Canine Scent Detection of an Invasive Wood-Boring Insect, the Brown Spruce Longhorn Beetle, *Tetropium fuscum*, in Laboratory Conditions

Environmental Science Undergraduate Honours Thesis

March 31, 2014

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Acknowledgements

I would first like to thank my supervisor, Dr. Simon Gadbois, for his support and guidance through the process of this research. I would like to acknowledge the project sponsors for their support: the Canadian Forest Service-Atlantic Forestry Centre, Forest Protection Limited and Sylvar Technologies Inc. A special thank you goes to Kate Van Rooyen and colleagues for their contributions to this project. I appreciate Maria DeNicola (Canid Behaviour Research Lab manager) and Fielding Montgomery (lab colleague) for their dedication and assistance. Thank you to all the dogs and their owners that participated in this research and to all the Canid Behaviour Research Lab volunteers for their commitment. I appreciate Dr. Shannon Sterling and Dr. Tarah Wright for their guidance as instructors in addition to their feedback on this thesis. Finally, I would like acknowledge the Dalhousie Environmental Science Department and the Environmental Science Honours students for making this journey an enjoyable experience.

Abstract

The brown spruce longhorn beetle (BSLB), *Tetropium fuscum*, native to Europe and Asia, is an alien wood-boring insect of quarantine significance with established populations in Atlantic Canada. In their non-native range, the BSLB kills spruce trees, primarily red spruce, *Picea rubens*, and is therefore a potential threat to the ecological integrity of forest habitats and the availability of resources for pulp and paper in North America. Collective efforts have been undertaken by the Canadian Forest Service (CFS) and the Canadian Food Inspection Agency (CFIA), including slow-the-spread programs to eradicate this invasive pest. Identification of infested trees is currently achieved by human visual ground surveys for characteristic signs and symptoms of tree trunks or pruned branches. Although usually reliable, false positives and misses occur, which lead to wasted human resources and unnecessary tree removal. Early detection of infestation is crucial to the management and eradication of this invasive insect.

The primary objective of this proof-of-concept study is to determine whether sniffer dogs are able to be trained to detect the BSLB in laboratory conditions. The application of trained sniffer dogs is anticipated to improve current BSLB management strategies by having a high positive predictive power as well as a low proportion of false alarms and misses regardless of the presence of physical/visual symptoms of infestation.

Three volunteer dogs were selected based on their motivation to work and ability to detect low saliency stimuli. Dogs were trained in psychophysical matching procedures of BSLB larvae. All the dogs have the ability to detect the larvae up to 100% accuracy in the presence of ecologically valid distracting stimuli. In addition to providing a potential novel avenue for forest pest management, the researchers anticipate applying the methodology to other invasive insect species, such as the emerald ash borer, *Agrilus planipennis*.

Keywords: brown spruce longhorn beetle, *Tetropium fuscum*, canine scent detection, forest pest management, invasive insect, sniffer dog

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Chapter 1.0: Introduction

1.1 Motivation for Research

The brown spruce longhorn beetle (BSLB), *Tetropium fuscum*, native to Europe and Asia, is an alien wood-boring insect of quarantine significance[1] with established populations in Atlantic Canada. (CFIA, 2012a). In their non-native range, the BSLB attacks and kills spruce trees (*Picea spp*.) and is therefore a potential threat to the ecological integrity of forest habitats and the availability of resources for pulp and paper in North America. Even though research and methods of eradication of the BSLB has considerably increased since its introduction to North America, limited accurate and effective methods for detecting and managing insect infestation remains a challenge for pest management authorities (Zahid et al., 2012). Early detection of infested trees is crucial to the management and eradication of this invasive beetle.

Dogs trained in scent detection have been applied to a wide array of disciplines ranging from security, forensics, medical research, security, wildlife conservation and insect pest detection (Helton, 2009a). The detection of invasive species appeals to the Canid Behaviour Research Team at Dalhousie University for environmental reasons, and is in keeping with the known ability of sniffer dogs in applied settings. Seeing as detection dogs have emerged as a valuable research tool for wildlife conservation, they are anticipated to improve and benefit current pest management strategies in that it would reduce the proportion of false identification of infested trees and the removal of healthy trees. Pest management strategies that incorporate sniffer dogs trained in the detection of the BSLB may provide an effective non-invasive detection strategy to help mitigate the adverse impacts posed by this damaging pest at the municipal, provincial and federal levels.

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[1] The BSLB are considered a pest of quarantine significance for the attack and infest healthy trees (Harrison & Smith, 2013).

1.2 Background and Environment Significance

1.2.1 Invasive Alien Species

Invasive alien species (IAS) are defined as plants, animals and other organisms that are non-native to an ecosystem that may cause substantial economic or environmental harm, or adversely impact human health (EA, 2013). Generally, pests are any organism that interferes with an anthropogenic endeavor or purpose (Freedman, 2010); or possesses characteristics that people consider as damaging or undesirable, such as feeding on agricultural crops (ECPA, 2013). More specifically, alien pests inflict more damage compared to indigenous species for they do not have natural enemies in their non-native range (NRC, 2013a). Consequently, IAS or alien pests, and their associated damage can potentially pose a threat to natural resources' capacity to provide economic, ecological and social benefits on a global scale (FAO, 2013). The impact of IAS varies considerably based on their severity or spread, the region infested and the availability of resources conducive to population growth (NRC, 2103b). The mountain pine beetle*, Dendroctonus ponderosae*, the Asian longhorned beetle *Anoplophora glabripennis*, the emerald ash borer, *Agrilus planipennis*, and the gypsy moth, *Lymantria dispar*, are all but a few examples of IAS that have threatened and severely depreciated ecological and commercial values of North American forests (NRC, 2103a).

Over the past five centuries, Canada has become host to an increasing amount of alien species, including the BSLB (Freedman, 2010). This increase is, in part, attributable to more efficient methods of free trade along with ecosystems becoming more vulnerable as a consequence of ecosystems being modified by human activities (NRC, 2013a).

1.2.2 Brown spruce longhorn beetle (BSLB)

The BSLB[2] in Europe, where the pest is widespread, is a secondary pest attacking previously stressed or dying trees, primarily Norway spruce, *Picea abies* (L.) Karst (Sweeney et al., 2004). In light of this knowledge, the BSLB are not considered as a destructive pest in their native range (CFIA, 2012b). Despite their ecological interactions in Europe, the BSLB pose a threat to North American forests.

In North America, established BSLB populations were first identified in red spruce (*Picea rubens*) trees near the port of Halifax in Point Pleasant Park, Halifax, NS in 1999 (Smith & Hurley, 2000).[3] The BSLB is thought to have been introduced to NS from wood packing material in cargo containers shipped from Europe. Upon further inspection, a naturalist's bark beetle survey of Point Pleasant Park in collected in 1990 revealed that the BSLB was misidentified as a NS native species of longhorn beetle, the eastern larch borer, *Cerambycidae*: *Tetropium cinnamopterum*. Thus, the BSLB has been present in NS for at least twenty years. This misidentification is attributable to the species' seemingly identical morphological appearance. The BSLB has been detected in at nine NS counties (Cunningham, 2010), as well as Kouchibouguac National Park, New Brunswick (Webster et al., 2009). The following image, provided by the Government of NS, represents areas within the province where the BSLB has been detected as of 2010 (Fig. 1).

[2] Adult BSLB have long flattened bodies that are brown or reddish brown in appearance and range from 0.8 to 1.8 cm in length (Humble & Smith, 2000). The BSLB get their name from their colouring, not the species of tree they infest.

[3] The BSLB was intercepted in wood packaging material at the Canadian ports of Montreal and Vancouver (Humble & Smith, 2000).

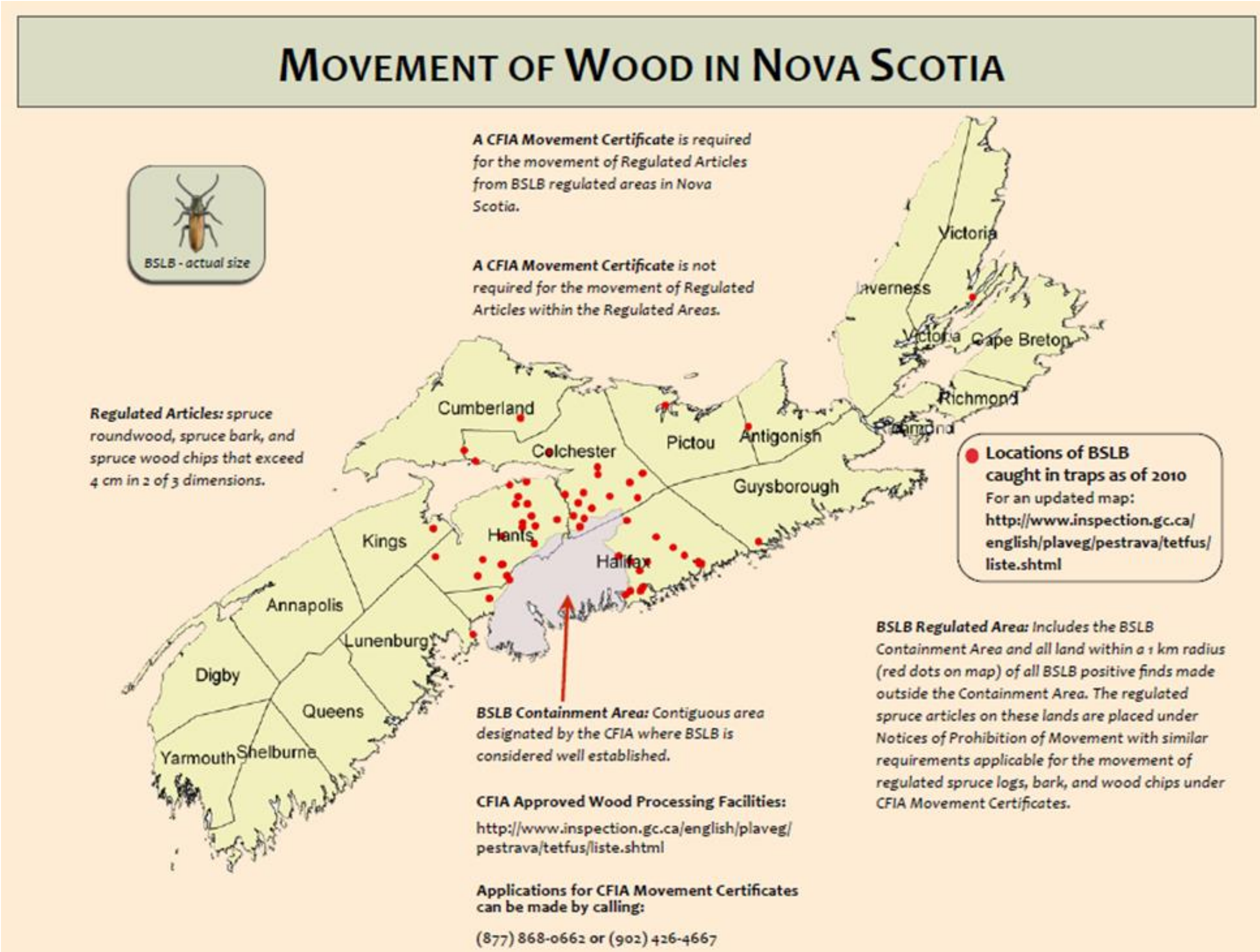

Figure 1: Map that displays the recorded locations of trapped BSLB, *T. fuscum*, in Nova Scotia, Canada. From the Government of Nova Scotia (2010).

Upon the introduction of the BSLB in North America, a growing number of exploratory investigations were made pertaining to host tree conditions optimal for BSLB infestation. In their non-native range, the Canadian Food Inspection Agency (CFIA) currently accept that the BSLB attack and infest healthy,[4,5] dying and recently fallen spruce trees, primarily red spruce (CFIA, 2012b), along with white spruce (*P. glauca* (Moench) Voss), black spruce (*P. mariana*

[4] Smith and Humble (2000) were first to suggest that BSLB, in NS, attack "apparently healthy" trees.
[5] "Healthy", is arbitrary in the literature. Hurley et al. (2006) indicated that a "healthy" tree is one that obtains a full green crown. However, O' Leary et al. (2002) state that a full green is not always indicative of tree health; for instance, a completely girdled trees may appear green for months until the roots begin to weaken and die.

(Mill.) B.S.P.) and Norway spruce (Smith & Humble, 2000). The BSLB will not, however, colonize all spruce trees for trees ten centimetres or greater in diameter are sought after (O'Leary et al., 2002). A collection of studies determined that experimentally stressed trees (i.e., cut and/or girdled) are generally more conducive to BSLB survival compared to healthy trees[6] (Flaherty et al., 2011; 2013). Flaherty et al. 2013 found that larval survival rate was reduced in healthy trees, due to induced host defenses.[7] Stressed trees characteristically contain higher or 'more balanced' nutrient content, including minerals, carbohydrates, and nitrogen (Flaherty et al., 2011). Stressed trees are suggested to supply more nutrients for larvae development, thereby facilitating higher BSLB survival rates. Interestingly, with the exposure of natural enemies (e.g., woodpeckers, *Picidae spp*.), Flaherty et al. (2011) discovered that survival was higher on healthy trees compared to those experimentally stressed, despite the known advantages that stressed trees offer to larval development.[8]

The BSLB can spread in one of two ways, either naturally or by anthropogenic movement (NRC, 2013c).[9] Inadvertent transportation of infested spruce logs, lumber, bark and wood chips as well as firewood from established or potential BSLB pest-ridden areas is the main pathway of pest introduction to an unestablished areas. Rhainds et al. (2011) provides evidence that the BSLB naturally spread slowly in the absence of anthropogenic influence given that adults often reproduce in close proximity to the host tree from which they emerged.[10] Although

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[6] Cut trees were 2.4-m logs from the base of each tree; the bark, phloem, and sapwood of girdled trees were cut to a depth of ~2.5 cm, 30 cm above the tree base; healthy trees were not manipulated (Flaherty et al., 2011).
[7] Flaherty et al. (2013) state that seasonal variation in the impact of bottom-up forces (host condition) has a significant impact on BSLB performance; timing of attack is critical for this beetle.
[8] The researcher observed that more woodpecker holes were found on girdled compared to healthy or cut trees. The density of Tetropium spp. or other bark or wood borers, may explain the predation by woodpeckers on physiological tree conditions (Flaherty et al., 2011).
[9] Anthropogenic movement may include transportation of infested spruce products.
[10] The distribution and relative abundance of the BSLB in relation to its site of introduction was investigated by luring the BSLB with male-produced pheromone traps and host volatiles over a three year time period.

this research is useful pertaining to BSLB spatial dynamics, in the event that infested spruce products are introduced to an unestablished area, the consequences could be severe (Hunt, 2006).

1.2.3 Negative Impacts Associated with the BSLB

A compilation of studies and governmental agencies have respectively investigated and examined the BSLB and the wide variety of impacts they pose. If the BSLB were to establish itself in North American spruce forests, it is apparent that they could have adverse impacts on ecological, economic and social levels (CFIA, 2012a).

Ecological

The ecological implications of an invasive insect outbreak are potentially, and can have a long lasting damaging effects on ecosystems. The primary cause of damage to a tree colonized by the BSLB occurs during the larval stage of development (O'Leary et al., 2002). Although the entire stem can be affected, the lower portion of the tree is typically the most heavily infested (Smith & Humble, 2000). Larvae feed upon and bore through the phloem, the living and food-conducting tissue of vascular plants located between the bark and wood forming L-shaped galleries.[11] As larvae feed on the phloem, nutrient transportation gradually becomes cut off, therefore tree quality and productivity is compromised (O'Leary et al., 2002). This form of biotic stress leads to the eventual death of the tree, which may take one to five years depending on the initial health of the tree prior to infestation as well as the intensity of re-infestation from year to year.

[11] Larvae bore horizontally into phloem for 2-4 cm, then vertically for 3-4 cm (NRC, 2013c)

Depending on the severity of a BSLB outbreak, the establishment of the BSLB could cause extensive tree mortality (CFIA, 2012b).[12] Should the BSLB become widespread, not only commercial forest stands are at risk, but also protected old-growth and urban forests are threatened. Tree mortality, in forested environments, can cause direct and indirect effects on nutrient cycling, decomposition rates, and wildlife habitat through changes of nutrient and organic matter distribution and ultimately through changes in species composition and forest structure (Hunt et al., 2006). High quantities of attacked individual trees or entire spruce stands in a given area, may subsequently lead to a decline in Caron stocks. In a non-commercial or natural forest, dead trees may remain as snags unless otherwise removed or replaced (Krasny & DiGregorio, 2001). Moreover, due to loss of needles and/or removal of infested spruce trees in a given location, canopy gaps may become more prominent. Canopy gaps alter light, moisture, and temperature intensities (Twery 1990; Stadler et al., 2006).[13] Community structure is ultimately altered, which gives rise to a ripple effect of ecological interactions and processes (Fig. 2).

[12] BSLB may significantly decrease if not eliminate entire mature red spruce stands given that this tree species do not seed until a mature age of 75 years old (O'Leary et al., 2002).
[13] Gap formation may sequentially lead to altered structure and successional trajectory of the landscape allowing for shade intolerant vegetation, like shrubs, that have less Carbon storage to dominate (Twery 1990; Stadler et al., 2006).

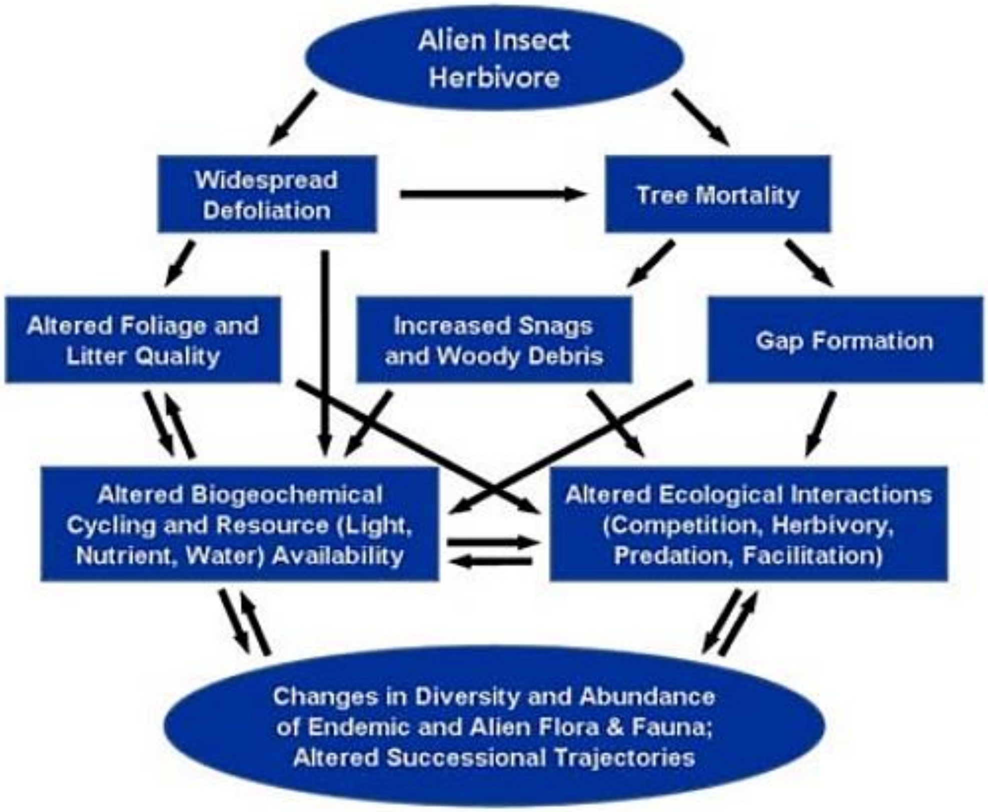

Figure 2: Generalized conceptual model depicting cascading direct and indirect effects of alien insect herbivores on ecological processes and interactions that ultimately impact community composition and successional trajectories in eastern North American forests. From Gandhi and Herms (2010).

IAS have been considered the second most significant threat to biodiversity after habitat loss and modification (CCFM, 2005). The BSLB may deprive native organisms of their natural habitat on which they depend of for survival, thus causing widespread damage to biodiversity of Canadian forests (CFIA, 2012b).[14] In the event that there was a reduction of these habitats, community structure and dynamics would change (Hunt et al., 2006).

Although, relatively little is known, the BSLB have been shown to exhibited interspecific competition between native beetle species in NS (Rhainds et al., 2011). Rhainds et al. (2011) studied the interspecific relationships between the ELB and the BSLB. BSLB and the ELB use

[14] Forests with densely populated spruce trees provide cover and shelter for a wide variety of wildlife species (Hunt et al., 2006).

the same host volatiles and male-produced pheromone for aggregation and mate encounters (Sweeney et al. 2004, 2010; Silk et al. 2007; Rhainds et al., 2011), thus their relative abundance can be monitored simultaneously. The abundance of the ELB increased with the distance to the site of introduction of the BSLB, inferring that competitive interactions exist between the two species. Rhainds et al. (2010, 2011) suggest that the BSLB may be a “superior competitor” potentially displacing the ELB.[15] The interspecific interaction between the BSLB and the ELB requires further investigation, in particular how the reoccurrence of the ELB hinders BSLB fitness.

Economic

The forestry industry is a predominant sector in the Canadian economy; it is the second largest exporter of primary forest products in the world (NRC, 2013d). In 2011, the forest industry accounted for $23.7 billion in Canada’s economy. In Canada, the prime tree species on forest land are spruce (53%), poplar (11.6%) and pine (9.3%) (NRC, 2013e). Canada is one of the world’s largest producers and exporters of softwood lumber for it accounts for 20% of the value of the country’s forest product exports.

In the event that either quantity or quality of commercial forest stands is compromised, this would lead to great economic loss to the forestry industry (CFIA, 2012b). The economic damage associated with IAS is estimated to be $20 billion in losses to the Canadian forest sector on an annual bases (EA, 2013). The adverse impacts associated with insect infestation,

[15] BSLB may displace the ELB in at least three ways. First, larvae development of the BSLB is likely to accelerate due to earlier adult emergence compared to the ELB, thereby reducing the risk of interspecific competition in the BSLB. Second, the BSLB can infest a broader range of host-tree conditions compared to the ELB. Third, the spatial associated between female and male ELB is relatively smaller in comparison to the BSLB; subsequently there is a potentially high risk of mating failures for the ELB.

specifically pertaining to BSLB infestation, can hinder the availability of resources for pulp and paper in North America, along with the competitiveness and the marketability of the forest industry (APEC, 2008). Red spruce, in particular, is commercially valuable conifer and a large contributor to the Maritime forestry industry as they are considered to be the most optimal lumber for sawing followed by pulpwood in the province (Government NS, 2006). Even though red spruce is restricted to Eastern North America, other spruce tree species are widely distributed across Canada (Smith & Humble, 2000), thus the likelihood of BSLB spreading throughout the range of its hosts is correspondingly high (Dobesberger, 2005).[16]

Social

On a social level, death or removal associated with BSLB infestation, could depress recreational, tourism and aesthetic values within urban or park-like settings (CFIA, 2012b). Trees are an important component for quality of life in the city for they provide shade, cleaner air in addition to aesthetic appeal to both city residents and tourists. The absence of trees in these settings, may therefore adversely impact those who intrinsically or inherently value the ecological services trees provide.

1.2.4 Mitigation Strategies

Upon the discovery of the BSLB in Atlantic Canada, collective efforts have been undertaken by a multi-agency task force led by the CFIA to mitigate the posed risks to the ecological integrity of Canada's forests and forestry sector (Sweeney et al., 2004). One of the agency's missions is to eradicate BSLB populations from Canada and to implement controls to

[16] Future costs related to stands of dead spruce on HRM lands and private land owners dealing with dead spruce trees may arise if effective control measures are not developed (HRM, 2008).

protect areas from quarantine pests. As of when cutting down trees was deemed ineffective in 2006, the CFIA has put greater emphasis supporting and investing in research providing alternative methods in the efforts to regulate and prevent further spread of the BSLB (CFIA, 2013a).[17] The CFIA, with the collaborative effort of federal, provincial and industry stakeholders, issued a Ministerial Order that enforces slow-the-spread programs to supersede emergency response strategies[18] in the efforts to regulate and prevent further spread of the BSLB (CFIA, 2013a).[19]

Monitoring of this pest is currently carried out using pheromone lures. Dr. Peter Silk and Jon Sweeney, of Natural Resources Canada, Canadian Forest Service have developed pheromone-based strategies using a BSLB aggregation pheromone, called 'fuscumol' (Silk et al., 2007; Sweeney et al., 2010),[20] for mass trapping and mating disruption[21] (NRC, 2009). Collectively, mass trapping and mating disruption strategies have led to a significant suppression of BSLB populations (NRC, 2013c).[22]

Collectively, these management strategies have helped suppress BSLB spatial spread and population establishment in North America. Management and control techniques, however, are not possible until detection has occurred. An extensive literature review on current BSLB detection strategies is provided in section 2.2.

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[17] Up until 2005, the CFIA removed over 6300 spruce trees in the efforts to eradicate BSLB populations (Henry et al., 2005)

[18] Cutting down trees constitute emergency response strategies.

[19] Generally, the current Order restricts movement and disposal of spruce logs and all types of firewood on areas where the BSLB has been detected or suspected to be, and during the high risk period when BSLB emerge from the host from April 30 until September 15 (CFIA, 2013d).

[20] Fuscumol attracts both sexes

[21] Fuscumol disrupts mating patterns by infusing the air with long-range sex pheromones in which males and females cannot locate each other (NRC, 2009).

[22] Generally speaking, pheromones are scents used for communication between animals of the same species (NRC, 2009). An insect may emit long-range (volatile) sex pheromones to attract a mate (Silk et al., 2011), or, an aggregation pheromone that communicates to others of its species that it has located a food source and needs help to overcome the tree's defences (NRC, 2009).

1.2.5 Sniffer Dogs

Dogs are not only man's best friend, they are also great sniffers! The science of working dogs is an emerging field of study. The use of sniffer dogs for the detection for species of interest has been evaluated at the Canid Research Behaviour lab, Dalhousie University. Dr. Simon Gadbois, an ethologist and neurobiologist who initially specialized in researching wild canids, started the Canid Research Behaviour Lab following the closure of the Canadian Centre for Wolf Research in 2007. The lab uses interdisciplinary integration of ethology (i.e., observational behavioural biology) and experimental animal psychology (animal learning and animal psychophysics[23]) to train dogs to survey local populations and habitat use of reptiles at risk in the field (Demontfaucon et al., 2009; Gadbois & Reeve, 2014). Extensive research at the Canid Behaviour Research Lab has focused on wild canine research along with the application of sniffer dogs as wildlife conservation assistants, helping human counterparts search and locate species of interest (e.g., coyotes, species-at-risk) in non-invasive ways (Gadbois & Reeve, 2014).

1.2.6 Canine olfactory psychophysics

Canine olfaction includes a number of sensory, perceptual and cognitive processes. The three most relevant (detection, discrimination, and identification) will be described here (Gadbois & Reeve, 2014).

- **Detection** is defined as the identification of one stimulus (e.g., grapefruit oil) or stimulus category (e.g., citrus essential oil) among background noise or interference.

[23] The study of the sensory processing of physical and chemical stimuli.

- **Discrimination** is defined as the identification of one stimulus or stimulus category in the presence of at least one other stimulus (the S- or negative stimulus), often similar (e.g., orange oil) or another category (e.g., floral oils).

- **Identification** requires explicit knowledge of the stimulus that requires a greater level of investigation. In the example of matching-to-sample tasks, it is indicative that the participant can perform identification tasks if the participant can explicitly identify the target as the same as the sample in the presence of (S-).

For any detection task there are four possible outcomes: (1) the target is present and the detector signals its presence; this is known as a hit, or a true positive; (2) the target is absent and the detector signals it as not present; this is referred to as a correct rejection or a true negative; (3) the target is physically present and the detector signals it is not present; this is called a miss or a false negative; (4) the target is not physically present and the detector signals it is present; this is known as a false alarm or a false positive (Helton, 2009b).

Accuracy, which conceptually encompasses sensitivity and specificity, is a key statistical measure of performance (Helton, 2009b). Sensitivity, also called the true positive rate, measures the proportion of actual positives which are correctly identified. Specificity, often called the true negative rate, measures the proportion of negatives which are correctly identified. While accuracy, with respect to sensitivity and specificity, is critical, it does not provide a complete measure of performance. It is important to consider that both false alarms and misses are equally important in assessing performance.

Positive predictive power (PPP) assesses the proportion of the dog's target-present responses that are correct whereas negative predictive power (NPP) assesses the proportion of the dog's target-absent responses that are correct (Helton, 2009b). Both of these measures are useful to compute the likelihood of a target's presence if a dog indicates the target is present and the likelihood that a target is not present if a dog indicates the target is absent. In other words, PPP and NPP measures how "predictive" the dog is.

Generally, performance, in the context of the current study, refers to a dog's accuracy rate with respect to sensitivity (hit rate) and specificity (correct rejection rate). Sniffer dogs are considered accurate recruits if the results show that they have the potential to supplement human counterparts in the efforts to optimize current BSLB detection strategies.

An extensive literature review on sniffer dog detection along with olfactory and cognitive processes is provided in section 2.3.

1.3 Summary of Knowledge Gaps

Knowledge gaps that pertain to the precise topics of the research questions correlate with current BSLB detection strategies in addition to canine olfaction and performances capabilities. Due to the relatively recent introduction of the BSLB in North America, it is a challenge to predict future impacts posed by the BSLB if it were to become widespread are evident in the literature. Therefore, pest management strategies that prevent further spread of this pest continue to be developed or improved.

The science of 'the working dog' is an emerging area of study (Helton, 2009a). Only until recently that research along with evaluation of canine behaviour, olfaction and performance started to be seriously considered for its potential applications.[24] How canine olfaction works and operates remains to be an inquisitive area of the behavioural sciences and neurosciences.

[24] Only two research studies evaluated canine intelligence between 1950 and 1995 (Hare & Woods, 2013).

Although working dogs have been increasingly recognized for their impressive cognitive and olfactory performances, applied canine olfaction, specifically pertaining to forestry insect pest management, is limited. Although, a select few examples of sniffer dog detection of forest insect pest in North America exist, no published studies to date have evaluated the use of sniffer dogs for the detection of the BSLB.

1.4 Study Overview

Under the supervision of Dalhousie professor Dr. Simon Gadbois, this experimental and descriptive study was conducted to determine whether sniffer dogs can be trained to detect larval BSLB. More broadly, BSLB is also used here as a model system for proof of concept. The ultimate objective of this study is to develop a novel pest management strategy to help eradicate and reduce the adverse ecological, commercial and social impacts posed by the BSLB using the acute scenting capability of dogs to detect BSLB infested trees. This study also intended to test the ability of sniffer dogs to identify and discriminate the BSLB from a morphologically and taxonomically similar NS native and non-invasive species of longhorn beetle, the eastern larch borer (ELB), *Coleoptera: Cerambycidae*: *Tetropium cinnamopterum*. Unfortunately, due to time constraints and larval supply of the ELB, this phase was not investigated formally and did not allow for explicit conclusions to be drawn. Furthermore, this study provides a conceptual framework supplying adequate information to describe and characterize the phenomena of the BSLB and the utilization of sniffer dogs in the context of its significance within environmental science.

1.4.1 Research Question

The research questions is:

Can sniffer dogs accurately detect larval brown spruce longhorn beetle, *Tetropium fuscum*, in the presence of ecologically valid distracting stimuli in laboratory conditions?

The hypothesis is:

Sniffer dogs, trained in detection, will be able to accurately detect larval brown spruce longhorn beetle, *Tetropium fuscum*, with respect to sensitivity and specificity, in the presence of ecologically valid distracting stimuli in laboratory conditions.

1.5 Study Approach

The research question is addressed using a classical methodology in animal psychophysics.[25] In order to determine whether sniffer dogs can accurately detect larval BSLB, investigators influenced outcomes using behavioural assessments and experimental methods. Evaluation of canine performance was achieved with the use of Signal Detection Theory (SDT). SDT can help evaluate a number of parameters such as accuracy with respect to sensitivity and specificity; it addresses (quantifies) detectability and discriminability (i.e.,, d'), and response biases (e.g., c). Response biases are informative as they can allow for an adjustment of training when specific dogs have an undesired bias (e.g., too many false alarms or misses).

All animals in this study were cared for in accordance with the recommendations of the Canadian Council on Animal Care (CCAC) and the policies of the local animal care committee (UCLA) at Dalhousie University. Dog sitters, walkers, handlers and trainers were all certified by the CCAC for lab work. All of the research conducted in the laboratory was non-invasive.

[25] Animal psychophysics is based on animal learning theory and studies sensory processing.

2.0 Literature Review

2.1 Introduction

An *a priori* content specific coding scheme, a form of qualitative analysis, was used to investigate the areas of interest in the literature.[26] The following main themes are assessed and analyzed in this literature review: 1) current BSLB detection strategies; 2) canine olfactory psychoethology with respect to scent detection; and 3) sniffer dog applications in insect pest management strategies[27].

2.2 Current BSLB Detection Strategies

Identification of BSLB infested trees greatly rely on human visual ground inspection for characteristic signs and symptoms of infestation, such as excessive resin flow, exit holes approximately 4 mm in diameter and progressive yellowing or browning of needles (CFS, 2009; Sweeney et al., 2007). As a consequence of removing a large quantity of trees, during preliminary eradication programs, it became more of a challenge to detect the presence of BSLB in trees with less obvious signs of infestation (Sweeney et al., 2007). Furthermore, detecting the presence of BSLB when low populations of BSLB exist in stands where the majority of spruce trees are infested with native bark beetles, has also shown to be a difficult endeavor (Harrison et al., 2004). As per communication with the Canadian Forest Service-Atlantic Forestry Centre (CFS-AFC), MacKinnon et al. have observed in their unpublished research that there is a correlation between "unexplained resin" flow and high BSLB catches,[28] but a poor correlation at

[26] For an *a priori* content specific coding scheme, the criteria is specified prior to reviewing the literature.
[27] Peer reviewed journal articles searched using Environmental Sciences and Pollution Management, Biological Abstracts, Web of Science and Google Scholar databases. 'Tetropium fuscum' OR brown spruce longhorn beetle' AND 'detection', 'sniffer dog' OR 'conservation dog' AND 'pest*' OR 'insect pest*' AND 'detection' were used to retrieve and review relevant citations.
[28] e.g., at sites like Hemlock Ravine Park, where trap catches of BSLB are high (30-40 BSLB per trap).

sites with low BSLB catches.[29] The data subsequently suggests that factors other than BSLB infestation may cause "unexplained resin" (i.e., not obviously due to BSLB infesting a host) in areas with low BSLB catches. Although visual surveys are usually reliable, falsely identifying and missing BSLB infested based on characteristic symptoms results in wasted human resources and unnecessary tree removal.[30]

Significant improvements in the detection of BSLB were achieved with the advances of pheromone traps (NRC, 2008). Pheromone traps, which are used as a mitigation measure, are deployed after the BSLB are first detected using these devices. Pheromone traps, therefore, serve as a dual approach, in which the BSLB can be mitigated and detected simultaneously in a given area.[31] These results provide for useful applications for trapping surveys of *Tetropium spp*., particularly surveys for early detection of other potentially invasive species, such as *T. castaneum* in North America. Although, pheromone traps are an effective and sensitive detection strategy, general areas with present BSLB populations are identified, rather than specific trees. Furthermore, pheromone-based strategies used for mass trapping and mate disruption are not exclusive to the BSLB, but also the ELB and other *Tetropium spp*.[32]

Another detection strategy is a wood-staining fungi in the genus *Ophiostoma* that has been associated with the galleries of *Tetropium spp*., including the BSLB (Jacobs et al., 2003; Harrison et al., 2004). A species of *Ophiostoma* that was isolated repeatedly from BSLB-infested trees was investigated (Jacobs et al., 2003). Harrison et al. (2004) claim that the presence of *Ophiostoma tetropii* is a strong indicator of whether BSLB is present in a stand. Based on this

[29] e.g., Glenholme, NS.
[30] More apparent signs of infestation were likely due to multiple years of re-infestation (Sweeney et al., 2007).
[31] Expanded surveys in 2007 and 2008 using synthetic fuscumol detected the BSLB at a total of 26 new locations beyond the containment zone boundary established in May 2007 (NRC, 2009).
[32] Native populations of *Tetropium spp.* (e.g., ELB) may be compromised

discovery, *Ophiostoma tetropii* was proposed as a useful BSLB detection tool. Jacobs et al. (2003), however, observed that *Ophiostoma tetropii* was only associated with BSLB in red spruce trees. Consequently, this detection tool may be limited to eastern North America where red spruce are abundant. While a potential detection tool, little research is provided in the literature on

2.2 Sniffer Dogs

Dogs have coexisted with humans for thousands of years (Helton, 2009a). Today, many humans have dogs as pets who are valued and appreciated for their companionships along with their individual personalities. Historically, dogs have been precedent of extensions of human senses and abilities that have allowed humans to be more effective and efficient performing tasks that can often impede human resources. Thus, opportunities for working dogs that take advantage of their acute senses, are intensively researched to improve performance and effectiveness of otherwise arduous tasks.

Dogs are recognized for their acute sense of smell and ability for detecting and discriminating between odors (Goldblatt et al., 2009). This stems from their evolution from wolves, an essential element of reproduction and survival. Humans' olfaction, or sense of smell, in comparison is relatively low to that of dogs. A dog's nose is approximately a hundred thousand to a million times more sensitive compared to a human.

In order for dogs to be effective 'workers', experience is necessary for dogs to be able to perform discrimination, identification and detection tasks (Helton, 2009d). Training sniffer dogs considerably varies with commands, scents, and different cues used for each dog; therefore specific learning tasks are based on combinations of these variables (Lit, 2009). Through experience and exposure to a specific odor, or, a selection of odors, dogs will maintain memory and perceptual learning to build the framework for olfactory perception (Goldblatt et al., 2009). It is therefore crucial that the formulated connections between perceptual learning and olfaction applications are followed.

Odorants are molecules that are defined by their physicochemical characteristics whereas an odor is a perception based on an accumulation of odorants (Goldblatt et al., 2009). Literature on scent detection demonstrates that olfactory thresholds in dogs are low even when a co-presented odorant with a higher concentration is present (Johnston, 1999). A detection threshold is the point where the stimulus can just be detected or just when the stimulus cannot be detected. In other words, the physical stimulus is not detectable below this limit. Johnston (1999) and William et al. (1997) determined a dog's limit of detection (absolute threshold) to be in the 10 parts per billion (ppb) range for benzoate, cyclohexanone, and nitroglycerin. In general, dogs are sensitive to scents, however the absolute threshold is not consistent for all odors; different odors will have a variety of different thresholds. Making general statements in relation to thresholds are therefore challenging to formulate. Furthermore, dogs have the ability to trail a scent to the location of the source (e.g., a person, animal) by detecting the difference in intensities of the scent in the direction where the entity travelled; to track a stationary odor (e.g., scat); and to search, which requires investigating the environment for the odor(s) of interest in an exploratory manner (Goldblatt et al., 2009). While searching for an olfactory cue, the sniffing rate increases up to 20 times per second (Steen et al., 2006). Moreover, it has been shown that there is a direct correlation between the difficulty of a task and the rate of sniffing, where the more difficult the task, the rate of sniffing increases and vice versa.

At Dalhousie University, the Canid Research Behaviour lab has trained dogs to survey local NS populations and habitat use of reptiles at risk in the field, which include the eastern ribbon snake, *Thamnophis sauritus sauritus*, and the wood turtle, *Glyptemys insculpta* (Demontfaucon et al., 2009). Sniffer dogs are valuable assistants in the field for they can locate the target more reliably compared to a human. The eastern ribbon snake, for example, camouflages itself within its environment, therefore making it difficult to visually detect. Trained dogs, combined with their sensitive nose and agility, can assist human counterparts to find the snakes by trailing its scent and indicating its location with a trained response.

Canine scent detection, specifically pertaining to insect pests, consist of but are not limited to, the detection of bed bugs, *Cimex spp*, (Pfiester et al., 2008) and termites, *Isoptera spp*. (Brooks *et al*., 2003). The study conducted by Brooks et al., 2003, claims that the mean accuracy (correct indications) of six trained dogs is 95.93% in locating Eastern subterranean termite workers and incorrectly indicate the presence of termites on an average of 2.69% (false positives). One of the six dogs score 100% for correct indications and 0% for false positives. Similarly, results from the study conducted by Pfiester et al. (2008) states that dogs trained to locate live bed bugs and viable bed bug eggs have a mean accuracy of 97.0%. Both Brooks et al. (2003) and Pfiester et al. (2008) provide promising results suggesting that trained sniffer dogs have the ability of accurately detecting damaging pests.

Studies most closely related to sniffer dog detection of the BSLB are those that investigated canine scent detection of other invasive forest insects in the United States, including the Asian longhorned beetle, *Anoplophora glabripennis* (Errico, 2012) and the emerald ash borer, *Agrilus planipennis*[33]. Sniffer dog detection of the Asian longhorned beetle (ALHB) (Errico, 2012) and the emerald ash borer (EAB) was investigated in separate proof of concept studies piloted by the United States Department of Agriculture's Animal & Plant Health Inspection Service (USDA-APHIS), and the Minnesota Department of Agriculture in partnership with Working Dogs for Conservation respectively. The objective of both studies is to help mitigate the adverse impacts posed by the invasive beetles with the application of sniffer dogs. Host trees present with characteristic signs of infestation; however, symptoms are not obvious in the early stages of infestation, therefore infested trees are often missed (Mohr, 2012 September 6). Only an abstract could be reviewed for the study pertaining to sniffer dog detection of the ALHB, subsequently inclusive information in relation to training procedures, performance measures and data analysis are limited. It states in the abstract that sniffer dogs were trained in laboratory and field conditions and are 80-90% successful in their ability to detect and respond to ALHB frass, sawdust-like excrement (Errico, 2012). Similarly, information pertaining to sniffer dog detection of the EAB is also limited, with only news articles reporting on its progress. News reports state that dogs were successfully trained with larval EAB placed on ash logs along with infested logs grounded up into mulch (Mohr, 2012 September 6). Unfortunately, officials state that insufficient funding has postponed sniffer dog detection of the ELB. Canine scent detection of the ALHB and the EAB in both of these piloted studies have independently demonstrated that sniffer dog can be a supplemental survey tool to be incorporated in an eradication program.

[33] No studies have been published concerning canine detection, only media attention has provided some insight. (Mohr, 2012 September 6), a news article is referenced.

Controversies concerning the application of sniffer dogs exist in the literature. Assigning the 'expertise' term to nonhuman animals, particularly dogs, continues to be disputed (Helton et al., 2009c). Applying the expertise term to nonhuman animals is arguable due to its implications "for animal consciousness, the relative contributions of nature and nurture to development, and the allocation of research funding to technology" (p. 18). Despite that some have criticised that underlying inferences pertaining to the behaviour and motivational state of working dogs' are anthropocentric, substantial evidence demonstrates that working dogs show clear signs motivation to perform tasks. Another issue that emerges is researchers stereotyping dog breeds' abilities. Since the domestication of dogs, people have artificially selected for desired traits, which has produced a large variety of dog breeds. Based only selected attributes and characteristics, breeds appear to differ in temperament. Trevelyan (1997) states that working dogs are neither reliable nor consistent and treat their job as a game when they were bored. However, Helton et al., (2009c) state that Trevelyan (1997) did not have supporting evidence for this claim. Although there has been skepticism pertaining to the reliability of working dogs, most of the criticism is a consequence of poor training procedures.

2.4 Knowledge Gaps

Although research is growing pertaining to the BSLB, biological features of the beetle and host symptoms of infestation continue to be investigated. Research on BSLB mitigation strategies persists for there is a demand for current and future research projects. Although there are a few other teams involved in this area of research (USDA-APHIS, Minnesota Department of Agriculture), there is limited research with regards to applied canine olfaction to detect forest pests. Furthermore, no studies to date have experimentally and under double-blind and controlled conditions evaluated the use of sniffer dogs for the detection of the BSLB.

The scientific analysis of the working dog is underdeveloped, subsequently large gaps exist in the literature (Helton, 2009b). Due to these gaps, problems that arise for agencies interested in assessing dog performance include inconsistencies in document performance, experimental conditions and limited information on training procedures. Despite the gaps of knowledge in how canine olfaction works, dogs have been successfully applied as sensitive detectors based on their impressive performance achievements.

3.0 Methods

3.1 Overview

To test whether sniffer dogs can detect the BSLB (and ultimately discriminate it from the ELB), a number of psychophysical methods were used, including:

1. Three alternative forced choice task (3AFC)
2. Open Matrix (3x3 and 4x4)
3. Go/No-Go (closed matrix)

These psychophysical methods comprised of matching-to-sample (MTS) procedures for the relevant target stimuli. The basic components of a MTS task are:

1) To present alternative choices simultaneously;

2) For the subject to identify the target stimulus; and

3) A response, indicating a choice (poke-and-hold)

With MTS, matrix and Go/No-Go training, researchers are were able to determine the detection ability related to accuracy (sensitivity and specificity) in the training of stiffer dogs for BSLB larvae

Training and data collection, in laboratory conditions, took place over a five month period from September 2013 to February 2014. Each of the psychophysical procedures were performed over an approximate timeline (Table 1). The listed procedures coincides with the order in which these procedures were performed.

Table 1: List of the consecutive psychological task performed by the sniffer dogs and the approximate time period required for completion. EVDS represents ecologically valid distracting stimuli.

Task	**Time Period**
3AFC Preliminary Training	6 weeks
3AFC w/ *T. fuscum* larvae	1-2 weeks
3x3 matrix (blank alts.) w/ *T. fuscum* larvae	1-2 weeks
3x3 matrix (EVDS) w/ *T. fuscum* larvae incl. S+ absent training	3-4 weeks
4x4 matrix (EVDS) w/ *T. fuscum* larvae	1 week
Go/No-Go (EVDS) w/ *T. fuscum* larvae	2 weeks

3.2 Sampling

This study incorporated systematic sampling of BSLB and ELB larvae as well as sniffer dog recruits. All larvae samples used in this experiment were supplied by the CFS-AFC in Fredericton, New Brunswick, from bolts (log/segment of tree) cut from infested spruce trees,[34] in established areas within the HRM, including Sandy Lake area, East Gore and Lake Major. Identification of BSLB and ELB larvae were assigned by the CFS-AFC. All shipments were accompanied with CFIA approved movement certificates. Bolts and larvae were securely

[34] Predominately red spruce.

contained in refrigerators at 3-5°C, when not in use. The following images are of a BSLB infested bolt that was is the process of being spliced in order to collect larvae (Fig. 3).

A B

Figure 3: Image of a *T. fuscum* infested white spruce, *P. glauca*, bolt in the process of being spliced to retrieve larvae. Image A gives a sense of scale of how deep horizontally larvae can be found in an infested tree or bolt (~2-4 cm). Image B, a close up of image A, shows a larva within a feeding tunnel (yellow circle).
Photo credit: Fielding Montgomery.

Recruiting posters requesting active dog breeds to partake in non-invasive research at the Canid Behaviour Research Lab were placed around Dalhousie Halifax campus, Point Pleasant Park and other prime areas where dog owners were likely to encounter the ad. Dog breeds that have been successful in past studies or have breed traits, characteristics and search strategies that were favourable to meet the particular criteria of the study were preferred. For example, scent processing dogs must have a high work ethic with sustained motivation with simple repetitive tasks (Gadbois & Reeve, 2014; Smith et al., 2003). Ten volunteer dogs, of various active dog breeds, were initially recruited in September 2013 to undergo preliminary training. All dogs were naïve, with no former experience with the study procedures. Three of these dogs, Flynn, Ros and Quiver, were selected to be trained in non-invasive matching procedures of BSLB based on their favourable traits and their ability to detect low saliency stimuli. Flynn is a five year old English shepherd, Ros is a three year old pure bred border collie and Quiver is an eight year old pure bred border collie. The dogs were trained two to three times a week. The training took place in the Canid Behaviour Research Lab at Dalhousie University with the assistance of qualified Canadian Council on Animal Care (CCAC) certified staff.

3.3 Stimuli

In order to train the dogs for the purpose of the experimental "in-vivo" manipulations, the following sources of olfactory stimuli were used:

1. live BSLB larvae
2. live ELB larvae

The following basic detection and discriminations were tested with the following procedures:

1. Detection task: BSLB vs. "nothing" (background noise)
2. Detection task: BSLB vs. Ecologically Valid Distractors (EVDS) (e.g. leaves, soil, bark)
3. Discrimination task: BSLB Larvae vs. ELB Larvae and EVDS
4. Discrimination task: BSLB Larvae vs. ELB Larvae

3.3.1 Stimulus Classifications

The following are descriptions of the types of stimuli that were used in the psychophysical methods:

- **Positive stimulus (S+)** – the target stimulus (e.g., BSLB larvae)
- **Negative stimulus (S-)** – this is a stimulus in which the researcher(s) assume the dogs may have difficulty discriminating against due to scent similarities (e.g., orange oil vs floral oil) (Gadbois & Reeve, 2014). Larval BSLB and ELB are expected to have similar

scents, therefore the ELB will be classified as S-. Incorporation of S- tests the dogs' ability to detect, discriminate, and identify the S+ among its presence.

- **Distractor stimuli**– ecological valid distracting stimuli (EVDS) including outdoor elements, such as leaves, grass, twigs, etc. (background noise) were used in attempt to replicate stimuli a dog may be exposed to in the field.
- **Blank or "nothing" –** (control); contains everything (i.e., background noise) the target containment has expect the S+

3.4 Jars/Containment

In order for the dogs to sniff and investigate stimuli, the latter were securely contained in a uniform jars or containers that prevented the stimulus from being damaged or contaminated. All glassware was washed then sprayed with an alcohol solution to ensure that any possible contaminants were removed prior to use. Cotton was initially used to protect/conceal stimuli; however small envelopes eventually replaced cotton for the latter were more effective in disguising the stimuli (look more uniform) and more efficient to move the stimuli between jars.

3.5 Preliminary Training

3.5.1 Clicker Training

Clicker training (Skinner, 1951; Pryor, 1999, 2005) is a positive reinforcement technique that stems from Pavlov's research in 1904 on classical conditioning.[35] This form of training is based on classical conditioning and creates an association between an unconditioned stimulus

[35] Clicker training has been adopted by many pet owners and professional trainers to teach a variety of tasks and tricks to animals including horses and dogs (Smith & Davis, 2008).

(food) and a conditioned stimulus (sound of the clicker) in order to shape a behaviour. After the association between the US and the CS is established, only the CS (sound of the clicker) is used to reinforce the dog.[36]

Once the dog was clicker trained, the clicker was then used as a reinforcer to shape the desired response that indicated to the handler that a dog has made a choice, which was at least a four second ‘poke-and-hold’ with the dog’s nose on top of or in the jar. The first step in shaping the behaviour was to perform informal sessions to ensure the dogs were interested in the jars that were used for the psychophysical methods. Food was placed in a jar and was positioned in different locations on the floor. The handler gave the verbal cue ‘go find’ to encourage the dog to approach the jar by gesturing towards it. As soon as the subject put their nose in or on the jar, the handler used the clicker and immediately gave a reward. Once the dog learned that ‘poking’ the jar was a positively reinforced behaviour, the jar was poked willingly. When the dog showed interest in the jar; the handler began delaying the click by fractions of a second at a time to encourage the dog to hold its nose on or in the jar. Depending on the progression of the dog, approximately two or three 3 to 5 minute sessions were completed before moving on to the next step: training the procedure.

3.5.2 Training the procedure

BSLB and ELB larvae were anticipated to be low saliency stimuli; therefore the dogs learned the basic procedures of the various psychophysical methods first with a relatively high

[36] The clicker is used as a conditioned reinforcer or secondary reinforcer (also called a ‘bridge’ by trainers such as Pryor, 1999, 2005) emitting a consistent distinct double-click sound which serves as an immediate signal to the dog that responding with a particular behaviour has earned a primary reinforcer (e.g., food) (Smith & Davis, 2008). In other words, the dog will learn that the sound predicts food (Skinner, 1951).

salient stimulus. The dogs were exposed to different saliencies of tea ranging from 15 minute steeped tea down to diluted five second tea. The idea behind this strategy was to train the dogs on a relatively strong scent that could be easily controlled; gradually decreasing the concentration of tea corresponded with a decrease in saliency. This method trained the dogs to detect low salient stimuli.

Steeped Tea concentrations

Diluted 5 second tea
5 seconds
15 seconds
30 seconds
45 seconds
1 minute
3 minutes
5 minutes
7 minutes
10 minutes
15 minutes

A consistent measurement of tea was used (i.e., either one TBSP of loose tea or a tea bag). Ten jars were used for each of the tea concentrations (Fig. 4). As soon as the boiling water came in contact with the tea, the timer started.

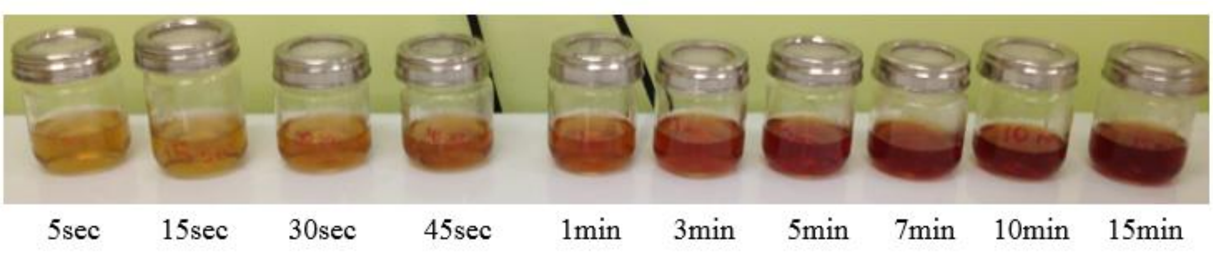

Figure 4: Gradient of tea concentrations ranging from 5 second to 15 minute steeped tea. Each concentration corresponds to a different saliency.

Diluted five second tea consisted of an exponential decrease or dilution (1/2^x) of five second tea compared to water. The first dilution was one half five second tea, one half water; the next dilution was one quarter five second tea, three quarters water and so forth until there was one sixteenth five second tea.

Training the method was first performed using a three alternative forced choice task.

3.6 Three Alternative Forced Choice (3AFC) task

3AFC is a multiple alternative forced choice test (mAFC) that stems from early works by Gustav Theodor Fechner who developed the two alternative forced choice task (2AFC), in which the participant is positively reinforced for responding to a target stimulus (S+) if it is the same as a sample stimulus. For the purposes of this study, the 2AFC was extended to a 3AFC in the efforts to eliminate the effects of side bias (DeCarlo, 2012) and in order to keep the dogs from guessing the position of the S+ by decreasing the probabilities of a hit; when the participant is presented with only two choices they have a 50% chance of being correct. Therefore, when there are three possible choices, the chances of being correct is reduced to 33%. One of three jars contained the S+. Initially, the two alternative choices were ‘blanks’. Although the alternative choices were ‘blanks’, all contained a component of background noise that was consistent, while one contained the S+. For example, cotton pads were used to absorb the tea, therefore all jars contained cotton pads so the only difference among the choices was the S+. The noise was assumed to be additive and independent of the strength of the stimulus.

Because the S+ was among alternative choices that were blank and/or contained background noise, it is a detection task. The dog first performed the 3FAC with one session with food as the S+. There were at least two trained lab members involved in each session including the handler and an individual that used the clicker, took notes, and randomized the location of the stimuli.

During 3AFC sessions, the handler gave a verbal cue “what is this?” to prompt the dog to sniff the sample followed by a command “go find” whilst gesturing to a line of jars (Fig. 5). The verbal cues were consistent for all the performed tasks. The sample is an olfactory cue that indicates to the subject what the researchers want them to find. Other contextual cues were also associated with the tasks performed in this study, such as the dog wearing a work vest to cue them that it was time for work. Given that the dogs were naïve to the tasks performed in this study, the handler was initially not blind in order to train and guide the dog to the S+, when appropriate, until the handler was confident that the dog could make choices on their own without any interventions from the handler. Another trained lab member clicked when the dog responded correctly. Early in training the dogs were holding for approximately one second, but as the trials and sessions progressed, the click was gradually delayed until the desired response was achieved (at least a four second poke-and-hold). After each trial, the hander and the dog exited the work room in order for someone to randomize the location of the stimulus. During those phases, sessions consisted of 10 trials. A performance score, with respect to sensitivity and specificity (correct responses) out of ten was recorded for every session the dog completed.

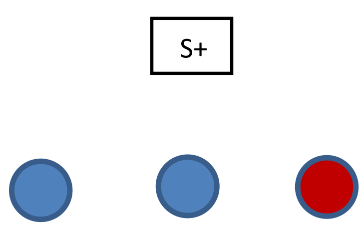

Figure 5: Illustration of a 3AFC task where the participant responded to one of three possible choices that contained the S+ (target stimulus). This is a matching-to-sample task in which the S+ was contained in the sample (the square) and one of the three jars (red circle).

To avoid biases, the S+ was placed randomly in equal portions in the three possible spatial positions: left, middle or right. Because there were ten trials during a session, the S+ would be allocated to two positions for three trials and the third position would have four trials. Furthermore, the S+ was not placed at one position for more than three consecutive trials; nor was the S+ placed in more than two consecutive back and forth patterns between positions (e.g., middle, left, middle, left). A six sided die or a randomizing list generator (Random.org) was used to obtain the random location of S+. The jars remained stationary and the various stimuli were randomly positioned within the jars in the matrix. If the dog responded incorrectly, the handler was able to determine if they were detecting a scent associated with the jar, or with a specific stimulus.

After a one to two sessions with food as the S+, a transition session was performed where food was paired with 15 minute steeped tea. A pipette was used to obtain the required amount of tea (5 ml). Cotton pads were used to absorb the tea in the jar. When the dog successfully detected the stimulus, where S+ was correctly indicated at approximately eight of the ten trials, the food was taken out for the next session. At this stage of preliminary training, the interpretation of the dog's response by the experimenter was sometimes subjective. Perhaps the dog was sniffing rather than poking and/or the clicker was used too quickly. With that said, the dog indicated in other ways that they found the S+, e.g., persistent pawing, licking or nudging. These behavioural responses indicated to the handler that they were making a choice even though the response on a four second poke-and-hold was not yet acquired. In addition, whoever was clicking often waited longer (postponed the click) to observe whether the dog returned to investigate the S+ after sniffing the alternative choices, which was a stronger indication that the dog has detected the stimulus. Training the poke-and-hold response took time. It was acceptable if the dog spent time at the alternative jars, but the dogs were encouraged to sniff and investigate all the alternatives. In order to shape the behaviour appropriately, it was important to observe whether the dogs were spending *more* time on the S+ before clicking.

The S+ for the next session was 15 minute tea.[37] The trials from then on were double blind, where neither the handler nor dog knew where the S+ was located. If and when the dog was confused, the handler intervened and guided and/or assisted the dog. If the dog indicated to the handler that they could detect the stimulus (approximately eight of the ten trials), the handler used personal judgement on whether to progress to the next tea concentration or to repeat the session. This was applicable for high concentrations of tea (3 minutes and above). The performance scores, in some cases, were an approximation due to the subjective interpretation of the responses. Please note that tea, in general, was used as a training tool, especially high concentrations. More formal trials began when the S+ was 1 minute steeped tea; all of the dogs were expected to indicate choices with clear responses (at least a four second poke-and-hold). A score of at least 80% for two consecutive sessions in a row or at least a score 90% (without ambiguous responses) must have been obtained before progressing to the next lowest concentration. Again, during this stage, tea was used at a training tool; however the handler required stronger evidence that the dogs could accurately detect lower tea concentrations prior to moving onto the next lowest concentration.

[37] 15 or 10 minute tea may have been used during this phase, both were considered high tea concentrations with a considerably strong saliency

BSLB larvae was eventually paired with five second tea where the latter was gradually diluted over successive sessions. Five larvae were placed in a glass vile so they did not come in contact with the tea (Fig. 6).

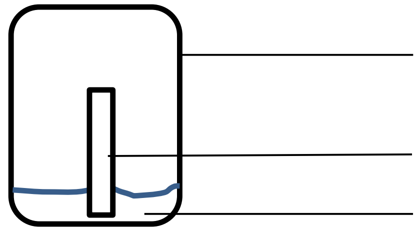

Figure 6: An illustration that shows how to pair *T. fuscum* larvae with tea.

Once the dogs successfully completed sessions (at least two consecutive performance scores above 80%, or a score of 90%) with larvae paired with one sixteenth five second diluted tea sessions, the tea was removed so only the larvae remained. Performance was anticipated to drop once the tea was removed, due to the change in stimulus composition. Three consecutive trials with a score of at least 80%, or two consecutive trials with a score of at least 90%, were achieved on 3AC sessions with BSLB larvae as the S+ before progressing to the next procedure: an open matrix. This convention was applied for all future steps in training when BSLB larvae was the S+. One of the three dogs, Flynn, met criteria on the 3AFC with larvae paired with frass (larvae excrement) due to time constraints (i.e., the two stimuli combined were of higher saliency).[38] Furthermore, reminder sessions with tea were re-introduced if a dog did not attain a score of 60% after two to three sessions in order to avoid the dogs becoming discouraged or confused with the task.

[38] Flynn was falling behind in the training process, compared to Ros and Quiver, where he was not detecting solely BSLB larvae; it was recommended to use less than a pinch of frass to avoid discouraging him. Frass is associated with the larvae stimulus, and therefore associated with BSLB infested trees.

A noteworthy difference in preliminary training procedures was applied to Quiver, for the reason that he advanced in training more quickly compared to Flynn and Ros and was, to some extent, a guinea pig to trial-and-error adjustments that occurred to meet the objective of this study. Note that this was only applicable during preliminary procedures with tea. It was advised to the handler that after two consecutive sessions scoring 80% on 5 second tea and two consecutive sessions scoring 70% on (1/2)[1] diluted 5 second tea, Quiver should be moved on to solely BSLB larvae as the S+. The suggestion of tea/BSLB pairing only applied to Flynn and Ros based on recommendations to improve the training procedures.

3.7 Open Matrix:

An open matrix is another MTS task where a squared numbers of jars are spatially presented on the floor in the form of a square. The psychophysical procedure using a matrix has been developed and internally tested by Dr. Gadbois (Gadbois & Reeve, 2014) in the lab in which this study was conducted.

The open matrix consisted of 3x3 (nine jars) and 4x4 (sixteen jars) arrangements where BSLB larvae (S+) were eventually in the presence of EVDS; and ELB larvae (S-). The size of the matrix is correlated with the difficulty of the task. Jars within the matrix were tightly arranged so the jars were just far enough apart without touching. This method encouraged the dog to pin-point the S+ in the proximity of alternative choices. Training procedures and commands remained the same as the 3AFC task, the main difference being in the number of jars and their spatial arrangement.

Initially, the alternative choices within the 3x3 matrix were blank. The matrix that obtained blank alternative choices still remained as a detection task. After meeting criteria with

BSLB larvae as S+, EVDS were introduced in to the matrix as background noise/distractors. Cotton covered the stimuli so all jars appeared uniform in order to eliminate any potential visual cues.

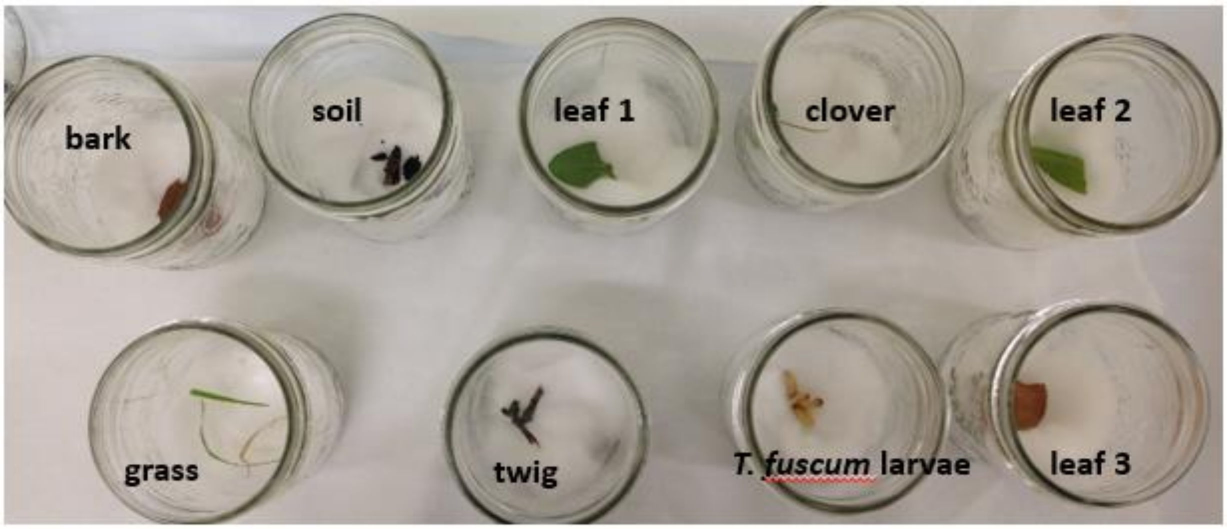

Figure 7: Ecologically valid distracting stimuli (EVDS). Leaf 1, 2, 3 are from different species of vegetation. *T.fuscum* larvae was the target stimulus (S+). *Note*: The bark is not associated with *T.fuscum* infested trees.

It was assumed, given the nature of the matrix, that the difficultly from a 3AFC to an open matrix task increased due to the increase in the amount alternative choices. Moreover, the addition of EVDS corresponds with increased interference leading to a decrease in perceptual memory of the S+ with every distractor stimulus the dog investigated. With this assumption, it was essential that the dogs were truly detecting BSLB larvae and not another scent that they may associate it with (i.e., smelling themselves on the jar that was left behind from the previous trial). To ensure that the dogs were detecting the BSLB, the jars remained stationary and the various stimuli were randomly positioned within the jars in the matrix. If the dog responded incorrectly, the handler was able to determine if they were detecting a scent associated with the jar, or, with a specific stimulus.

3.8 S+ Absent Trials

In preparation for discrimination and Go/No-Go tasks, S+ absent trials were introduced within open matrix sessions as part of the training process and a dog met criteria on a 3x3 matrix with EVDS present. The response that was trained to indicate to the handler that the dog did not detect S+ was to sit. Training the absent response (sit) was achieved by means of informal sessions with the S+ among a random spatial arrangement of several jars that were either blank or contained EVDS. The handler informally randomized the positions of the jars and randomly removed and introduced the S+. Once the dog sniffed all of the choices when the S+ was absent, the handler gave the verbal cue "sit" then used the clicker when the dog sat. The dog learned that responding "no" (sitting) when the S+ was absent would also be rewarded. Depending on the dog, this took approximately three to five 5 minute sessions to train the S+ absent response. Once the dog was confidently making correct "yes, present" or "no, absent" choices without assistance, absent S+ trials were formally introduced in open 3x3 and 4x4 matrix sessions.

3.9 Go/No-Go

The Go/No-Go task required a dog to locate a single scent source in which the dogs responded with either "yes, present" or "no, absent" on a large number of trials (Lit, 2009). The "yes, present" response continued to be at least a four second poke-and-hold response whereas the "no, absent" response was to sit. This task required performance of a desired response in the presence of the S+ and a desired response when alternate stimuli (EVDS and/or S-) were present with the absence of S+. Essentially, this was a closed-matrix procedure where an open top cone, 19.05cm wide at the base and 15.24cm in height, was placed over a mixture of stimuli contained in envelopes (Fig. 8). Where there was a single scent source for this task, the four possible responses outcomes in a detection task could be more clearly observed and recorded for every trial. The number of S+ absent trials in a session gradually increased to attain equal (50/50) proportions of S+ present and S+ absent trials. The number of trials increased from 10 up to approximately 16 within a session owing to the fast nature of how this task was performed, and to collect as much data as possible within the time allotted.[39]

Figure 8: Apparatus used (cone) for the Go/No-Go task with dimensions indicated.

3.10 Preliminary Discrimination Tasks

Each dog was exposed to two discrimination tests that were performed in December 2013 and January 2014 respectively. Flynn, Ros and Quiver were first exposed to S- in a 3x3 open matrix in the presence of EVDS; where the second exposure was in a 3AFC task in which

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[39] *Note*: Extra S+ present trials were occasionally added to the end of the session if Flynn was considerably conservative in his decision bias, due to a high miss rate, to encourage a criterion closer to the desired ideal participant, and to prevent Flynn from being discouraged on the task.

both S+ and S- were present in every trial, and the third alternative was a control (empty envelope). As noted previously, the results of this tasks could not be formally evaluated.

3.11 Response Strengthening: Variable Ratios reinforcement schedule

Response strengthening trials consisted of variably reinforcing a correct response using a variable ratio (VR), which was achieved using Random.org. This ratio was introduced to encourage the dog to increase the strength of the response and to increase the resistance to extinction. Increasing the VR schedule implies that the frequency of positive reinforcement decreases. For example, a VR of 2 implies that positive reinforcement would be given after an average of two trials. VR of 3 implies that positive reinforcement would be given after an average of three trials so and so forth. If the dog chose or responded incorrectly, the reinforcement was shifted down to the proceeding trial in order to maintain the VR. At least two sessions at a particular VR were performed throughout the training process before it increased; increasing the VR was gradual in order for the dog to adjust to the change.

Although a VR is useful, judgement calls were made on whether or not to apply a VR if a particular dog performed better when rewarded every correct trial. The sniffer dog recruits had different levels of motivation, and as a result there was often trial-and-error to find which schedule of reinforcement worked best for each dog. Consequently, Flynn and Quiver ended-up being on continuous reinforcement and Ros was on a VR5.[40]

[40] Ros performed better when she was rewarded less frequently; she tended to guess more if the VR was low.

4.0 Results

4.1 Data Analysis

For the purposes of data analysis, only sessions where BSLB was the S+ were included. If a dog's response during any trials was ambiguous to the handler (i.e., poking the cone on an S+ absent trial for approximately equal amount of time as a S+ present trial before sitting to indicate its absence) it was not included in data analysis with the purpose of avoiding biasing the results. Moreover, if a noticeable change in sniffer dog performance occurred due to a likely explanatory variable e.g., contamination of a jar, switching from cotton to envelopes, distractions interfering with performance, were also not included.[41]

Data collected from the Go/No-Go task was analyzed using Signal Detection Theory (SDT). Data analysis is based on a total of 217 trials that were performed over 16 sessions during a two week period starting on January 31, 2014. For more information on SDT parameters with respect to accuracy, PPP and NPP can be found in section *1.2.6*. Other important dependent variables were extracted from SDT: d', a parametric measure of detectability, and the criterion (c). d' is a measure of the distance between the means of the signal (S+ present) and the noise (S+ absent) distribution curves (Gadbois, 2013). A d' of at least 2.0 is suggests that the dog can accurately detect whether or not the S+ is present (i.e., low proportion of false alarms and misses). A d' of 0 indicates that a dog is performing at chance, and a d' of 4 represents a perfect discrimination[42]. c is a measure of bias which indicates if the dog was more likely to make conservative or liberal decisions based on the ratio of hits to false

[41] During the occasional session, a dog would flag the same jar, regardless of the stimulus it contained, its position or wiping the jars with an alcohol solution between trials.

[42] Not that there is not theoretical limit to d'. But beyond d'=4, the two curves do not overlap, they are fully separated.

alarms (Gadbois, 2013). c is the distance from the actual threshold to the ideal participant (Gadbois, 2013). The sign (+, -) of c reveals the participant's strategy: when c=0, they are an ideal participant; when c is negative, the participant is *liberal* (i.e., responds "yes" more often than the ideal participant); when c is positive, the participant is *conservative* (i.e., responds "no" more often than the ideal participant). In other words, if the criterion is considerably positive, the miss rate is correspondingly high (Type II error), whereas if the criterion is considerably negative, the false alarm rate is correspondingly high (Type I error). A good detector has a strong perceptual sensitivity and that does not have a highly-favoured decision bias (Helton, 2009). For the purposes of this project, an ideal participant is desired. The ideal participant minimizes both the probability of a miss and of a false alarm (Gadbois, 2013). *If*, for example, the participant happens to be either conservative or liberal, a slightly liberal participant is preferred over a conservative dog for the purposes of BSLB detection in the field, for the reason that misses are costly both ecologically and economically for management strategies in the long term (Sweeney, 2007). The cost of a high false alarm rate would likely lead to excessive false identification of non-infested trees, leading to excessive unnecessary removal of non-infested trees; a high false alarm rate would consequently defeat the purposes of this study.

For the purposes of the Go/No-Go task, a dog is considered an accurate candidate, if a high accuracy; a high PPP and NPP; a d' of ~ ≥2; and c close to 0 is attained overall and/or during his most recent performance (Table 3). All of these measure, therefore, collectively provide the bases of judgment for whether Flynn is an accurate candidate. Flynn's overall performance takes into account all of the sessions performed for this procedure. Flynn's most recent performance is arguably more representative of his true potential (i.e., a more truthful depiction of how accurate he is). Flynn's most recent performance is subdivided to show his performance, 1) over the last sessions performed where at least a 80% performance score (correct responses) was consecutively achieved (4 sessions), and 2) his performance during his last day of performing this task (2 sessions). It is important to note that d' estimates are unreliable and not considered when false alarms or misses are 0, therefore d' cannot be relied on to suggest that a dog is an accurate recruit in these specific instances.[43] This is true, unless only percentage correct is presented, or the equivalent of giving sensitivity rate and specificity rate.

The following values that are relevant to data analysis have been included in the results and were calculated as follows:

- Hits (%) = Sensitivity = Hits/(Hits + Misses) x 100%
- Correct Rejections (%) = Specificity = Correct Rejections/(False Alarms + Correct Rejections) x 100%
- False Alarms (%) = False Alarms/(False Alarms + Correct Rejections) x 100%
- Misses (%) = Miss/(Misses + Hits) x 100%
- Positive Predictive Power (PPP) (%) = Hits/(Hits + False Alarms) x 100%
- Negative Predictive Power (NPP) (%)= Correct Rejections/(Correct Rejections + Misses) x 100%
- d' and c (decision biases) were calculated using the d' Calculator © a program courtesy of the psychologically department of Memorial University, Newfoundland.

[43] d' may be high (≥3) when the false alarm rate or miss rate is 0.

S = signal or stimulus

Response → Signal ↓	**"YES"** Says S is there	**"NO"** Says S is not there
PRESENT S is there	**HIT =** # of hits -------------------- # of Present trials	**MISS =** # of misses -------------------- # of Present trials
ABSENT S is not there	**FALSE ALARM =** **# of false alarms** **--------------------** **# of Absent trials**	**CORRECT REJECTION =** # of correct rejections -------------------- # of Absent trials

Figure 9: A chart that represents the four possible outcomes of a detection task that correlates to whether he signal or stimulus (S) is present or absent. The equations to calculate the portions of each response has also been provided in this chart (Gadbois, 2013).

4.2 Performance Scores in Detection Tasks

The range of performance scores, with respect to sensitivity and specificity, from a possible minimum of 0% to a maximum score of 100% during a session on a task (3AFC, 3x3 matrix without and with EVDS, 4x4 matrix with EVDS and Go/No-Go with EVDS) as well as the mean performance score for the corresponding task Flynn, Ros and Quiver performed has been tabulated (Table 2). The mean and range of the performance scores takes into consideration the transfer of learning from the previous task. The summary of performance scores is also presented in the form of two bar graphs, both display the mean performance score where one includes error bars representing the range of performance scores for the corresponding matching tasks (Fig. 10), where another includes error bars +/- the standard deviation (Fig. 11). The standard deviation shows how much variation or dispersion from the mean performance score exist. All of the dogs completed training for the 3AFC, 3x3 matrix with and without EVDS. Due to time constraints, however, only Flynn advanced in training performing sessions on 4x4 matrix and Go/No-Go matching tasks.

All the dogs do not exceed a performance score of 50% during their first exposure to solely BSLB larvae. As expected a drop in performance was observed when tea was removed from the tea-larvae pairing procedures.[44] It took the sniffer dogs a period of one to two weeks to meet criterion on the 3AFC task where only BSLB larvae remained as S+ (Table 2). Mean performance scores tend to improve with repeated exposure to BSLB larvae (Table 2, Fig. 10, 11).

Results show that all the sniffer dogs have the potential to detect BSLB larvae at 100% accuracy in the presence of EVDS (Table 2, Fig. 10, 11). Generally, performance scores range from 10% to 100%. When considering all the dogs' ranges in performance scores, there are only two instances were 100% is not attained on a task: Quiver on the 3x3 matrix without the presence of EVDS and Flynn on the 4x4 matrix (the maximum is 90% for both cases). For Flynn, the mean performance score improves with each consecutive task, increasing from 54.0% in the 3AFC to 90.0% in the 4x4 matrix task with EVDS present in the latter; with the exception of a lower mean score of 83.5% for the succeeding Go/No-Go task. On the other hand, Ros and Quiver's lowest mean performance scores are 58.7% and 59.3% respectively on a 3x3 matrix rather than the 3AFC. Both Ros and Quiver's highest mean performance score is reached on the 3x3 matrix with EVDS, scoring 77.1% and 73.9% respectively. Flynn also obtained his highest mean performance score at this phase in training with 86.9%; though his mean score improved to

[44] When diluted tea (i.e., 1/16th part 5 sec steeped compared to water) was removed from the tea-larvae pairing procedures.

90% on the 4x4 matrix with EVDS present.[45] All the dogs' mean performance scores improve from the 3x3 matrix with blank alternative choices to the 3x3 matrix with the introduction of ENVS.

Performance scores sometimes vary from day to day in the lab, even in the span of one day, and on a trial by trial bases. For example, on Oct. 21, 2013 Ros performed two sessions on a 3AFC with BSLB larvae as S+ scoring 90.0% and 100.0% consecutively. On the next day of trials, which took place on Oct. 23, 2013, Ros performed four sessions on the same task, however, performance scores began at 40% and increased up to 100% over the course of the day which demonstrates considerable variation in performance.

[45] *Note*: Flynn completed only two session on the 4x4 matrix with EVDS due to time constraints.

Table 2: Summary of sniffer dog performance. Range and mean +/- the standard deviation for matching procedures performed in laboratory conditions. Procedures were either preformed with blank (background noise) or ecologically valid distracting stimuli (EVDS) as alternative choices. Target stimulus (S+): *T. fuscum* larvae.

Task	**Flynn Performance Scores (%)**		**Ros Performance Scores (%)**		**Quiver Performance Scores (%)**	
	Range	*Mean*	*Range*	*Mean*	*Range*	*Mean*
3AFC	10-100	54.0 ± 32. 1	40-100	75.5 ± 24.6	30-100	66.3 ± 18.8
3x3 Matrix	10-100	66.7 ± 49.3	10-100	58.0 ± 37.0	20-90	59.3 ± 29.1
3x3 Matrix with EVDS	70-100	86.9 ± 10.1	30-100	77.1 ± 24.9	40-100	73.9 ± 21.9
4x4 Matrix with EVDS	90	90.0 ± 0	-	-	-	-
Go-No-Go with EVDS	65-100	83.5 ± 10.6	-	-	-	-

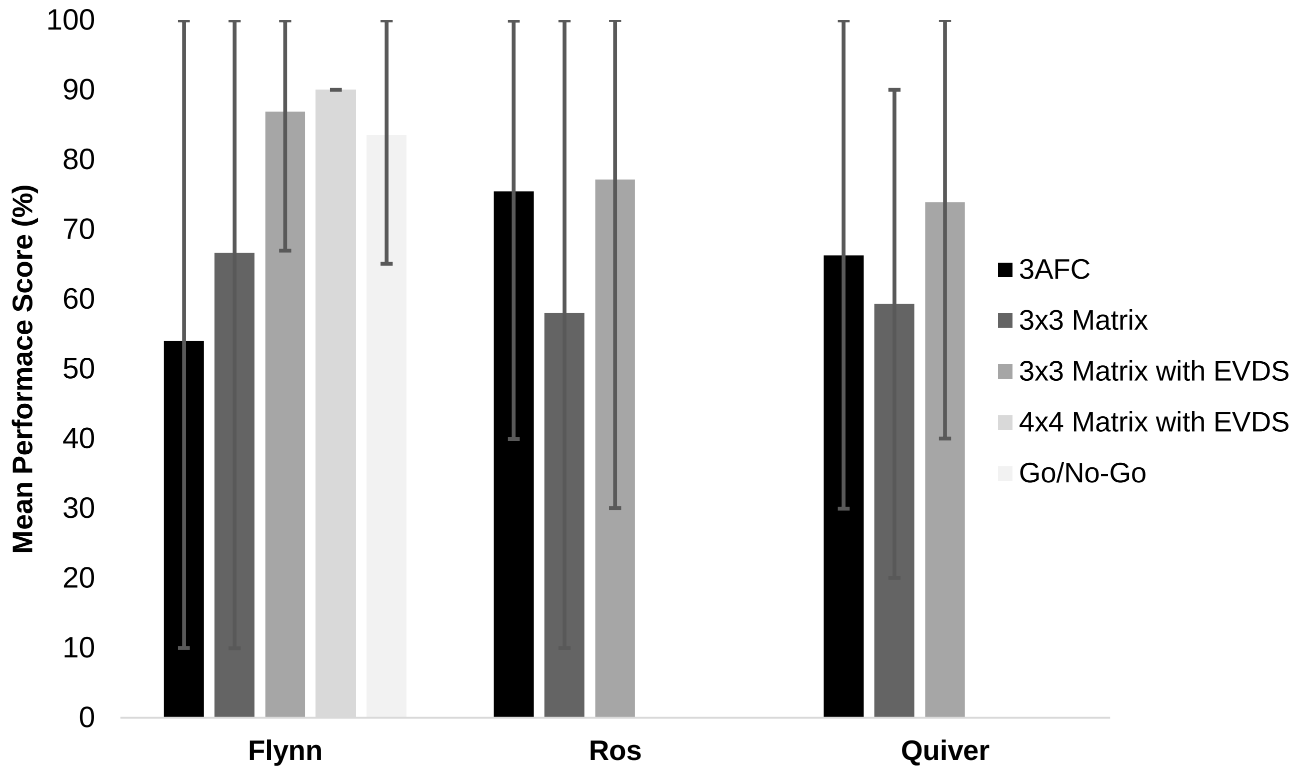

Figure 10: Summary of sniffer dog performance in laboratory conditions. The error bars represent the range (max and min) of scores for performed tasks. Procedures were either preformed with blank (background noise) or ecologically valid distracting stimuli (EVDS) as alternative choices. Target stimulus (S+): *T. fuscum* larvae.

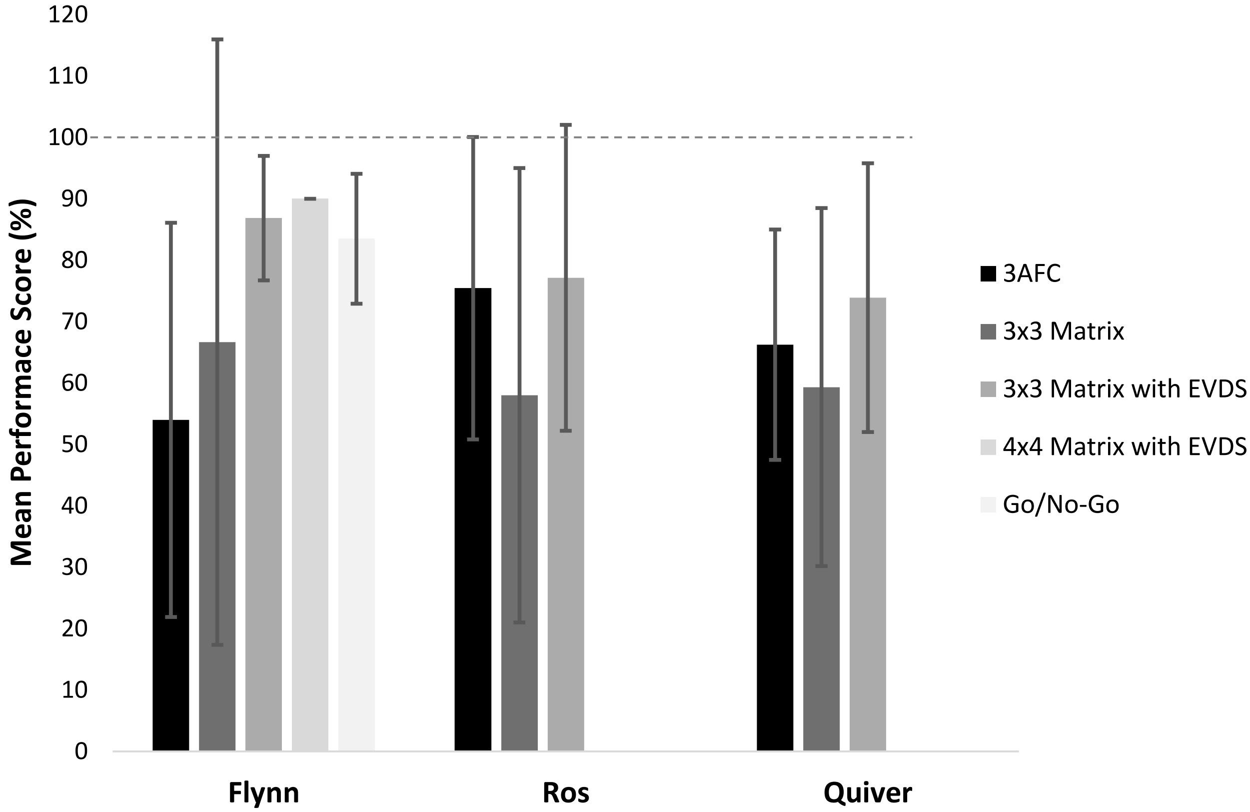

Figure 11: Summary of sniffer dog performance in laboratory conditions. The error bars represent +/- the standard deviation. Procedures were either preformed with blank (background noise) or ecologically valid distracting stimuli (EVDS) as alternative choices. The dashed line represents the maximum performance score that could be attained 100%. Target stimulus (S+): *T. fuscum* larvae.

4.3 Go/No-Go: Signal Detection Theory (SDT)

Flynn is considered an accurate sniffer dog recruit based on his performance in the Go/No-Go task, which is most apparent based on his results during his most recent performance (Table 3). In the Go/No-Go task, Flynn's overall accuracy, with respect to sensitivity and specificity, is 78.2% and 89.2% respectively; and the overall PPP and NPP values are 91.0% and 80.0% correspondingly. In addition, the overall d' is 2.02; and the decision bias (c) is 0.23, which is indicative of a slightly conservative decision maker (Table 3). Based on his performance in the four most recent sessions the sensitivity and specificity rates are 90.6% and 87.1 % respectively; the values of PPP and NPP are 88.0% and 90.0% correspondingly. The d' is 2.45; and the decision bias (c) is -0.09.[46] Although the c is indicative of a slightly conservative decision maker, the value is notably close to desired ideal participant. Even though PPP dropped from 91.0% to 88.0%, a higher d' value and a c closer to 0 is indicative that Flynn's ability to detect whether the S+ is present or absent improves (lower proportion of misses and/or false alarms). There is a 12.4% increase in sensitivity rate (or 12.4 decrease in miss rate) between overall performance and the performance calibrated over the last four sessions. Finally, the last two sessions produce sensitivity and specificity rates equating to 93.8% and 86.7% respectively; and PPP and NPP values of 88.0% and 90.0% correspondingly. The d' is 2.65 and the decision bias (c) is -0.21, and therefore a slightly liberal decision maker. Even though, PPP and NPP remained the same in comparison to the evaluated performances over the last four sessions, d' increased by 0.20 to 2.65, due to a 3.2% increase in sensitivity rate (reduction in miss rate).

[46] The four most recent sessions consist of consecutive sessions with a performance score of at least of 80% (i.e., correct responses).

Performance, with respect to accuracy, fluctuates to some degree between consecutive sessions on the Go/No-Go task (Table 3). There is greater variation in sensitivity rate compared to specificity rate. For example, fluctuation is apparent between the seventh and eighth sessions, where sensitivity rate drops from 100.0% to 50.0% in the respective sessions. It is noted that the percentage of S+ absent trials in the seventh session was 40% whereas the eighth session initially consisted of seven of S+ absent trials (46.7%), closer to the desired 50% S+ present and S+ absent trials. The same condition, where there was a significant decrease in the sensitivity, occurred from the tenth session to the eleventh session. In the respective sessions, sensitivity rate dropped from 75.0% to 33.3% and correct rejection remained at 100%.[47] With successive sessions where S+ present and S+ absent were in equal portions, Flynn's sensitivity rate increased to 100% by session 15.

Some of the derived parameters evaluating performance in the Go/No-Go task do not improve with experience as expected (Table 3). When comparing overall experience to most recent performance (last four and last two sessions), NPP and d' and accuracy, as it pertains to sensitivity increases, and c decreases (i.e., closer to 0), thereby these performance parameters improve (Table 3). Conversely, PPP as well as accuracy, as it relates to specificity, decrease, thus these aspects of performance moderately worsen. Having said that, however, when considering all the parameters collectively (accuracy, NPP, PPP, d' and c), performance improves over time.

[47] The latter session consisted of initially sixteen trials in order for there to be 50% S+ present and S+ absent trials; one S+ present trial was added to the end of the session due to the conservative decision making as before.

Table 3: Summary of Flynn's performance on the Go/No-Go task in laboratory conditions. EVDS were present in all trials. The tabulated parameters were calculated using Signal Detection Theory. d' and A' represent a parametric and non-parametric measure of detectability respectively; c represents the criterion (decision bias). Target stimulus (S+): *T. fuscum* larvae.

Date	Session	Trials	S+ Absent Trials	Hits (%)	False Alarms (%)	Correct Rejections (%)	Misses (%)	PPP (%)	NPP (%)	d'	c (-/+)	Decision Bias
Jan.31/14	1	10	3	71.4	0.0	100.0	28.6	100.0	60.0	4.87	1.87	Conservative
Feb.3/14	2	10	3	71.4	33.3	66.7	28.6	83.0	50.0	1.00	-0.07	Liberal
Feb.5/14	3	10	3	85.7	33.3	66.7	14.3	86.0	70.0	1.50	-0.32	Liberal
	4	10	3	100.0	0.0	100.0	0.0	100.0	100.0	8.60	0.00	Neutral
	5	10	3	85.7	0.0	100.0	14.3	100.0	80.0	5.37	1.62	Conservative
	6	10	4	100.0	25.0	75.0	0.0	86.0	100.0	4.97	-1.81	Liberal
Feb.7/14	7	15	6	100.0	16.7	83.3	0.0	90.0	100.0	5.27	-1.67	Liberal
	8	17	7	50.0	0.0	100.0	50.0	100.0	60.0	4.30	2.15	Conservative
	9	15	7	75.0	0.0	100.0	25.0	100.0	80.0	4.97	1.81	Conservative
Feb.10/14	10	15	7	75.0	0.0	100.0	25.0	100.0	80.0	4.97	1.81	Conservative
	11	17	8	33.3	0.0	100.0	66.7	100.0	60.0	3.87	2.37	Conservative
Feb.12/14	12	15	8	57.1	25.0	75.0	42.9	67.0	70.0	0.85	0.25	Conservative
	13	16	8	87.5	0.0	100.0	12.5	100.0	90.0	5.45	1.58	Conservative
	14	16	8	87.5	25.0	75.0	12.5	78.0	90.0	1.83	-0.24	Liberal
Feb.14/14	15	15	7	100.0	14.3	85.7	0.0	89.0	100.0	5.37	-1.62	Liberal
	16	16	8	87.5	12.5	87.5	12.5	88.0	90.0	2.30	0.00	Neutral
Performance Overall (All Sessions)		217	93	78.2	10.8	89.2	21.8	91.0	80.0	2.02	0.23	Conservative
Performance Last 4 Sessions		63	31	90.6	12.9	87.1	9.4	88.0	90.0	2.45	-0.09	Liberal
***Performance Last Day (2 Sessions)**		31	15	93.8	13.3	86.7	6.2	88.0	90.0	2.65	-0.21	Liberal

4.4 Discrimination Performance

If you are interested in reviewing the outcomes of the preliminary trials, the breakdown in performance for both exposures has been provided in the appendix (8.5)

4.5 Search Patterns

All the sniffer dog recruits demonstrated unique ways of searching for S+. Quiver developed a distinct systematic approach in his search patterns, tending to investigate the jars in the same order during trials. During the initial stages of this behaviour, Quiver would often sit (S+ absent response) without smelling S+ in one of the various choices that were left uninvestigated. In these situations, the handler initially recorded the trial as an incorrect, which impeded corresponding performance scores. When the behaviour became persistent, it was suggested that the handler should encourage Quiver to complete the matrix if he sat before investigating all the S+ absent choices or sitting before S+ was investigated. Based on these changes in procedural approaches, some of the performance scores, where sitting prematurely, may not be an accurate depiction of Quiver's performance abilities prior to the scoring modification, which consequently may have compromised mean performance score during matrix procedures to some degree. Flynn and Ros, on the other hand, usually completed the matrix before making a response, and were therefore more systematic in their searches. Rather than investigating a specific order of jars as Quiver did, Flynn and Ros tended to search the jars randomly.

4.6 Lab Conditions

In lab conditions, physicochemical features, such as wind movement and terrain are negligible; however temperature and relative humidity of the work space are applicable. The lab, in which the current study was conducted, remained at room temperature (~21°C+ /- 2°C) through the duration of the study; however, humidity was informally observed to differ over the course of the five month period, especially during the winter season. Note that temperature and humidity were not recorded and are extraneous (not confounding) variables affecting performance.

5.0 Discussion

Over the course of five months, the results show that all the sniffer dogs selected to participate in this study have the potential to detect and signal BSLB larvae in the presence of EVDS, at a success rate of up to 100% accuracy. Based on Flynn's overall performance scores, more evidently in his most recent performance, he is the most promising sniffer dog and is considered an accurate candidate in the evaluated parameters using SDT (Table 3).

5.1 Generalizations and Trends in the Observations

5.1.1 Saliency of BSLB Larvae

BSLB larvae are likely to be a low salient stimulus for the following reasons: when diluted steeped tea was removed from the tea/BSLB pairing as the S+, performances decreases and it takes a period of one to two weeks for the sniffer dogs meet criteria on the 3AFC. However, the intensity of the stimuli remains unclear and detectability is not just about saliency. It was realized after the completion of preliminary training, that the cotton only in the jar containing the S+ was dampened with the tea/diluted tea mixture whereas the cotton in the 'blank' alternatives remained unscathed. The moisture content, coupled with the possible difference in odor properties in dampened cotton, may be an explanatory variables that contributed to the notable decline in performance after the tea was removed from preliminary procedures. In other words, when the tea was removed, the saliency of S+ may have significantly been reduced, or the odor perception may have been altered between the respective target stimuli. In hindsight, the 'blank' alternatives during preliminary training should have been dampened with water to ensure only the S+ itself was being detected. Nevertheless, over time the dogs seemingly adjusted to the method and detected the assumedly low saliency larvae up to 100% accuracy.

5.1.2 Sniffer Dog Performance

A clear trend presented in the results is that sniffer dog performance detecting BSLB larvae as an olfactory in each of the performed tasks all the dogs were exposed to, improved with time and experience which led to their ability to detect BSLB larvae up to 100% accuracy. This trend is apparent when observing Flynn's mean performance score generally increases over time with each consecutive task (Table 2, Fig. 10, 11).[48] Moreover, mean sniffer dog performance scores improve from the initial 3AFC procedure to the most recent task performed (Fig. 10, 11). This trend is consistent with findings in the literature on olfactory learning, including dogs. In addition, a collection of studies have shown that with experience, the threshold of detection decreases where the participant is able to detect stimuli at lower threshold compared to preliminary testing (Goldblatt et al., 2009).

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[48] Flynn's mean performance score increased from 54.0% on the 3AFC to a maximum mean performance score of 90.0% on the 4x4 matrix with EVDS present (Fig. 14).

5.1.3 Performance between consecutive tasks

As expected, in cases where sniffer dog performance drops between tasks, this is due to transfer of learning. Broadly, by making the task more challenging, performance may be hindered (Helton, 2009d). It is expressed in the literature, that as the number of choices increases (e.g., from a 3AFC to a 3x3 matrix with 9 alternative choices), there is an inverse relationship with the probability of a dog making the correct choice (DeCarlo, 2012). More information on the likelihood of a subject choosing S+ correctly, see 3.7 in the Methods section.

Preliminary performance scores on the 3x3 matrix with EVDS were expected to decrease compared to the latest session performed on the 3x3 matrix without distractors, due to the increase of perceptual interference of EVDS. During preliminary sessions on the 3x3 matrix with EVDS, performance did initially decrease as expected, yet the dogs met criteria on the 3x3 matrix procedure with the introduction of EVDS within a shorter temporal scale compared to making criteria on a 3x3 matrix with 'blank' alternative choice after progressing from the 3AFC task. Moreover, even though the chances of being correct decreases from a 3x3 to a 4x4 matrix, mean performance improves. Both of these examples provide evidence that the experience obtained from the previous task laid the foundation for the consecutive procedure.

Unlike Ros and Quiver, Flynn's mean performance does not drop from the 3AFC task trials to the 3x3 matrix (Table 2, Fig. 10, 11). Once possible explanation for this occurrence is that he was exposed to and met criteria on a 2x2 matrix with BSLB as S+ as a transitional step between the two procedures due to three initial poor performance scores on the 3x3 matrix. The handler acknowledged that Flynn expressed confusion and/or difficulty on the 3x3 matrix and thought it would be appropriate to introduce a transitional step. Flynn performed seven consecutive sessions on a 2x2 matrix before 3x3 matrix training continued. All dogs were exposed to a 2x2 matrix if the dogs performed poorly on the 3x3 matrix to avoid confusing and discouraging the dog, but Ros and Quiver were exposed to a 2x2 considerably later in their 3x3 matrix training (i.e., after three weeks since the initial 3x3 matrix exposure for Ros, and six weeks since the initial 3x3 matrix exposure for Quiver). In other words, Flynn gained experience on 2x2 matrix within preliminary training on the 3x3 matrix compared to Ros and Quiver, which likely contributed to the increase in Flynn's mean performance from the 3AFC to the 3x3 matrix procedure.

Moreover, the difficulty of the task is a probable factor that explains why Flynn's mean performance score decreases in the Go/No-Go task compared to the 3x3 and 4x4 matrix procedures with EVDS present (Table 2, Fig. 10, 11). This task was anticipated to be more challenging due to the novelty of the cone used to cover the stimuli, and Flynn was tested on whether he could detect the BSLB larvae within a mixture of eight EVDS. Experimental studies that investigated odor mixtures provide evidence that odor masking increases the difficulty of odor perception of humans, in which the presence of one odor impedes the perceived intensity of another when both are simultaneous presented together in a mixture (Laing & Francis, 1989). Continued experience, yet again, overcomes this olfactory limitation of being able to detect an odor from a rich olfactory background. As of 2009, only one published paper by Waggoner et al., (1998) investigated detection by dogs of odors in the presence of maskers. Waggoner et al. (1998) found that high concentrations of the masking odour were needed to reduce the detection of the target chemical. In the present study, the EVDS were anticipated to be more salient compared to the larvae,[49] which relates to Waggoner et al.'s findings. The ability to detect S+,

[49] The researchers could smell odors from the EVDS (e.g., leaves, soil); but could not smell an odor from the larvae; however how a dog perceives the odor may be different (i.e., more acute sense of smell).

however, is most likely contingent on the saliency or intensity of the S+ and the odor mixtures. In reality, there are numerous distracting and/or masking scents in the field that a dog would encounter while searching for BSLB larvae.

5*.1.4 Performance within tasks*

While variations between consecutive tasks occur, it is also interesting to note the variations in performance from session to session *within* a task. Specifically looking at the derived parameters from SDT, most varied from session to session during the Go/No-Go task (Table 3). Declines in sensitivity rates are observed after a 100% sensitivity rate was obtained, but interestingly the specificity increases during the respective sessions.[50] The proportion of S+ absent trials between successive sessions is a possible explanatory variable that influenced variations in Flynn's performance. Subsequently, based on anecdotal evidence, it is suggested that as S+ present and S+ absent trials became closer to 50/50 proportions, Flynn became more conservative in his decision making, and perhaps guessing that the S+ was absent due to the improved chances of being correct. This trend is consistent in the literature (DeCarlo, 2012).

Some of the evaluated parameters derived from SDT did not improve with experience as expected (Table 3). When comparing overall experience to most recent performance (last four and last two sessions), NPP and d' and accuracy increased, and the proportion of misses decreased. Thus, these performance parameters improved over time (Table 3). Conversely, the false alarm rate increased, and PPP as well as accuracy, as it relates to specificity, decreased, thus these aspects of performance moderately worsened. Having said that, however, when considering all the parameters collectively, performance improved with experience. When

[50] The sensitivity rate drops from 100% in the seventh session to 50% in the eighth session, however the specificity increases from 83.3% to 100% between the respective sessions.

assessing the decision bias (c), Flynn was initially a slightly conservative and later on became a slightly liberal decision maker.[51] As previously noted, the gradual change to a 1:1 ratio of S+ present and S+ absent may have affected his decision bias and therefore altered the respective performance parameters.

5.1.6 Search Patterns

On an individual level, the sniffer dog recruits each presented with unique ways of searching for the S+. The order in which Ros and Flynn searched the jars were observed to be random, unlike Quiver, representative of different search strategies. A possible explanation why Flynn and Ros investigated the choices randomly might be due the random placement of S+ from trial to trial. Quiver might have developed a search pattern investigating the same order of jars and often made a choice part way through the matrix, perhaps because he was rewarded more often among those choices. More on the observed search patterns can be found in 4 in the results. Sniffer dog search patterns have been documented in the literature. With searching experience, "a dog will develop an underlying sense of the likeliness that a target is present in a certain location" (Helton, 2009, p. 95). For example, research by Gazit, Goldblatt, and Terkel (2005) provided evidence to suggest that dogs develop search patterns that betters their chances or likelihood of being correct based on the outcome of a previous searched path.

5.17 BSLB and ELB Larvae Discrimination

Although data is insufficient, there are possible variables that may explain why the sniffer dogs were not able to naturally discriminate between the two *Tetropium* species during

[51] Flynn's c overall is 0.23, but when considering Flynn's most recent performance his c is -0.21.

preliminary trials. In the current study, it was anticipated that larvae from both beetle species would have similar odors and saliencies, therefore dogs were expected to have difficulties discriminating between the two stimuli resulting in poor performance. Anecdotal evidence suggests that this is the case. The ability to discriminate between odors improves with experience (Goldblatt et al., 2009). In the unlikely event that ELB were reared from a BSLB infested bolt and/or larvae were misidentified from the supplier, these conditions would compromise the dogs' ability to discriminate between the two stimuli as they may have been mixed together. Please note that this is unlikely, but an important scenario to consider nonetheless.

5.2 Influences of Sniffer Dog Performance in Lab Conditions

Although, underlying causes and mechanisms explained some of the trends and patterns of performance, there are many other factors that may affect perception of an odor and performance. In lab conditions, physicochemical features, such as wind movement and terrain are negligible; however temperature and relative humidity of the work space do apply. Although temperature and humidity are considered extraneous variables influencing performance, they might be a possible factor to consider when evaluating performance. A high relative humidity is conducive for scent perception as available moisture in its nasal membranes enables the scent to be carried to the odor receptor sites and increases the rate compounds are volatilized (Vander Wall, 1998). Conversely, over time, exposure to low humidity can dry out and inflame the mucous membrane lining the respiratory tract (Keck, 2000).

Contamination is a likely explanatory variable that gives rise to skewed sniffer dog performance. The possible sources of contamination are vast. For instance, the handler may not have touched the jars uniformly, the jars may not have been washed properly, the dog may put its scent on the stimulus, etc. As a dog's olfactory mechanisms are considerably more sensitive compared to humans, inadvertent contamination of the apparatus and/or stimuli can occur and the handler or lab assistants may not be aware of the contamination (Goldblatt et al., 2009).[52] The researchers suggested the commonality between the persistently flagged jars was that they acquired more of the dog's scent from poking the jar, which may have been perceived as an olfactory cue to locate the S+. After identifying that scenario, the researchers put the dog's scent on each of the jars prior to a session to ensure the scent was uniformly distributed.[53] For more details on measures taken to avoid contamination, see section *3.5*. The complications and hindrances associated with contamination with non-target odors in olfactory procedures have been addressed in an increasing number of studies (Goldblatt et al., 2009). Moreover, if the contaminants maybe more salient that the target stimulus and perhaps may mask or the target odor. This is supported by unpublished research concerning explosive odor detection by rats (Goldblatt et al., 2009).[54]

Some dogs may have more experience or different skill sets compared to others, which may also have an effect on performance. Although Ros and Quiver were naïve to the tasks presented in this study, they both had former lab experience performing other matching-to-sample tasks. Ros has been exposed to the Canid Behaviour Research Lab since she was a puppy and has performed various psychophysical tasks. Quiver was recruited over the summer of 2013 and performed other matching-to-sample tasks that were not a part of the present study. Rather

[52] During the occasional session, each of the sniffer dogs would persistently flag the same jar, regardless of the stimulus it contained, its position or wiping the jars with an alcohol solution between trials.
[53] Note that sessions where contamination of the stimulus containment was possible were not included in data analysis.
[54] Secondary reference.

than lab experience, Flynn had previous experience searching for species at risk, include wood turtles*,* *Glyptemys insculpta*, in the field. The dogs' past experiences and acquired skill sets may have influenced their overall sniffing and searching performance, and thereby providing for better candidates to meet the objectives of this study. Border collies, like Ros and Quiver, tend to be the most common, and arguably some of the best suited dog breeds for lab-based studies (Gadbois and Reeve, 2014). Based on past and current anecdotal evidence, in the Canid Behaviour Research Lab, border collies have demonstrated a high motivation and eagerness to work regardless of the task's repetitiveness; they appear to enjoy the stimulation and challenges that the lab offers. Not to say that other breeds are not suitable for lab work, but due to a combination of interested border collies owners that express keen interest to volunteer their dog for a good cause and favourable breed traits and temperaments makes them more likely to be potential suitable candidates.

Dogs, like humans, have 'off days' and present with uncharacteristic dispositions in their behaviour. Canine behaviour is complex, and is continuously being researched and becoming more prominent in the literature. There are a myriad of factors and variables that may be at play that motivates an animal to behave in a particular way. All the dogs that participated in this study belong to members of the community, therefore the environment in which they are exposed to when not in the lab are dissimilar. How an owner trains, disciplines and interacts with their companion is subjective. Moreover, the dog may have been tired due to a morning run, or sick from something that it ate. Dogs cannot communicate with humans in a way that we clearly understand, but settle depositions and nuances in their behaviour, although often subtle, can suggest to their human counterparts as to what their needs are or why they are behaving or performing in a way that is uncharacteristic. As an example, one day Quiver was having an 'off day' in terms of performance: his disposition was different than usual, he was not as eager to go to the experimental room and often lingered around the exit door of the lab. Later that day, the researchers discovered that he ate something with a plastic wrapper causing him to be sick. In addition, other important attributes ought to be considered when evaluating individual performance include "work ethic, general morphology, agility and athleticism, general health, and nerve strength/mental fortitude" (Hurt & Smith, 2009).

Due to the psychological nature of this study, as it pertains to canine cognition and olfactory perception, it is a challenge to provide all the reasons as to why the dogs performed as they do. Justifying why variations in performance occurred is a challenge. There are many questions pertaining to canine behaviour and olfactory mechanisms that remain unanswered in the literature.

5.3 Agreement with previous work

Although the current study involved a unique approach based on experimental methods used in animal psychophysics (the study of sensory perception and neuroscience in animals) and animal learning theory; similar results are found in the literature. The findings in this study, as it pertains to sniffer dog detection of an insect pest, is consistent with other studies, including the detection bed bugs, *Cimex spp,* (Pfiester et al., 2008); termites, *Isoptera spp*. (Brooks *et al*., 2003); the Asian longhorned beetle, *Anoplophora glabripennis* (Errico, 2012) and the emerald ash borer, *Agrilus planipennis* (Mohr, 2012 Setember 6). For more detailed descriptions of the respective studies, see section 2.8 in the literature review.

5.4 Practical Implications of Study

The application of trained sniffer dogs is anticipated to improve current BSLB management strategies by having a high positive predictive power and a high negative predictive power regardless of the presence of physical/visual symptoms of infestation. Thus, sniffer dogs may provide a more accurate census of level of infestation in a region; and are likely to minimize associated costs without compromising the mobility of field teams. Similarly, Troy Kimoto, a Plant Health Survey Biologist with the CFIA, as per communication, suggested that sniffer dogs could be utilized at receiving and importing nurseries (e.g., for the domestic sale of ash), at Canada Border Services Agency (CBSA) wood packaging detection facilities across Canada where overseas pallets, crates, boxes, etc., are examined by CBSA. Although a great opportunity, the application of sniffer dogs at CBSA facilities is currently unfeasible due to limited financial resources.

Collectively, the sponsors (CFS-AFC, Forest Protection Limited and Sylvar Technologies Inc.) consider this study to be an opportunity to improve current BSLB detection strategies on which potential management procedures can be based. One of CFS-AFC's primary research areas is to develop and assess effective detection and management strategies to mitigate and alleviate native and alien forest pests (NRC, 2013d). Forest Protection Limited is a privately owned company whose priority is to protect forests with services such as fire management, aerial surveys and pest management (FPL, 2004). FPL persists in investing in research and technology to ensure that challenges are adequately addressed with the most effective tools and measures. Sylvar Technology Inc. develops biological control products including environmentally safe baculovirus bio-pesticide for the forest insect pest control (Sylvar Technology Inc., 2006). Sylvar Techonology Inc. is interested in the projected outcomes of this study so trained sniffer dogs could be used to identify BSLB infested trees and/or areas that can selectively treated with insecticides; however insecticides specific to the BSLB are yet to be developed. The Canid Behaviour Research Lab is currently working with the project sponsors and planning to maintain a research and development collaboration. Note that the CFS-AFC offers logistical and operational support for the purposes of this study.

5.5 Study Limitations

There are some inherent limitations to a study of this nature:

- The availability of sniffer dogs was dependent on the convenience of dog owners. If the owner was not able to bring in their dog (e.g., vacation, an inconvenience in their work schedule) this led to setbacks the dog's training and/or limited the amount of data collected.
- Methodological procedures performed 'in vitro' conditions could not truly mimic the natural habitat of the BSLB.
- Methodological procedures were dependent on the availability of BSLB and ELB larvae, which were supplied by the CFS-AFC.
- Training and data collection for the lab component took place from September 2013 to February 2014. Time constraints limited quantity of data collected.

6.0 Conclusion

6.1 Research topics and Research Question

This proof-of-concept study was conducted to determine whether sniffer dogs can accurately detect larval BSLB in laboratory conditions. The ultimate goal, with sufficient research, is to demonstrate to governmental agencies and/or private companies (e.g., Sylvar Technologies, Forest Protection Limited) that the adoption and use of sniffer dogs is a non-invasive and effective detection strategy for management programs of the BSLB at the municipal, provincial and federal levels. The study sought to answer the following question:

> Can sniffer dogs reliably detect the brown spruce longhorn beetle, *Tetropium fuscum*, in the presence of ecologically valid distracting stimuli in laboratory conditions?

6.2 Main Finding of the Study

The main finding of this study is that sniffer dog detection of BSLB larvae, in laboratory conditions, is successful. The results show that all the sniffer dog recruits have the ability to detect BSLB larvae, up to100% accuracy, on a selection of psychophysical methods comprising of matching procedures. Based on Flynn's overall performance scores, more evidently in his most recent performance, he is considered the most promising sniffer dog and a reliable candidate in the evaluated parameters using Signal Detection Theory. Although, this is the first study that has evaluated sniffer dog detection of BSLB, successful canine scent detection of a stimulus of interest is consistent with the findings in the literature.

6.3 Study Limitations

There are noteworthy limitations that arose in this study. Time constraints hastened training procedures and limited data collection. The availability of sniffer dog recruits was dependant on convenience for the dog owners; dogs unable to perform their scheduled days led to setbacks in their training and/or limited the amount of data collected. Another key limitation inherent to this study was that samples of BSLB and ELB larvae were contingent on the available supply from CFS-AFC. Shortages in larvae supply, particularly ELB larvae, led to insufficient data collection for discrimination tasks. Further research is required to thoroughly evaluate sniffer dog detection between BSLB and ELB larvae. In addition, researchers can use this study to revise and refine the original survey design, and recruit more naïve or inexperienced dogs to improve the validity of the research findings, as it pertains the canine detection of BSLB larvae.

6.4 Future Research

Upon completion of data collection, an undergraduate honours student in the biology department of Dalhousie University, Fielding Montgomery, evaluated sniffer dog performance in transition training from lab to field conditions with the recruits from the current study. Essentially, the study is an extension of the current studies progress to train dogs to be able to detect and identify BSLB colonized trees in the field.

Success rates of trained sniffer dogs and human visual surveying for positive (BSLB present) and negative (BSLB absent) identification of BSLB infested trees can be compared. The success rate of each method can be determined using either emergence traps on the tree trunks or incubating bolts. Surveying time and a cost-benefit analysis of the two respective methods can be analysed. Preliminary field training may be evaluated on previously tested sites within HRM where BSLB infested trees have been positively identified. The Canid Behaviour Research Lab is open to the possibility that the sniffer dogs may not contribute to a sufficient reduction of cost (e.g., monetary, human resources). The lab will work closely with its partners to identify ways to optimize their use and assess their financial and environmental impact.

The Canid Behaviour Research Lab will continue he training of scent canines with already trained field dogs, as well as naive dogs (new recruits). It is important to note that in further projects a number of manipulations can be used in order to identify the nature or source of the volatiles used by the dogs (e.g., fuscumol, other pheromones or semiochemicals, life stages, insect/wood as a system, biochemical response from the tree). This work could be done in collaboration with chemists, biologists and biochemists at Dalhousie University, Sylvar Technologies, and governmental agencies.

Future projects could apply the current study's methodology to evaluate sniffer dog detection of other spreading threats to Canadian forests, such as the emerald ash borer, *Agrilus planipennis.*

6.5 Final Statement

The proposed project may have positive implications on ecological, economic and social levels. The application of sniffer dogs may be an effective tool providing an early detection strategy for BSLB management in the efforts to supress and limit further spread and establishment of BSLB in that it would serve to restore and maintain the ecological integrity of North American forests.

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8.0 APPENDIX

8.1 Timeline

Timeline of preliminary experimentation to ensure the completion of the 4902 honors thesis project

Preliminary Experimentation	August 2013	September 2013	October 2013	November 2013	December 2013
Sniffer Dog recruits					
Preliminary Training					

Timeline of actual experimentation to ensure the completion of the 4902 honors thesis project

Actual Experimentation	November 2013	December 2013	January 2014	February 2014	March 2014	April 2014
Data Collection						
Data Analysis						
Thesis Write-Up						
First Draft of Thesis Submission						
Final Thesis Submission						
Corrected Thesis Submission						
Honours Qualifying Exam						

8.2 Ethics Review Application

DALHOUSIE UNIVERSITY
Inspiring Minds

FORM C

UNIVERSITY COMMITTEE ON LABORATORY ANIMALS

Application to use INVERTEBRATES, tissues obtained at NECROPSY or obtained from a SLAUGHTERHOUSE

FORM C - INFORMATION

*** This application is for investigators using invertebrate species for research or teaching, for which the Canadian Council on Animal Care does not require detailed information. This includes the use of eggs, protozoa or other single-celled organisms; experiments involving containment, incision or other invasive procedures on metazoa. It is the expectation of the CCAC that animals of all species, including invertebrates used in research or teaching projects will be treated humanely including the use of anesthetic or analgesic agents when appropriate with rapid and humane forms of euthanasia.

*** This category excludes higher invertebrates with complex nervous systems (ie. all cephalopods) - for these species, detailed information on the FORM A application is required.

*** This application may also be used for investigators wishing to use tissues obtained at necropsy or from a slaughterhouse.

Information requested on this form will enable the office for the University Committee on Laboratory Animals to sign investigator's grant application forms to indicate that the UCLA has approved the proposed use of invertebrates, tissues obtained at necropsy or from slaughterhouse as required by many granting agencies.

Please send electronic version and one signed paper copy to UCLA secretary.

Approval of this application will extend the project for a two year period. At the end of this period you will be required to re-submit another Form C.

Leslie Lord
Secretary - UCLA
1390 LeMarchant St.
Dalhousie University

PHONE: 494-1270
FAX 494-1516
EMAIL: leslie.lord@dal.ca

8.3 Ethics Protocol Approval on Laboratory Animals

DALHOUSIE UNIVERSITY

Inspiring Minds

NOTICE OF PROTOCOL APPROVAL
UNIVERSITY COMMITTEE ON LABORATORY ANIMALS

Protocol Number:	12-085
Investigator:	Simon Gadbois
Expiry Date:	Aug 1, 2014
Category/Level:	B
Title of Study:	Can Sniffer dogs Detect Tetropium Fuscum and Discriminate it from Tetropium Connamopterium in Laboratory and Field Conditions?:
Species:	dogs

Leslie Lord
Secretary – University Committee on Laboratory Animals
Dalhousie University
1390 LeMarchant St. Halifax, N.S. 902-494-1270
leslie.lord@dal.ca
WEBSITE: http://animalethics.dal.ca

Leslie Lord

IMPORTANT FUNDING INFORMATION:

In compliance with granting agency and Dalhousie University policy, the Office of Research Services in not permitted to release funding instalments into research accounts until documentation of all necessary approvals are submitted to Research Services (ie. Human ethics, animal ethics, biohazard and radiation permits).

*** To ensure the research funds related to this animal protocol are released, please fill out the information below , sign and Fax the entire sheet to the Research Office.

- Dalhousie University Research Office FAX 494-1595
- IWK Research Office FAX 470-6767

If this protocol covers more than one research project, include information on all related grants.

Name of Principal Investigator:

Granting Agency(ies):

Granting Agency (ies) Award #:

Dalhousie Account # (if known) to which this protocol applies:

Signature of Grant Holder:

8.4 Brown spruce longhorn beetle and eastern larch borer Identification Guide

(Supplied by the Canadian Forest Service-Atlantic Forestry Centre (CFS-AFC)

Slab #91

T. cinnamopterum larvae. Note that the Urogomphi have no space between them and come from the same base.

T. cinnamopterum adult. Note the dimpled look of the pronotum - no raised bumps. These can appear much more shiny than T. fuscum.

Sexing Tetropium is the same regardless of species. This is a female - note the "duck-bill" appearance of the last segment of the abdomen. In T. cinnamopterum, this segment will often appear darker (closer to black) than T. fuscum.
In males, there is a second segment. This last segment will appear to hinge when the male is given a gentle squeeze. Similarly, when the female is gently squeezed she will often stick out her ovipositor.

8.5 Performance Scores in Discrimination Tasks

A breakdown of sniffer dog performance for both exposures has been provided. Note that the sessions in the discrimination tasks generally consist of fewer trials, this was done to avoid confusing the dog for the S+ and S- were anticipated to be similar stimuli.

4.3.1 First ELB (S-) Exposure: 3x3 Matrix with EVDS

Flynn

Flynn's performance score is 100%, based on a session with six trials. For the first four trials, S+ (two BSLB larvae) was correctly indicated (hit), whilst in the presence of EVDS. Only two larvae were used due to a limited supply of S-. S- (two ELB larvae) was introduced on the fifth trial with no S+ present. Flynn investigated S- twice along with various EVDS and sat indicating that S+ was absent (correct rejection). On the six trial, where S+ and S- were both present, S+ was chosen; however it was ambiguous to the handler whether S- was also investigated (i.e., the dog's nose quickly passed over S-). In this exposure, preliminary results suggest that Flynn could naturally discriminate between S+ and S-.

Ros

Ros's performance score is an estimation of 80%, based on a session with five trials. For the first three trials, S+ was correctly indicated (hit), whilst in the presence of EVDS. S- was introduced on the fifth trial with no S+ present. During this trial, Ros first investigated an EVDS, followed by S- which was investigated then she continued on smelling other EVDS then returned to S-, and chose it. On the fifth trial, S+ and S- were both present. Here, four EVDS were investigated, followed by S-. Ros sat after sniffing S- (correct rejection); Ros did not investigate S+, therefore sitting would be the correct response if S+ was not yet investigated.

The last trial was marked as correct, but as Ros did not find S+ in the matrix, the overall score was therefore an estimation.

Quiver

Quiver's performance score is 54%, based on a session with thirteen trials. For the first five trials, S+ was correctly indicated (hit), whilst in the presence of EVDS. S- was present on sixth and seventh trial with no S+ present, and S- was indicated both trials. The eighth and ninth trial, S- was removed, and Quiver correctly indicated S+ both trials. S- was presented again in the tenth trial, without S+ present, and S- was chosen. On the eleventh trial, S+ was present without S-, and Quiver correctly signalled S+. Both S+ and S- were present on the twelfth trial; Quiver sniffed both S+ as well as S- and interestingly chose S-. To conclude the session, S- was removed from the matrix, Quiver investigated all of the choices including S+ and sat. During this last trial, it was noted that Quiver appeared anxious and was not sniffing as thoroughly (i.e., rushing through the matrix). During this exposure, it was apparent that Quiver could not naturally discriminate between S+ from S-; the only correct trials were if S+ was in the matrix without S-.

4.3.2 Second ELB (S-) Exposure: 3AFC

Flynn

Flynn's performance score is 33%, based on a session with six trials. In the first trial, Flynn sniffed all three choices and chose S-. For the first and second trials, only S+ was investigated, and was chosen (hit). In the third and fourth trials, only S- was sniffed, and was chosen. During the fifth trial, Flynn investigated all three choices, and went back to S- to choose it. In the last trial, Flynn chose S- without sniffing S+.

Ros

Ros's performance score is 40%, based on a session with five trials. In the first trial, Ros sniffed each of the three choices, and selected S-. In the second trial, Ros investigated S+ then S- in which the later was chosen. Ros signaled S+ in the third trial (hit), before sniffing S-. During the fourth trial, Ros investigated S+ then S- in which the later was chosen, which also occurred in the second trial. On the last trial, Ros sniffed all three choices with S- being the last, and S- was signalled. It was interesting to note that Ros, similar to Flynn, chose S- in most trials, even if S+ was investigated.

Quiver

Quiver's performance score is 40%, based on a session with five trials. In the first trial, Quiver sniffed each of the choices and selected S+ (hit). For the next two trials, Quiver signalled S- before S+ was investigated. On the fourth trial, S+ was the only choice investigated and it was chosen (hit). On the last trial, S- was chosen after sniffing all of the choices.