten biodiversity (Braithwaite et al. 1989; Musil 1993), and cause damaging economic effects for land managers in western North America. Early detection and containment or eradication of new invasions can slow their spread (Moody and Mack 1988), prevent future weed problems (Hobbs and Humphries 1995), and reduce ultimate management costs (Higgins et al. 2000). Eradication requires near-perfect control for many years, yet finding juvenile and small adult plants is challenging (Tomley and

Panetta 2002), and sampling becomes increasingly difficult on large sites as management reduces weed density over time (Panetta 2007). The detection of rare individuals, or those occurring in low abundance in an area of occupancy (Gaston 1997), is a common problem but is critical for invasive plant eradication and important for accurate and unbiased occupancy estimates of rare native species (MacKenzie et al. 2002).

Domestic dogs, trained to search for the presence of specific odors and referred to as detection dogs, might provide an effective and reliable detection method for rare plants based on their ability to cover large areas thoroughly (Killam 1990) and to accurately discriminate specific odors (Williams and Johnston 2002). For example, detection dogs with associated handlers have been used to locate buried land mines (McLean 2003), human remains (Killam 1990), cadavers (Rebmann et al. 2000), desert tortoises

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Interpretive Summary

Monitoring invasive plant populations is an essential aspect of the eradication process to locate and remove every individual. Eradication is difficult because small, obscure plants often escape detection and reproduce, increasing the time needed for extirpation. Our study investigated the performance of trained dogs in comparison to human surveyors, the standard detection method, to search for and locate rare spotted knapweed targets over multiple seasons. We found detection dogs were as accurate as humans at identifying large-size spotted knapweed infestations (0.52 m^3) and more accurate than humans (94 vs. 78%) with medium infestations (0.13 m^3). Dog accuracy was much better than humans (67 vs. 35%) with small infestations (plants; 0.02 m^3). In addition, dogs were able to identify small infestations from greater distances than humans. Humans were more selective to larger knapweed targets with stronger visual signals, causing a sampling bias against the detection of small plant targets. Our study demonstrates detection dogs can contribute to effective search protocols by thoroughly covering large areas and increasing the probability small and inconspicuous plant targets will be detected.

(*Gopherus agassizii* C.; Cablk and Heaton 2006), San Joaquin kit fox (*Vulpes macrotis mutica* Merriam) scat (Smith et al. 2003), and the presence of black-footed ferrets (*Mustela nigripes* Audubon and Bachman; Reindl-Thompson et al. 2006). Detection dogs are probably best noted for their use in the field as hunting dogs and in search and rescue to locate missing people or avalanche and disaster victims (Syrotuck 1972). Detection dogs typically search an area by working back and forth in a serpentine pattern while continuously sampling small amounts of odor. When the dog detects a trace of the target scent it follows the odor gradient to its source and gives a trained alert using a reward-based system.

Given that all plant species produce blends of volatile organic compounds with distinctive odors, it seems likely that dogs could be trained to identify the unique signature odors of a plant species or genus in the field. We conducted this study to determine whether dogs could be trained to search for and locate an invasive plant species in a natural setting. We chose spotted knapweed (*Centaurea stoebe* L.) as the experimental model based on its availability, ecological and economic importance, and the precedence established by the successful invasion of western North America (Callaway and Vivanco 2007). The objectives of this study were to test the hypotheses that dogs (1) detect rare spotted knapweed with equal or better accuracy than humans, and (2) detect rare spotted knapweed at greater detection distances than humans.

Materials and Methods

Scent Discrimination and Search Training. Dogs were trained to discriminate the odor of spotted knapweed in the



Figure 1. Field-collected spotted knapweed (left), crested wheatgrass (middle), and soil (right), planted in single hollow, concrete bricks attached to a wood base.

framework of signal-detection theory and operant conditioning (Lieberman 1999). The training procedure consisted of discrete trials with one of two explicit stimuli (signal plus noise or noise alone; Green and Swets 1966). Four to six hollow, concrete bricks were placed 3 m (9 ft) apart in a line. Target knapweed plants (*signal*) were placed in one brick, and the remaining bricks contained nontarget (*noise*) crested wheatgrass plants [*Agropyron cristatum* (L.) Gaertn.], natural field soil, or were left empty (Figure 1). A second hollow, concrete brick was stacked on top of the filled concrete bricks to prevent visual cues to the dogs. Each dog sampled the odor (off-leash) from the concrete bricks. Handlers were initially aware of the target location, which was randomized for each trial. A previously trained alert response (scratching or sitting) to the target odor was rewarded with play and praise. Missed targets resulted in task repetition, and false-positives (alert response to target odor absence) resulted in verbal correction with task repetition. Blank trials without the target odor (*noise alone*) were randomly included at low frequencies (2 or 3 out of 10 trials), based on handler discretion to prevent canine expectations of a reward on every occasion. Once the dogs were competent at discriminating between knapweed targets and controls based on $\geq 80\%$ correct-response scores, they advanced to blind trials, where the handlers were unaware of the identity of each brick. Blind trials ensured unintended cues from the handlers were not influencing the odor-selection process by the dogs.

Search training was conducted in flat, open perennial grasslands. Natural-occurring knapweed plants or fresh-cut knapweed material (about 30 to 50 g [1.1 to 1.8 oz] of leaves and stems) was placed at random locations within the search area. Handlers were initially aware of knapweed target locations to evaluate dog scenting behavior. Missed targets resulted in a repeated search of the target area until the dog responded with an alert response. Later, blank searches (noise-alone trials) were introduced to enforce an expectation targets can be absent. Blind trials were also included, during which the handlers were unaware of target locations.

Table 1. Range and means (\pm SE) for weather conditions at study site between 6:00 and 10:00 A.M. in (a) September 17 to 29, 2005, (b) May 15 to 25, 2006, (c) July 10 to 23, 2006, and (d) September 9 to 26, 2006.

Season	Temperature		Relative humidity		Wind speed	
	Range	Mean (SE)	Range	Mean (SE)	Range	Mean (SE)
	C		%		m/s	
a. Fall 2005	-1.7, 39	6.8 (0.7)	31, 97	74 (2.1)	0, 5	1.8 (0.2)
b. Spring 2006	6, 24	15 (0.6)	90, 98	95 (0.3)	0, 8.6	1.9 (0.3)
c. Summer 2006	8.3, 25	17 (0.6)	27, 94	54 (2.0)	0, 3.4	1.8 (0.1)
d. Fall 2006	-0.6, 17	7.1 (0.4)	30, 97	73 (1.7)	0, 6.9	2.1 (0.2)

Dog and Human Surveyors. One mixed-breed Shepherd and two German Shepherds were selected to participate in this study with their handlers. The dogs varied in detection work experience. *Nightmare* was a 4-yr-old female with 1 yr of spotted knapweed scent training.¹ *Tsavo* was a 6-yr-old male with 3 yr of carnivore scat-detection training, and *Rio* was a 9-yr-old male with 6 yr of carnivore scat-detection training.² Twelve human surveyors participated in this study. New surveyor groups consisting of three people were replaced for each of the four sets of seasonal trials. This was done to avoid bias associated with knowledge gained of the experimental site from prior searches. Surveyors had approximately 2 yr of spotted knapweed detection experience before the study. All surveyors were similar in backgrounds, allowing for generalization to experienced surveyors.

Field Experiment Site and Test Procedure. The field experiment was conducted in Gallatin County near Belgrade, MT (45°46'N, 111°9'W; 1,360 m elevation), in a 67-ha (166-ac) dryland pasture dominated by crested wheatgrass and seasonally grazed by cattle in the summer and fall. The mean annual temperature is 5.5 C (41.9 F), annual precipitation is 35.7 cm (14.1 in), and the frost-free period is 115 d. The field experiment consisted of four sets of seasonal trials that occurred in fall 2005 (September 17 to 29), spring 2006 (May 15 to 25), summer 2006 (July 10 to 23), and fall 2006 (September 9 to 26). Seven sampling units (rectangular area; 0.5 ha) were defined within the pasture according to the spatial distribution of natural knapweed targets and experiment-site limitations, including fence lines. Thirteen targets were present among the seven sampling units as five individual plants and eight infestations (multiple plants) for the first three seasonal trials. One plant target died after the summer seasonal trial; thus, the total number of targets in the last seasonal trial was 12. Target size, density, and percentage of grass cover were characterized three times (May, July, September) in 2006. Mean (\pm SE) height and width of knapweed plants were 20 cm (\pm 0.9) and 29 cm (\pm 1.2), respectively. Mean infestation area, or the smallest area occupied by an

infestation including the outermost plants, was 8.3 m² (\pm 5.1)(89 ft²). The large variance in infestation area was due to differences in patch size and quality, e.g., infestations ranged from 3 plants 1.0 m⁻² patch to 25 plants 42 m⁻² patch. Mean infestation density was 9.6 plants infestation⁻¹ (SE = 2.9). Mean interseasonal canopy cover and grass height were 53% (SE = 1.1) and 12 cm (SE = 0.3), respectively. Ambient temperature, relative humidity, and wind speed data were obtained from a Federal Aviation Administration automated weather station located 3.2 km (2.0 mi) from the study site. The range and average of weather conditions among seasonal trials are given in Table 1.

Human surveyors and dogs with their original handlers independently performed line-transect sampling for stationary populations (Anderson et al. 1979) using a census-based approach. Parallel transect lines were established at 5-m intervals and defined with survey flags. The narrow-transect width (10 m) provided a high probability of target detection and ensured sites were adequately searched in similar time periods to reduce sampling bias related to quick or incomplete search efforts. Dogs were off-leash and performed the search on their own and ahead of the handlers (~10 to 15 m). The role of the handlers was to interpret dog behavior and to control for dog-related and weather-related sampling bias by ensuring adequate coverage of the sampling units by the dogs and observing air currents that affect odor availability, much like a person operating vapor sensor equipment. Handlers were not aware of target locations and instructed not to look for targets so dog performance would not be biased. Dog trials were videotaped to document scent detection and eliminate observer bias. Dog and human surveyors searched between 6:00 and 10:00 A.M. Trials were not conducted during inclement weather. Dogs were deployed at 24-h intervals to allow for dispersion of any odor cues.

Parameters Measured and Statistical Analyses. Knapweed target size was indexed based on the volume of a cylinder ($\pi \times r^2 \times ht$) that would completely cover each plant. Infestation volume was calculated by summing the

volume of individual plants. The 13 targets were classified into three size categories with the k -means algorithm for clustering (Tou and Gonzalez 1974) using MATLAB (Version 7.4) statistics toolbox.³

Signal detection theory (Green and Swets 1966) was applied to assess the binary decision making for subjects (yes, there was a signal; no, there was not a signal) and its relationship to signal presence or absence. The purpose of analyzing response outcomes was to evaluate response bias and the ability of subjects to discern between signal and noise. Four possible response outcomes for the detection task included *true positive* (TP) or hit, *false positive* (FP; Type I error) or false alert, *false negative* (FN; Type II error) or miss, and *true negative* (TN) or correct rejection to target absence. Dog and human trials were scored for sensitivity (accuracy or recall), precision, and F measure. These performance measures are from detection theory (receiver operating characteristic [ROC] analysis; Egan 1975) and commonly used in information-retrieval analysis because the scores do not require true negative data (Han and Kamber 2006). In ROC analysis, accuracy is the proportion of TP and TN results (Fawcett 2006). Our experiment did not address true negatives. Therefore, we defined accuracy as the hit rate using the formula for sensitivity (S_n), which refers to the proportion of TP divided by the sum of TP and FN. Allowances are not made for chance agreement, however, when false positives are commonly subtracted from the hit rate. To evaluate random error and qualify accuracy, we accounted for false positives in our precision and F measure scores. Precision (Prec) was defined as the proportion of TP divided by the sum of TP and FP. Accuracy was also evaluated using Type II F measure values, or the combined performance of precision and sensitivity [= $2(Prec \times S_n)/(Prec + S_n)$]. Sensitivity and F measure refer to the discriminative ability of the method to detect a signal from background noise (Macmillian and Creelman 2005). Confidence intervals (95% CI) for sensitivity, precision, and F measure were estimated using standard methods for proportions.

Because detection is a binary task, we treated this experiment as a series of independent Bernoulli trials of success and failure (i.e., either the target is detected or not), which results in a binomial distribution. On each trial, the probability of success is p , which yields value 1, and the probability of failure is $q = 1 - p$, which yields value 0, and $p + q = 1$. However, based on the lack of an a priori binomial probability, we calculated the exact probabilities (P values) of human performance (1) equaling or bettering dog (human \geq dog, conservative statistic), and (2) bettering dog (human $>$ dog, less-conservative statistic) over four seasonal trials and among target sizes by full permutation tests (Good 1994) with probability p . The probability of dog performance (1) equaling or bettering human (dog \geq human), and (2) bettering human (dog $>$

Table 2. Knapweed target characteristics and size category as defined by k -means clustering.

Target	No. of plants	Volume (SE)	Size category ^a
		m ³	
1	1	0.006 (0.002)	S
2	1	0.053 (0.031)	S
3	1	0.025 (0.015)	S
4	6	0.106 (0.030)	M
5	22	0.720 (0.252)	L
6	4	0.141 (0.034)	M
7	4	0.437 (0.132)	L
8	6	0.138 (0.018)	M
9	1	0.014 (0.007)	S
10	23	0.390 (0.107)	L
11	3	0.004 (0.001)	S
12	1	0.012 (0.007)	S
13	9	0.025 (0.029)	S

^a Abbreviations: S, small (0.004–0.053 m³); M, medium (0.106–0.141 m³); and L, large (0.39–0.72 m³).

human) was determined with the probability $1 - p$. Therefore, the probability that humans outperform dogs is one minus the probability they will not outperform dogs.

Detection distances, or the length between the surveyor and target, were measured to compare the distance over which the target signals were detectable to dogs and humans. Distances were determined at first visual detection for humans and first scent detection for dogs. The dog detection point was verified with video and defined where a noticeable difference in search behavior was observed, e.g., changes in pace, direction, and sniffing behavior. Detection distances were divided into three groups (near, intermediate, and far) by discretization using equal-frequency binning (Grzymala-Busse 2002). Permutation and discretization was implemented with MATLAB (Version 7.4)³ scripts.

Results and Discussion

Knapweed targets were divided into small (0.02 ± 0.01 m³ [0.71 ± 0.35 ft³]; $n = 7$), medium (0.13 ± 0.01 m³; $n = 3$), and large (0.52 ± 0.10 m³; $n = 3$) categories by k -means clustering (Table 2). High variability in target volume resulted from an increase in aboveground growth between the spring and summer trials and a reduction in aboveground biomass from browsing by cattle between the summer and fall trials. Target 9 died between the end of the summer trials (July 23) and the beginning of the fall trials (September 9).

The summary of response outcomes (defined above) show the Bernoulli trial scores (Table 3) for hit or miss and indicate the dog method detected more targets than the

Table 3. Summary of response outcomes by dog and human surveyors to target presence ($n = 153$; TP and FN) and absence (FP).^a

Surveyor	Response	Season												Total
		Fall 2005			Spring 2006			Summer 2006			Fall 2006			
		D ₁	D ₂	D ₃	D ₁	D ₂	D ₃	D ₁	D ₂	D ₃	D ₁	D ₂	D ₃	
Dog	TP	11	10	11	9	8	10	12	10	12	10	10	11	124
	FN	2	3	2	4	5	3	1	3	1	2	2	1	29
	FP	0	2	0	1	0	1	1	0	0	0	2	1	8
		H ₁	H ₂	H ₃	H ₁	H ₂	H ₃	H ₁	H ₂	H ₃	H ₁	H ₂	H ₃	
Human	TP	7	9	7	6	5	7	9	8	9	8	7	8	90
	FN	6	4	6	7	8	6	4	5	4	4	5	4	63
	FP	0	0	0	0	0	0	0	0	0	0	0	0	0

^aAbbreviations: TP, true positive (hit); FN, false negative (miss); FP, false positive (false alert); D₁, dog 1, Nightmare; D₂, dog 2, Tsavo; D₃, dog 3, Rio; H₁, human 1; H₂, human 2; H₃, human 3.

human method (TP = 124 vs. 90, respectively). Interdog variability in performance was largely due to natural differences in sampling behavior and temperament traits. For instance, although Tsavo displayed obedience and enthusiasm to work, he gave more false alerts (FP = 4) and less hits (TP = 38) than the other two dogs (Table 3). We attributed mistakes and lower accuracy to distraction by ground squirrels (*Spermophilus* spp.), resulting from high prey drive to chase and catch small animals. Rio performed slightly better than Nightmare as a result of two additional hits (TP = 44 vs. 42, respectively) due to his slow searching behavior (discussed later). Both dogs had two false alerts (FP). Overall accuracy (81%) for the dog method was greater than the human method (59%). Dog accuracy for small (67%) and medium (94%) size targets was better than human accuracy (35 and 78%, respectively) but similar for large targets (Table 4). Overall F measure (mean, 86%; 95% CI, 85 to 87%) of the dog method was greater than the human method (mean, 74%; 95% CI, 72 to 75%). Overall precision of the dogs was lower (mean, 94%; 95% CI, 93 to 95%) than humans (100%), indicating both methods have high precision and, thus, low response bias or minimum systematic errors. The accuracy and F measure scores suggest the dog method has

more discriminative ability for knapweed than the human method. Dog performance is subject to odor availability as a function of handler skills (Gutzwiller 1990). Our design minimized handler error and influence, so performance could be attributed to dogs. Odor availability was controlled over seasonal trials by maintaining a constant search strategy (i.e., parallel line-transect searches), conducting searches in the early morning hours, and selecting similar sampling units (flat ground with short vegetative cover; see Materials and Methods).

The probabilities of dog equaling or outperforming human (conservative measure) and dog outperforming human (less-conservative measure) are shown in Table 5. The conservative results (dog \geq human) show the detection performance of dogs is equal to or better than humans among target sizes and over seasons, except in one observation (medium-size target, fall 2006), where dogs were better than humans about half of the time [49.8% ; $(1 - 0.502) \times 100$] because of odor evaporation resulting from marginal weather conditions (i.e., high heat and low moisture). Overall, these conservative results suggest dogs significantly outperform humans with small and medium size targets and perform equally well with large targets over seasons. More specifically, dog accuracy for large targets (100%) was as good as human accuracy (94%) and much better than humans for medium (94 vs. 78%) and small (67 vs. 35%) size targets. An interesting finding is that dogs were consistently more accurate than humans with important small targets at 81% probability and better. The less-conservative results (dog $>$ human) show dogs outperform humans at least 92.7% [$(1 - 0.073) \times 100$] of the time among target sizes and over seasons, except again in the previously mentioned observation (medium size target, fall 2006), where dogs outperformed humans at 87.3% probability [$(1 - 0.127) \times 100$]. Overall, these

Table 4. Summary of dog and human surveyor accuracy scores by target size.

Surveyor	Target size category			Overall accuracy
	Small	Medium	Large	
	mean % (95% CI)			
Dog	67.1 (2.50)	94.4 (2.06)	100.0	81.1 (1.49)
Human	34.7 (1.56)	77.8 (3.44)	94.4 (2.06)	58.9 (1.59)

Table 5. Probability ($1 - p$) of dog equaling or outperforming human (dog \geq human) and dog outperforming human (dog $>$ human) for three target size categories (small, medium, and large) over seasonal trials.

Season	Target size category					
	Small		Medium		Large	
	Dog \geq human	Dog $>$ human	Dog \geq human	Dog $>$ human	Dog \geq human	Dog $>$ human
Fall 2005	0.981	0.998	0.748	0.998	0.00 ^a	0.998
Spring 2006	0.806	0.927	0.811	0.967	0.748	0.998
Summer 2006	0.996	0.998	0.00 ^a	0.998	0.00 ^a	0.998
Fall 2006	0.981	0.998	0.498	0.873	0.00 ^a	0.998

^aValue of 0.00 means dog and human performances were equal.

less-conservative results indicate dogs consistently outperform humans over seasons and among target sizes. In particular, we found dogs were more accurate than humans with small targets (67 vs. 35%, respectively) at 93% probability and better. The moderately high sensitivity (81%) and F measure (86%) of the dog method compared with the human method (sensitivity, 59%; F measure, 74%) explain our observations on better dog performance, i.e., better dog accuracy was due to the ability of canines to discriminate target signals, including small or low-level signals, from background noise (explained below) given the experimental conditions. In a similar study with desert tortoises, Cablk and Heaton (2006) found dogs detected smaller tortoises (30 mm [1.2 in] midline carapace length [MCL]) compared with humans (110 mm MCL). In our study, dog and human surveyors show high precision (94% and 100%, respectively). Dog precision was lower than humans because of eight false alerts (FP) by dogs compared with none for humans (Table 3) resulting from corrections in perceptual judgment before a response. We attributed false alerts by dogs to natural factors, such as scent pools remote from the source (Rebmann et al. 2000) and behavioral variations. Although dogs gave a higher level of false positives (Type I error), which may incur a cost (e.g., credibility loss), the risk is acceptable to the lower level of false negatives (Type II error) that have higher relative costs when missed plants reproduce.

The ability of dogs to locate small knapweed plants that escape detection by humans is because dogs use olfaction to identify targets, whereas humans rely on vision. Dogs possess hundreds of different receptor types organized by similarity to encode specific proteins, which then converge upon two precise loci in the olfactory bulb (Buck 1996; Buck and Axel 1991; Mombaerts et al. 1996) to increase signal-to-noise ratios by averaging out uncorrelated noise via postsynaptic summation (Laurent 1999). The ability to reduce input noise might help explain why dogs can detect odor concentrations of about 0.001 ppmv (or 1 part per billion; Waggoner et al. 1998). In contrast, mammalian vision systems process only a subset of all visual

information by three peripheral receptor types (i.e., red, blue, and green; Young–Helmholtz theory) and are strongly affected by noise (Tanner and Swets 1954). It is interesting that humans were more selective to larger targets, whereas small targets were frequently under-sampled. We believe humans were most successful with larger targets because of stronger visual signals that likely surpassed the background noise of surrounding vegetation. We reason dogs were less affected by noise and, therefore, sensitive to each target size, including small plants with low signal strength.

Dog and human surveyor performance was influenced by seasonal effects on knapweed targets and site conditions. We believe lower performance by dogs and humans in the spring trials compared with the summer and fall trials (Table 5) was due to difficulties in perceiving plant targets. During the spring trials, all vegetation was uniform in size and color, and targets were less visually conspicuous to humans compared with other seasons. In the case of dogs, target odors were likely trapped by dense vegetation or counteracted by the comparatively high levels of volatile compounds produced by the actively growing vegetation (which reduced target odor intensity or masked target odor quality). That is, high levels of extraneous plant odors, which are somewhat chemically similar to knapweed, might have interfered with perception of the target odor by neural inhibition or by competition for olfactory receptor sites (Cain and Drexler 1974; O'Connell and Akers 1989). During the summer and fall trials, knapweed plants were more noticeable to humans because they remained green with purple flowers against background grasses that were dormant and yellow. Cattle grazing also improved the detectability of targets by reducing the aboveground biomass of perennial grasses to increase target visibility and odor transport. These factors together likely acted to improve both dog and human performance.

Distances of detected targets ranged from 1.0 to 62.2 m for dogs ($n = 124$) and 0.4 to 31.1 m for humans ($n = 90$). Dog and human surveyors detected targets from distances > 20 m in 19 of 52 (37%) and 2 of 18 (11%)

Table 6. Mean (\pm SE) for detection distance of dog and human surveyors grouped by target size.

Target size	Dog		Human	
	Distance	Frequency	Distance	Frequency
	m		m	
	mean (SE)		mean (SE)	
Small	8.5 (1.3)	54	4.6 (0.6)	28
Medium	12.8 (2.8)	34	4.5 (0.5)	28
Large	12.6 (1.9)	36	7.4 (1.1)	34

cases, respectively. We observed dog detection distances > 50 m were rare (3 of 52 cases; 6%). Target detections ($n = 214$) were grouped into three distance bins of approximately equal size ($n \approx 71$). Near, intermediate, and far distance bins correspond to distances of < 3.7 m, 3.8 to 7.8 m, and > 7.9 m. The mean values of these frequencies, i.e., detection distances, were grouped by target size (Table 6) and plotted as histograms (Figure 2). From the constructed histograms, the estimated frequency of detected targets by target size was visually evaluated for each distance bin. The characteristic feature of these histograms is the difference in detected targets, in particular small targets, identified at far distances by dogs and humans. Out of 25 small target detections in the far distance category (> 7.9 m), there were 20 detections made by dogs (80%) and only 5 detections made by humans (20%). These results suggest dogs are better at detecting small targets at greater distances than humans are at detecting them. More specifically, dogs found 54 small knapweed targets of which 37% were detected at far distances, and humans found 28 small targets of which 17% were detected at far distances. These observations further validate (1) the high sensitivity of the dog's olfactory system to detect weak signals, even as these signals are dissipated with distance; and (2) the low sensitivity of the human vision system which requires small targets be proximate before they can be detected, if they are identified at all. The range of distances over which knapweed targets were detectable to dogs (1.0 to 62.2 m) given the site conditions, i.e., short vegetative cover and flat ground, is similar to other dog studies involving desert tortoise detection (0.5 to 62.8 m) in open desert-shrub habitat (Cablak et al. 2008). Although the dogs in our study were trained to indicate the source of the target odor, i.e., the knapweed plant, future detection distance studies might consider training dogs to indicate detection at the first whiff of the target odor for quantitative measures of detection distance.

Dog teams have considerable potential for invasive plant eradication by improving accuracy for small, infrequent plants, which are undersampled by human surveyors. Monitoring should, therefore, rely on multiple methods to sample populations satisfactorily. We reason detection dogs

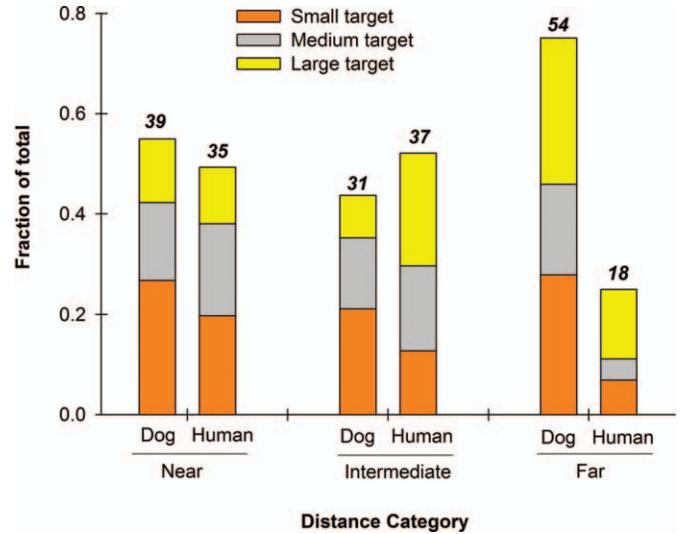


Figure 2. Target size distribution of successful detections at near (0.4 to 3.7 m; $n = 74$), intermediate (3.8 to 7.8 m; $n = 68$), and far (7.9 to 62.2 m; $n = 72$) distance categories for dog and human surveyors. Numbers above stacked bars indicate the sum of counts for each.

can provide a useful auxiliary system to human surveyors, particularly in areas known to contain low-density plants that require high levels of detection. The frequency and temporal spacing of surveys and numbers of humans and dogs in a search team will fluctuate based on accuracy requirements, individual performances, site conditions and search area, target detectability, detection rates, and the length of the prereproductive phase of the plant species (Panetta 2007; Panetta and Timmins 2004).

Future studies should focus on the control of environmental and biological variables that affect the accuracy of rare-plant detection by dogs, like search duration, vegetation, weather, and behavioral traits. Although mean (\pm SE) search duration of dogs (24 ± 0.9 min) was similar to humans (26 ± 1.1 min), dogs were considerably more effective because more plants were detected per unit of time and area. Our sampling units were located on flat ground in grazed grasslands that were relatively structurally uniform. Future studies in structurally complex sites (multiple plant species and canopy layers) might result in longer search times and lower accuracy because dense vegetation would likely impede the transport of target plant odors. Weather factors also affect odor availability and detection in response to wind, temperature, and moisture (Gutzwiller 1990; Phelan and Webb 2003; Syrotuck 1972), and drive discontinuous odor signals in field settings (Bach and Phelan 2003). Strategies to minimize dog-induced detection bias (misses or Type II errors) include searching in the early morning hours to control for daily weather cycles when solar radiation frequency and evaporation potential is low. Odor availability

is greatest with high soil-moisture content and relative humidity, cool air and soil temperature, and low wind velocities (Phelan and Webb 2003).

Search accuracy might also be improved and detection bias reduced by selecting slow-searching dogs with the ability to concentrate in field settings. For instance, Rio had a slower mean (\pm SE) search time (31 ± 1.5 min) than Nightmare (19 ± 0.9 min) and Tsavo (23 ± 1.1 min) and was responsible for 80% (four out of five) of the unique interseasonal detections by dogs. Because olfaction is a low-bandwidth sense that occurs on a relatively slow timescale, when compared with vision (Laurent 1999), searching at slow speeds permits longer odor exposures and better discrimination accuracy for weak signals (Rinberg 2006). High levels of concentration needed to search at slow speeds are the preferred sampling behavior of dogs trained to detect land mines (Bach and Phelan 2003) and may have been important in this study because spotted knapweed roots were found to release odors detectable to dogs (K. M. Goodwin et al., unpublished data). We believe the techniques described in this study are applicable to the arena of rare and endangered plant species detection. Visual surveys for these plants often reflect only adult life stages, whereas nonflowering plants and inconspicuous life stages can often be overlooked under intense searches (Palmer 1987). Clearly, the scent training and field search techniques we employed could easily be extended to detection of other plant species and represents an exciting opportunity for future research.

Sources of Materials

¹ Rocky Mountain Command Dogs, Virginia City, MT.

² Working Dogs for Conservation Foundation, Three Forks, MT.

³ MATLAB, Version 7.4, statistical software, The Math Works, Natick, MA.

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CHAPTER 2

CANINES TRAINED TO LOCATE SPOTTED KNAPWEED USING AERIAL
TISSUE CAN ACCURATELY DETECT VOLATILE COMPOUNDS FROM THE
ROOTSIntroduction

Invasive plants can displace native species, affecting ecosystem processes (Dukes and Mooney 2004) and threatening biodiversity (Wilcove et al. 1998). Early control of new populations is desirable because it allows herbicide treatment with reduced nontarget effects to the ecosystem and increases the likelihood of eradication. Successful eradication is difficult to attain due to inadequate detection rates (Regan et al. 2006) for relatively obscure targets including juvenile and small adult plants (Tomley and Panetta 2002). Recently, it has been demonstrated (Goodwin et al. 2010) that domestic dogs (*Canis familiaris* L.) can be trained to search for and detect rare spotted knapweed (*Centaurea stoebe* L.) incursions in field settings with greater overall accuracy than human surveyors. Spotted knapweed is an invasive plant, listed as a noxious weed in 11 western states (USDA, NRCS 2010), which has spread throughout the western United States with serious impacts on rangelands (DiTomaso 2000). Superior dog performance in detecting this weed was attributed to their olfactory ability to identify small plants with greater accuracy and probability than humans who relied on vision. Trained dogs might improve detection rates for small plant targets but little is known about how dogs detect spotted knapweed within a plant community.

Previously described training protocols that enabled dogs to search for and locate spotted knapweed in the field typically used fresh-cut vegetative tissue samples as scent targets (Goodwin et al. 2010). The rationale for this decision was based on the belief that foliage derived odors would play a dominant role in canine knapweed detection. However, it became apparent during training sessions that root-derived odors diffusing through the soil might also play a role in the process of detection. In particular, we observed a dog that exhibited a positive response to knapweed odor employ deep, 'hard sniffs' when digging deeper in the soil profile containing dead knapweed roots. This behavior occurred on two separate occasions. 'Hard sniffing' has been found to increase air flow and the rate of collection of odor molecules of interest by olfactory receptors (Settles 2005). Given that dogs trained to search for and locate spotted knapweed foliage within a plant community are also able to detect knapweed root material, it is likely they utilize root volatiles in the detection of knapweed targets.

The first objective of this study was to test the hypothesis that root volatiles contribute to spotted knapweed detection by trained dogs. An additional component of this objective was to characterize the volatile composition of spotted knapweed foliage and root material to evaluate the above and below ground odor profiles. This would aid in understanding the ability of the dogs to identify underground root tissue that they had not been specifically trained to detect.

Materials and Methods

Detection of Root Odors

Dogs: One 4-yr-old female mixed-breed Shepherd and two male German Shepherds, 6-yr-old and 9-yr-old, respectively, were trained to search for and locate naturally-occurring spotted knapweed plants in field settings described by Goodwin et al. (2010). The behavioral responses of the dogs to odors from bare and intact spotted knapweed roots were measured in a controlled setting.

Odor Sources: Five spotted knapweed plants at the flowering/seed maturation stage and five crested wheatgrass, *Agropyron cristatum* (L.) Gaertn., plants at the vegetative regrowth stage were collected in mid-August 2006 from random sites in Gallatin County near Belgrade, MT (45° 46'N, 111° 9'W; 1360 m elevation). Crested wheatgrass was selected for inclusion because it was the dominant plant species at the field site, and its previous use as a negative control in scent discrimination training. Plants were transplanted to 20-cm diameter (3.5 L) plastic pots containing a pasteurized potting soil. Soil was collected from the locations where the plants were obtained and mixed with the potting soil at a rate of 2:1. This blend of soil was evenly distributed among identical 20-cm diameter plastic pots ($n = 5$). The plants and field soil were held for one week under greenhouse conditions (natural light, daily low temperatures ~10°C, daily high temperatures ~23°C). This procedure yielded odor sources for two experiments using dead spotted knapweed roots.

Experiment 1 – Dog Detection of Exposed, Bare Root Material: The objective of this experiment was to test the hypothesis that dogs can correctly select bare spotted knapweed root material more successfully than would be expected by chance alone. Randomly selected knapweed ($n = 3$) and wheatgrass ($n = 3$) plants were prepared from the experimental array described above by separating the above ground and root tissue. Root tissue was washed with distilled water, and air-dried (25°C) for three weeks. Washed, air-dried knapweed (~ 25 g) and wheatgrass (~32 g) root material was placed inside hollow concrete bricks (approximately 20 cm³ with a 12 cm² opening). Four concrete bricks were used in each trial. One brick contained dry knapweed roots (25.2 g), two bricks contained dry wheatgrass roots (30.6 and 32.7 g) and one brick was empty. Dehydrated roots were moistened by misting with ~1 ml of distilled water 15 hours prior to the start of the odor response trial.

Experiment 2 – Dog Detection of Buried Root Material: The objective of this experiment was to test the hypothesis that dogs can correctly select intact spotted knapweed root material more successfully than would be expected by chance alone. Knapweed ($n = 1$) and wheatgrass ($n = 2$) plants were randomly selected from the experimental array and transplanted as whole plants to single hollow concrete bricks. A brick also contained natural field soil integrated with potting soil ($n = 1$). Plants were maintained for 3 days under greenhouse conditions, as was the brick containing the soil mixture. Water and sunlight were withdrawn for three weeks to kill the plants. Aerial tissue was removed without disturbing the soil or damaging the roots, dried at 50°C for 5

days, and weighed. Dried knapweed aboveground biomass was 3.8 g. Mean (\pm SE) weight of dried aboveground biomass of wheatgrass was 7.3 ± 1.7 g. Each brick contained approximately 1.8 kg of soil. The aim was to construct odor sampling positions that simulated the odor of dry intact roots of knapweed ($n = 1$) and wheatgrass ($n = 2$) without foliage as might occur in a natural setting, and also to provide a soil only control ($n = 1$). The surface of the dehydrated soil in each brick was moistened by misting with ~ 1 ml of distilled water 15 hours prior to the start of the experiment.

Experimental Setting and Procedure: The study was conducted in a livestock building (20 x 60 m) unfamiliar to the dogs with a dirt floor at the Gallatin County Fairgrounds (45°69'N, 111°4'W) in Bozeman, MT on 18 September 2006 between 9:00 and 11:30 A.M. The building was not climate controlled, and mean (\pm SE) temperature and humidity in the building during the experiments were $16 \pm 0.5^\circ\text{C}$ and $32 \pm 1.0\%$, respectively. Two randomized single blind odor response experiments were conducted to evaluate the ability of the foliage trained dogs to identify spotted knapweed root odors. One target (brick with knapweed roots) was placed among three controls (empty bricks or bricks with wheatgrass roots) to create a four alternative-forced-choice task (Green and Swets 1966).

A single test consisted of one unleashed dog independently sampling the odor of each brick, arranged in a line with at least 3 m between each brick, until an alert response was given. Each dog performed ten tests for each experiment. Alert responses to target odor presence were positively reinforced by the handler by play and praise. Other

responses were not recognized. We evaluated performance in the context of signal detection theory (Green and Swets 1966) to examine the association between the presence and absence of a signal and the positive and negative decision making of the dogs. The correct or incorrect response (binary outcome) of each dog to each brick (unit of analysis) was recorded and included the following responses to (1) target presence: *true positive* (TP) or hit and *false negative* (FN; Type II error) or miss, and (2) target absence: *true negative* (TN) or correct rejection and *false positive* (FP; Type I error), which is false alert, or an incorrect indication that the target signal is present. Dog deployment order was randomized within each experiment. The location of the target brick randomly changed for each new test. For all tests, the target was present at one of four locations.

Data Analysis: In our binomial experiment, each choice (i.e., each brick) resulted in either a success (correct response: true positive or true negative) or failure (incorrect response: false negative or false positive) and the probability that a trial results in a success is the same for each trial. The outcome measured was the mean success rate per dog, compared with an expected value of 1 in 4 (25%). Maximum likelihood techniques for estimation and bootstrap methods were applied to construct 95% confidence intervals for the parameter estimator, i.e., success estimates of the dogs, given the small dataset and the absence of prior knowledge about the distribution of the underlying population (Efron and Tibshiriani 1993). To determine whether there was a systematic improvement

in dog performance over the ten tests, confidence intervals for the first five tests were estimated and the overlap was evaluated for all tests combined for each experiment.

Volatile Collection and Chemical Profile Analysis

Intact Plants: Spotted knapweed plants at the flowering/seed maturation stage (~11 g above ground tissue, fresh wt) were collected from random sites in Gallatin County near Belgrade, MT in early-September 2005. Five knapweed plants were transplanted to 20-cm diameter (3.5 L) plastic pots filled with a pasteurized potting soil mixture and maintained in greenhouse conditions as previously described for three weeks. Volatiles were collected over an 8 hr sampling interval using an automated ‘push-pull’ headspace collection system (Weaver et al. 2009). Briefly, whole plants were individually enclosed in glass collection chambers (4 cm diameter, 80 cm long) with a hole at the base of each plant that was sealed with a flexible Teflon sleeve. Purified, humidified air was pumped into the bottom of the chamber through a ChemThread no. 7 inlet at a rate of 1.0 L/min. Air was pulled through the chamber at the same rate into a collection trap, 6.35 mm OD x 76 mm long (Analytical Research Systems, Gainesville, FL), that contained 30 mg SuperQ adsorbent (Alltech, Deerfield, IL). The volatile collection trap was attached to the top of the chamber using a rubber O-ring and a No. 7 ChemThread cap. Air flow and pressure were regulated in the chambers by a balanced compressed air delivery system leading through the trap to a stable vacuum setting which was regulated by large capacity pump (Heath and Manukian 1994). Before use, all glassware was thoroughly cleaned and then rinsed with analytical grade acetone and

hexane, and stored at 150°C to prevent condensation of ambient volatiles. Empty chambers were used as controls. Volatile collections were repeated twice.

Fresh and Dry Roots: Fourteen spotted knapweed plants at the flowering/seed maturation stage were selected and identified with survey flags in early October 2006 at random sites in Gallatin County near Belgrade, MT. The aerial tissue was cut off at the root crown. Twenty four hours later the roots were gently removed from the soil and carefully washed with distilled water. The root crowns were wrapped with aluminum foil to minimize collection of volatiles from any aerial tissue remaining on the root crown. Volatiles were collected from seven roots simultaneously for 22 hours. The volatile collection apparatus is described in Daisy et al. (2002). Briefly, the detached roots were individually placed in 3.5 cm diameter, 53 cm long horizontal glass volatile collection chambers. Volatile collection traps were fitted on one end with the O-ring ChemThread assembly. All other conditions of collection were identical to those used with intact plants. Collections were repeated twice with seven roots. Fresh roots were 11 cm (± 1.3) long and 2.1 cm (± 0.3) wide and the mean weight was 7.8 g (± 1.1). Uprooting alters the integrity of the root and causes physical damage to the tissue, which in addition to cutting or even gentle handling, changes the chemical profile that is normally emitted (Piesik et al. 2006). Therefore, the fresh root material was dried at room temperature (25°C) for six weeks and the volatile collection procedure was repeated on the dried roots for 22 hours. Mean weight of dry knapweed roots was 3.6 g (± 0.6). Volatiles were collected from the

dry roots to characterize these compounds in relation to those released by roots that were freshly extracted from the soil.

Analysis and Identification of Volatiles: Compounds were eluted from the volatile collection traps using 200 μ l of hexane. An internal standard of 7.34 ng of (*E*)-2-nonene was added to each elution. Samples were stored at -30°C until analysis. Eluted extracts were analyzed on a Hewlett Packard 5973 mass spectrometer interfaced to a Hewlett Packard 6890N gas chromatograph fitted with a 30 m HP-5MS capillary column. The GC-MS oven temperature was initially set at 50°C for four minutes. The temperature was then ramped at 5°C per minute to 160°C, and then 25°C per minute to 280°C. The identity of the compounds was initially determined using National Institute of Standards and Technology (NIST) Version 1.7a library matches. Compounds of interest were quantified by comparison of individual peak areas to those of the internal standard and reported as production rates in ng/hr. Where possible, spectral matches were confirmed using the retention time and mass spectra of authentic standards.

Results

Odor Response Experiments

The summary of response outcomes to the presence and absence of spotted knapweed root volatiles are shown in Table 1. Compared to a 25% expectation if selection were random, the dogs correctly selected the bare root target in 29 of 30 cases, which gave a mean success rate of 96.7%. Using maximum likelihood estimation the

95% confidence intervals (CI) were 90.1 and 100% and 76.7 to 100% using bootstrap methods. The untrained dogs correctly selected the intact root target in 26 of 30 cases, which gave a mean success rate of 86.7% with 95% CI of 74.3 and 99.0% with maximum likelihood estimation and 65.4 to 93.3% using bootstrap methods. In the first five tests, the dogs correctly selected the bare root target in 14 of 15 cases, which gave a mean success rate of 93.3% with 95% CI of 80.3 and 100% and correctly selected the intact root target in 12 of 15 cases, which gave a mean success rate of 80.0% with 95% CI of 59.1 and 100%. The confidence intervals for the first five tests overlapped those for all tests combined in each experiment, so the performance of the dogs did not differ at a probability <0.05 . This suggests that there was no systematic increase in canine performance over the test interval.

Volatile Collections

The GC-MS analyses of the volatiles eluted from the collection traps yielded 28 identifiable compounds (Table 2) in spotted knapweed, fresh roots and the same roots after drying. The main constituents of the foliage were found to be (*Z*)-3-hexenyl acetate, **8**, β -cis-ocimene, **11**, and β -cubebene, **19**, which were not present in the roots. The main constituents of the fresh and dry roots were found to be junipene, **21**, β -caryophyllene, **16**, and thujopsene, **20**. Junipene and thujopsene were absent from the foliage, however, β -caryophyllene was the most common compound among the plant tissues. Although there was overlap in the minor compounds (**1** – **6** in Table 2) from the foliage and roots, this varied by the type of root tissue (fresh or dry). The volatile profiles of the foliage

and roots were different both qualitatively and quantitatively, especially for the dominant compounds from each tissue.

Discussion

The dogs demonstrated accurate detection performance in both odor response experiments with a high level of true positives (97% bare roots, 87% intact roots) and true negatives, and a low level of false positives and false negatives. This greatly exceeds a 25% expected true positive response by chance and indicates that root odor is a component of the odor detection signature dogs use to detect knapweed targets. The ability of the dogs to recognize bare root material (97%) was better than intact root material (87%), which was probably the result of more odor availability to the dogs due to better odor dispersion compared with the intact roots. Although the intact roots may have released the same quality and amount of vapor as the bare roots, the soil likely reduced odor transport rates due to sorption (Bach and Phelan 2003), or the ability of soil particles to hold molecules. This likely resulted in less odor availability to the dogs and slightly lower detection rates compared with the bare root targets. These findings indicate root volatiles play a role in the process of spotted knapweed detection by dogs.

The foliage and roots of spotted knapweed produce volatile profiles containing many constituents. The three most abundant constituents of released by knapweed foliage were determined to be (*Z*)-3-hexenyl acetate, **8**, β -cis-ocimene, **11**, and β -cubebene, **19** (Table 2). Although the foliage compounds reported in Table 2 were in general agreement with the results from other *Centaurea* studies for intact leaves, flowers, and

stems (Buttery et al. 1986, Binder et al. 1990a and 1990b, Andersson et al. 2002, Senatore et al. 2003, Norouzi-Arasi et al. 2006, Beck et al. 2008), it is difficult to generalize the volatile composition and quantities released due to differences in sampling and analytical methods.

To our knowledge, this is the first report of the volatile profile from spotted knapweed roots. Although the extracts of spotted knapweed root exudates have been the subject of several phytotoxic studies using racemic catechin (Ridenour and Callaway 2001, Bais et al. 2002 and 2003, Weir et al. 2003, Perry et al. 2005, Tharayil and Triebwasser 2010) and later dismissed as allelochemicals by several studies (Blair et al. 2005, 2006, 2009; Tharayil et al. 2008, Duke et al. 2009), the chemical profile of the root odors have not been characterized to date. The main constituents of the fresh and dry roots were found to be junipene, **21**, β -caryophyllene, **16**, and thujopsene, **20**. These compounds are sesquiterpenes that result during fungi and bacteria metabolism (Korpi et al. 2009) on the root surface of plants. The root volatiles were collected under non-sterile conditions so the biological source of these components is not known. Also, the roots were severed and it is expected that wounding dramatically increased the rate of volatile emission from the roots. The response to wounding was corrected by air drying the fresh roots for six weeks and collecting the volatiles again. The volatiles released by the dry roots might better represent the constituents associated with spotted knapweed roots in natural settings. Volatile profiles of roots can be altered by biotic factors that increase volatile release, such as degradation or attack by microorganisms (Vančura et al. 1977) and below-ground herbivory (van Tol et al. 2001, Neveu et al. 2002, Aratchige et al.

2004, Rasmann et al. 2005, Ali et al. 2010). Therefore, the results obtained in this study should be viewed cautiously due to potential wound induction of volatiles. A more informative approach to evaluate volatile composition from roots might involve experiments conducted under sterile conditions using hydroponic or sand culture.

The odor profile released by spotted knapweed plants and their roots are complex mixtures of many different compounds (Table 2), many of which are ubiquitous in plants. These volatile signals must be discriminated by a trained dog against a similar (and complex) odor background in the plant community. Location of a plant target in a field setting by a detection dog might involve using only a few compounds for target detection as separate signals (Harper et al. 2005) or mixtures of a variety of compounds (Williams et al. 1997). The task of isolating the chemicals that produce an active response to detection dogs is difficult (Furton and Myers 2001) and initially requires behavioral and field experiments using isolated volatiles (Lorenzo et al. 2003). When a detection dog alerts to a spotted knapweed plant, we do not know what the dog is responding to. It is not practical to evaluate the large number of different single compounds, multicomponent mixtures or to assess blend perceptions of plants in the canine olfactory system. Therefore, our findings describe the general relatedness of the principal components of the odor profile of spotted knapweed foliage and roots (Table 2). Although the attributes of the odor signatures are not known, these findings demonstrate that dogs trained to detect spotted knapweed plants recognize root odors that are dissimilar to those associated with the initial foliar training aid (Table 1). This suggests that the volatiles associated with the roots were discriminated by the dogs when detecting knapweed plants

in field settings. We attribute untrained, positive responses to root odor that is dissimilar to foliage odor in composition and quantity (Table 2) to learned behavior associated with stimulus generalization (Rilling 1977). We believe the reinforcement of correct detection responses to multiple knapweed plants with different odor signatures under field conditions made the odorant cues broader than from foliage alone. Possibly, the dogs used root volatiles to confirm natural targets in field settings.

Canine detection success relies on the availability of target odors, which in turn depend on chemical properties and other factors that may not be held constant, such as source release rates and local environmental conditions (Göth et al. 2003). It is reasonable to suggest that below ground volatiles could play an important role because these signals can produce a stronger and more reliable odor than the foliage. For example, in Table 2 the combined release rates of the dry root volatiles were almost tenfold greater compared with the foliage and almost eightyfold greater for β -caryophyllene, **16**, a compound released by all tissue. Below ground volatiles may also exhibit greater persistence and uniformity in comparison to foliage volatiles that are in direct contact with the atmosphere (Peñuelas and Llusía 2001). The availability of below ground odor to dogs relies on transport to the soil surface which is influenced by climatic factors, source release rates, and chemical properties such as volatility, water solubility, soil sorption, plus local soil conditions such as composition, temperature, moisture and microbes (Rovira 1969, Webb and Phelan 2000, Phelan and Barnett 2001). In the case of the root and foliar associated compound β -caryophyllene (**16**, Table 2), Rasmann et al.

(2005) report the suitability of this constituent as a below ground signal to nematodes because it rapidly diffuses in moist sand and has both chemical stability and motility.

Although it is difficult to determine the active volatile components that dogs use to locate plant targets in the field, the volatility of the scent and the search manners observed for the dogs in our previous study might provide insight into the mechanisms of plant detection. For instance, it was reasoned the dogs used high volatility compounds with low molecular weights produced by the foliage as airborne scent (Wilson and Bossert 1963). These diffuse over greater distances and could be readily detected as they rapidly searched a broad area with elevated heads (Goodwin et al. 2010). Detection of a trace of the target scent resulted in upwind movement by the dogs which followed the odor gradient to the plant source with lowered heads, perhaps using low volatility compounds that were produced by the foliage and accumulated on the soil surface or that were produced by the roots and transported to the soil surface.

Conclusions

The ability of search dogs to cover large areas thoroughly (Killam 1990) and detect small knapweed plants with higher accuracy than human surveyors (67 vs. 34%; Goodwin et al. 2010) can improve the efficiency and accuracy of plant detection. Furthermore, the ability of dogs to use below ground odor to detect plants might also improve search protocols by expanding seasonal coverage that targets plants outside the flowering period. Plant monitoring usually targets flowering periods to improve the visual conspicuousness of plants and increase the probability of locating plants that

would otherwise be difficult to detect. Although sites should be surveyed from early spring to late fall to locate nonflowering plants and inconspicuous life cycle stages, such as juveniles and plants with underground dormancy, these plants are frequently missed despite intensive searches (Alexander et al. 1997). The selection of slow-searching dogs with the ability to concentrate and focus their search in field settings can provide good discrimination accuracy for buried targets, such as landmines (Bach and Phelan 2003) and potentially other below ground targets including dormant plants as tubers or root volatiles (roots: the hidden half of the plant) that might be present when foliage vapor is not. Awareness of the role of below ground odor in plant detection provides new areas for research to increase our knowledge about rare or exotic plant surveys.

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Table 2.1. Summary of response outcomes to bare root ($n = 30$) and intact root ($n = 30$) target presence (TP and FN) and absence (TN and FP).^a

Response	Bare root			Total
	D ₁	D ₂	D ₃	
TP	10	10	9	29
FN	0	0	1	1
TN	23	16	19	58
FP	0	1	0	1
Intact root				
TP	10	7	9	26
FN	0	3	1	4
TN	21	20	22	63
FP	0	0	1	1

^a Abbreviations: TP, true positive (hit); FN, false negative (miss); TN, true negative (correct rejection); FP, false positive (false alert); D₁, dog 1, Nightmare; D₂, dog 2, Tsavo; D₃, dog 3, Rio.

Table 2.2. Volatile components of spotted knapweed

Compd	Identification	Release rate, ng/h					
		Foliage		Fresh root		Dry root	
		mean	SE	mean	SE	mean	SE
1	3-hexanone	0.15	0.01	.	.	0.37	0.05
2	3-hexanol	0.27	0.02	0.46	0.14	0.54	0.07
3	1-hexanol	1.20	0.23	0.40	0.11	.	.
4	heptanal	1.61	0.33	0.07	0.01	0.18	0.02
5	benzaldehyde	0.92	0.17	0.13	0.06	.	.
6	6-methyl-5-hepten-2-one	0.71	0.12	0.31	0.07	0.43	0.07
7	β -pinene	5.43	1.14
8	(<i>Z</i>)-3-hexenyl acetate	16.11	7.16
9	2-ethyl-1-hexanol	1.36	0.20	0.68	0.12	1.46	0.16
10	benzeneacetaldehyde	0.98	0.27	0.10	0.02	.	.
11	β -cis-ocimene	12.38	5.11
12	acetophenone	1.88	0.74	.	.	0.51	0.07
13	nonanal	10.99	2.10	0.48	0.09	2.24	0.30
14	(<i>E</i>)-2-nonenal	8.70	1.96
15	decanal	1.94	0.30	.	.	1.52	0.20
16	β -caryophyllene	2.15	0.82	390.57	51.39	170.25	31.05
17	α -bergamotene	.	.	200.92	40.63	74.42	19.73
18	γ -elemene	.	.	26.41	4.22	18.41	4.13
19	β -cubebene	13.59	6.49
20	thujopsene	.	.	264.31	29.62	85.20	21.72
21	junipene	.	.	519.73	85.12	345.58	132.97
22	β -eudesmene	.	.	140.10	17.62	62.94	14.65
23	1-pentadecene	.	.	44.61	20.87	21.82	4.84
24	β -bisabolene	.	.	8.80	1.30	3.48	0.99
25	caryophyllene oxide	.	.	95.80	12.45	58.83	10.78
26	hexadecane	10.80	1.61
27	1-heptadecene	.	.	10.71	1.53	.	.
28	octadecane	2.54	0.64	.	.	3.59	0.48