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AVIAN FORAGING RESPONSE TO JACK PINE (*PINUS BANKSIANA*) VOLATILE CHEMICALS

By

Katie L. Bjornen

THESIS

Submitted to

Northern Michigan University

In partial fulfillment of the requirements

For the degree of

MASTER OF SCIENCE

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Abstract

AVIAN FORAGING RESPONSE TO JACK PINE (*PINUS BANKSIANA*) VOLATILE CHEMICALS

By

Katie L. Bjornen

Birds benefit trees and other plants by removing herbivores that damage the plant and impede its growth. They can significantly increase the growth and biomass of the trees by foraging on the herbivores that harm plants, and the prey items eaten in turn benefits foraging birds by providing sustenance. This interaction becomes complex when tree volatile chemical communication is added. Trees regularly release volatile organic chemicals (referred to as VOCs) as part of metabolism. The particular VOCs released by a tree can depend on several factors, including water availability, time of year, and damage type (e.g., mechanical damage or insect damage). Therefore, the VOC profile released by a tree has the potential to encode the type of agent causing plant damage, and potentially the infestation level of a species of foraging insect. If birds can sense these VOCs, they could determine which trees will be the most efficient to forage on. I tested this interaction in the jack pine (*Pinus banksiana*) forests in the Upper Peninsula of Michigan. I measured VOCs released from trees that were favored by foraging insectivorous and omnivorous birds and compared them to VOC measurements from trees that had no avian foraging. Specifically, I focused on both the difference in the α -pinene to β -pinene ratio between preferred and non-preferred trees and the overall VOC composition of preferred and non-preferred trees. I found significant differences in the VOCs released between preferred and non-preferred trees. These differences were consistent with prior research on the differences between insect-damaged and non-insect damaged trees, suggesting the birds were indeed foraging on trees with more insects.

Keywords: Volatile organic compounds, avian foraging behavior, olfaction, jack pine.

Introduction and Study Site

Avian Foraging Decisions

The movement of animals could be driven by many factors, or movement could be random. Foraging is a necessary activity for many organisms to sustain themselves, and optimal foraging theory predicts foraging movements should not be random such that net energy gain will always be maximized (Charnov 1976). For birds, directing foraging efforts to habitats and microhabitats with abundant and easily-acquired food resources is one important way to maximize the energetic trade-off (McNamara 1987). On a large scale, this is relatively obvious, since hummingbirds don't try to procure nectar from conifer flowers, osprey don't try to catch fish in the desert, and oystercatchers don't look for mollusks in the trees. But even within preferred habitats (i.e. a flower patch for hummingbirds, lake/river surface for osprey, shorelines for oystercatchers), birds are likely using more fine-scale cues to direct their foraging efforts toward specific microhabitats to minimize the energetic costs and increase their foraging efficiency (Todd and Cowie 1990). Fine scale cues can be used by insectivorous birds to detect microhabitats (e.g., individual trees) with higher levels of insect abundance, leading to shorter foraging time per food item acquired. Individual trees with higher insect levels could be detected by the birds using vision or olfaction. Thus, if birds are foraging optimally to maximize the amount of prey found per unit of energy invested in hunting and procuring that prey item, we might expect birds to direct their foraging energy to microhabitats, or more specifically trees, with higher density of insects.

Plants and Phytophagous Insects

Phytophagous insects can impart significant damage to their plant hosts, costing plants energy (Holmes et al. 1979). Because of the damage caused by herbivorous insects, many plants use direct and indirect methods of defense to prevent or limit herbivory (War et al. 2012). One method of direct defense by plants involves the plant increasing the secretion of a bitter or toxic chemical that prevents

insects from ingesting the plant material, or the chemical reduces the nutritional value of the tissue and renders the plant less appealing (War et al. 2012). A classic example of direct chemical defense is shown by mustard plants (Trachyophyta:Capparales), which respond to herbivory by degrading glucosinolates to compounds that are toxic to common insect herbivores such as the southern army worm (*Spodoptera eridania*) (Blau et al. 1978). A method of indirect defense by a plant involves the plant releasing volatile organic compounds (hereafter referred to as VOCs) in response to an insect predation event, and those VOCs serve as chemical attractants to predatory insects (Keeling and Bohlmann 2006, Heil 2008, Gols et al. 2011) or other predators (Amo et al. 2013). Several species of parasitic and predatory insects can detect VOCs released by trees that are under attack by phytophagous insects (Llusà and Peñuelas 2001). The ability to detect the VOCs allows the parasitic and predatory insects to focus effort on trees with higher phytophagous insect abundance, increasing their foraging efficiency and (although unintentionally) assisting the tree in limiting the damage caused by phytophagous insects (Price et al. 1980). An example of this VOC detection occurs in apple trees (*Malus sp.*) which when damaged by a phytophagous spider-mite (*Panonychus ulmi*), release VOCs that attract the predatory mite (*Amblyseius andersoni*) whose primary prey is the spider-mite (Llusà and Peñuelas 2001). Females of the parasitic wasp, *Cotesia marginiventris*, preferentially respond to and orient toward the specific VOC blends released by maize plants when they are infested with lepidopteran larvae (Fontana et al. 2011). These VOC signals emitted by the plants have been termed a plants' "cry for help" (Dicke 2009; Mantyla et al. 2011).

Avian Detection of VOCs

The avian foraging hypothesis posits that birds respond to the VOCs released by plants much like predatory insects respond to them. Recent research supports this hypothesis. Mäntylä et al. (2004) demonstrated a preference in Willow Warblers (*Phylloscopus trochilus*) for foraging on insect-damaged

birches (*Betula pubescens*), but it was unclear if the birds were using visual or olfactory cues (Mäntylä et al. 2004). In a controlled aviary setting, Amo et al. (2013) showed that Great Tits (*Parus major*) preferentially moved towards insect-infested apple trees (*Malus sp.*) over non-infested trees, although the test birds were unable to see either type of tree. Amo et al. (2013) was among the first to disentangle visual from olfactory cues, firmly indicating that Great Tits are able to use some cue other than visual cues for microhabitat selection. More recently, two species of tits (*Parus major*, *Cyanistes caeruleus*) were attracted to Scots pine (*Pinus sylvestris*) that had been infested by pine sawfly larvae (*Neodiprion sp.*) even when the larvae were not visible to the birds (Mäntylä et al. 2016). Importantly, Mäntylä et al. (2016) also showed that there were significant differences in the quantity and composition of VOCs released by the infested trees and non-infested trees.

Avian Olfaction

The avian olfactory foraging hypothesis relies on the assumption that birds can sense olfactory cues. Although it has been largely assumed that birds have poor senses of smell, a series of more recent studies suggest the ability to detect chemical cues is more widespread than previously imagined. We now know birds appear to use olfaction for navigation, for foraging, and for individual recognition (Krause et al. 2012; Gagliardo 2013; Amo et al. 2013; Mihailova et al. 2014). Early studies (e.g., Niimura and Nei 2005) reported a limited number of functioning olfactory receptor genes in Red Jungle Fowl (*Gallus gallus*), and this helped fuel suspicions that birds may not have extensive chemical detection abilities. But a more recent study found evidence of a much higher proportion of functioning olfactory receptor genes in a diverse group of bird species (Steiger et al. 2008). They also suggest that a small proportion of functioning olfactory receptor genes may not be indicative of a lesser olfactory ability, but rather an ability to sense only niche-specific odors (Steiger et al. 2008).

Study Overview

Except for the 2016 study on Scots pine (*Pinus sylvestris*), and a field study on mountain birch (*Betula pubescens*??) in 2015, studies of the avian olfactory foraging hypothesis have focused on VOCs released by deciduous trees and detected by birds in aviary conditions (Koski et al. 2015; Mäntylä et al. 2016). Although studies of VOC response to insects have primarily focused on agriculturally important crops and deciduous trees, research of conifer VOCs does exist and is growing (Table 1) (Wallin and Raffa 1999; Huber et al. 2004; Gao et al. 2005; Blande et al. 2009; Mäntylä et al. 2016; Semiz et al. 2017).

Given the evidence that conifers release VOCs in response to phytophagous insect attack and that birds are able to detect VOC cues and subsequently redirect their foraging effort in response to this detection, I set out to test – under field conditions – the hypothesis that birds use VOCs released by ecologically and economically important conifer trees as olfactory cues for foraging decisions. I approached this problem in two different ways. The first was to document foraging efforts by insectivorous and omnivorous birds on jack pines (*Pinus banksiana*) and compare the VOC signal of trees that had no foraging to the signal of trees with foraging preference. For the second experiment, I treated trees with three different VOCs and observed foraging by birds on these trees. These two parts are detailed in chapters two and three respectively.

Study Site

I performed this study on a jack pine (*Pinus banksiana*) forested tract in Marquette County, in the Upper Peninsula of Michigan, USA. Jack pines are found throughout the northern US and Canada in well drained soils. Many of these forests are regularly managed by the United States Forest Service, private companies and county governments for paper production. The species is fast-growing, and susceptible to infestations of jack pine budworm (*Choristoneura pinus*). The jack pine budworm is a

micro-lepidopteran that emerges in May and forages on the pollen cones and surrounding needles until pupation in early August. Their populations cycle between years of high abundance (and severe damage to jack pine forests) followed by 10-15 years where there are low population numbers. During outbreaks when budworm numbers are high, infestations can extensively damage jack pine trees, causing “top kill” and weakening the tree. Thus secondarily, an infested tree becomes more susceptible to disease and further insect infestation, such as pine bark beetle (*Ips sp.*) (Wallin and Raffa 1999; Conway et al. 1999). In the study area, insect population levels appeared to be moderate to low and there was no visible top kill or signs of jack pine budworm. The pine spittlebug (*Aphrophora cribrate*) were active on >90% of the trees.

Jack pines are important habitat for several insectivorous and omnivorous passerine species, ruffed grouse (*Bonasa umbellus*), and are the sole habitat for the endangered Kirtland’s Warbler (*Setophaga kirtlandii*), an insectivorous bird species that is now largely extirpated from Michigan’s Upper Peninsula. Kirtland’s Warblers require very specific habitat for breeding: young, homogenous jack pines growing closely together, with regular gaps in the jack pine matrix (Donner et al. 2008). In pre-colonial times, these sorts of jack pine habitats were historically maintained by regular forest fires and other disturbance on the landscape. With the advent of fire suppression, lack of habitat drove the bird to a limited range in the northern Lower Peninsula of Michigan, where jack pines are now managed specifically to support the breeding of Kirtland’s Warblers (Walkinshaw 1983). In recent years, male Kirtland’s Warblers have been noted on this study site, suggesting these birds may be attempting to reclaim some of their former breeding grounds.

I conducted my research on a forested tract owned by Marquette County in Sands Township. The area is forested with jack pines that are managed for pulp production. These pines are planted in even height stands of approximately 10-30-acres. The soil is primarily sand, and it contains dense undergrowth dominated by wild blueberry (*Vaccinium sp.*). I focused on stands of trees in the age range

of 5-15 years. The jack pine habitats in this area are used by several insectivorous species of birds, including; Clay-colored Sparrows (*Spizella pallida*), Chipping Sparrows (*Spizella passerina*), Dark-eyed Juncos (*Junco hyemalis*), Nashville Warblers (*Leiothlypis ruficapilla*), Black-capped Chickadees (*Poecile atricapillus*), Palm Warblers (*Setophaga palmarum*), Yellow-rumped Warblers (*Setophaga coronata*), Hermit Thrushes (*Catharus guttatus*), Brown Thrashers (*Toxostoma rufum*), White-breasted Nuthatches (*Sitta carolinensis*) and the endangered Kirtland's Warbler (*Setophaga kirtlandii*).

Figures



Fig. 1 Study area in the north-central portion of the Upper Peninsula of Michigan. Site (black square) is county-managed jack pine forests in stands of varying ages from seedling to approximately 30-year-old stands

Tables

Table 1 Studies of coniferous tree VOC response to insect depredation. Pinenes are frequently the foremost response VOC in conifer studies to date

| Tree Species | Phytophagous Insect Species | VOC response | Study |
|--|---|--|-------------------------|
| Jack Pine (<i>Pinus banksiana</i>) | Jack Pine Budworm (<i>Choristoneura pinus</i>) | Decrease in α -pinene to β -pinene ratio with defoliation. Also found increase of α -pinene in moderately defoliated trees (decrease in lightly) | (Wallin and Raffa 1999) |
| Jack Pine (<i>Pinus banksiana</i>) | Jack Pine Budworm (<i>Choristoneura pinus</i>) | Increase in α -pinene, β -pinene, and limonene. | (Lazebnik 2012) |
| Scots Pine (<i>Pinus sylvestris</i>) | Pine Sawfly larvae (<i>Diprionidae</i>) | Increase in α -pinene, β -phellandrene, α -coepene, β -farnesene, bicycloelemene, α -humulene and α -amorphene plus more. | (Mäntylä et al. 2016) |
| Norway Spruce (<i>Picea abies</i>) | Pine Weevil (<i>Hylobius abietis</i>) | Increase in α -pinene, β -pinene, myrcene, camphene and linalool | (Blande et al. 2009) |
| Turkish Pine (<i>Pinus brutia</i>) | Pine Weevil (<i>Hylobius abietis</i>) | Increase in α -pinene, β -pinene, 3-carene, limonene, myrcene, α -terpineol and β -phellandrene plus others. | (Semiz et al. 2017) |
| Grand Fir (<i>Abies grandis</i>) | Mechanical Wounding | Immediate increase of limonene, and delayed increase of both a and b pinene. | (Steele et al. 1998) |
| White Spruce (<i>Picea glauca</i>) | White Pine Weevil (<i>Pissodes strobi</i>) | Increase in limonene and sabinene hydrate and a decrease in myrcene found in the bark of trees damaged by weevils (or weevil simulation). | (Tomlin et al. 2000) |
| Sitka Spruce (<i>Picea sitchensis</i>) | White Pine Weevil (<i>Pissodes strobi</i>) | Increase of both a and b pinene in the xylem in response to weevil damage. | (McKay et al. 2003) |
| Lodgepole Pine (<i>Pinus contorta</i>) | Mountain Pine Beetle (<i>Dendroctonus ponderosae</i>) | Increase of b-pinene in trees that survived pine beetle attack but varied by location. | (Clark et al. 2012) |

Chapter Two

Introduction

Olfactory Foraging Hypothesis

When moving through a forest, birds may forage in a seemingly infinite number of places. Optimal foraging theory states that birds should be able to maximize their efficiency by investing foraging time on portions of the habitat that are more likely to contain food resources. There are many variables that affect individual birds as they select foraging spots, including the exposure to predators, the probability of finding quality food, social interactions, and species-specific dietary preferences (Bartumeus and Catalan 2009; Tsurim et al. 2010; McMahon and Marples 2017). To direct foraging efforts more efficiently, birds could use visual, aural, tactile, or olfactory cues when evaluating the probability of finding quality food. Raptors are adept at using their excellent vision in hunting (Lind et al. 2013), American Robins use auditory cues to detect worms under the soil, and sandpipers detect pressure gradient changes caused by prey buried in the sand (Montgomerie and Weatherhead 1997; Piersma et al. 1998). Insectivorous birds foraging on trees could be using indirect visual cues such as damage on chewed leaves or branches that shows evidence of disease or death. For example, damaged branches of mountain birch (*Betula occidentalis*) reflect less light when phytophagous insects are removed by the observer post feeding (Mäntylä et al. 2008).

The olfactory foraging hypothesis (discussed in chapter one) is that birds are able to sense volatile organic compounds (referred to as VOCs) released by trees that have been attacked by phytophagous insects, and the birds then direct their foraging efforts toward those trees (Mäntylä et al.

2004; Amo et al. 2013; Mäntylä et al. 2016). Mäntylä et al (2004) first provided evidence for this hypothesis in observing the response of Willow Warblers (*Phylloscopus trochilus*) to sawfly-infested mountain birches (*Betula occidentalis*). They found birds preferentially foraged on trees with insect damage (with the insects removed) but were unable to determine whether the birds responded to olfactory or indirect visual cues (Mäntylä et al. 2004). A later study, Amo et al (2013), demonstrated the use of olfaction by foraging birds by preventing captive great tits (*Parus major*) from being able to visually inspect infested and non-infested apple trees (*Malus pumilla*), yet they showed birds preferred foraging on infested apple trees.

VOCs and Insect Damage

Plants respond to insect damage by releasing hormones for endogenous signaling, directing other physiological pathways to produce VOCs of an altered composition from that which they normally produce (Hudgins et al. 2003; Mäntylä et al. 2014). VOC responses to insect damage can vary widely. Some plants vary the signal when damage is caused from insect feeding when compared to the signal in response to simple mechanical damage. Relatedly, the suites of VOCs released can vary with the developmental phases of both the trees and insects involved (Arimura et al. 2009). Trees can be extremely sensitive to insect feeding, some even responding to simple oviposition by insects (Hilker and Meiners 2010). The response occurs quickly, usually in a matter of hours (War et al. 2012). Specific compound types commonly released in response to insect damage are monoterpenes and sesquiterpenes (Lerdau 1997; Geron et al. 2000). Conifers are particularly well-known for their use of these compounds in direct defense against phytophagous insect attack (Trapp and Croteau 2001). VOCs released by conifers also may function as indirect defense by attracting predators of the infesting phytophagous insects, but only one study has focused on avian foraging in response to the VOC signal

from conifers. My study centers on jack pines (*Pinus banksiana*) and their potential release of VOCs as an indirect defense mechanism. Jack pines are known to release various VOCs, including α -pinene, β -pinene, β -myrcene, limonene, α -terpinene, α -phellandrene and 3-carene (Wallin and Raffa 1999; Lazebnik 2012). I examine whether the individual jack pines that received the greatest foraging efforts by insectivorous and omnivorous birds were releasing demonstrably different VOC signal.

Study

To determine if insectivorous and omnivorous birds preferred foraging on trees that released a different composition of VOCs, I observed foraging birds in the jack pine system of Northern Michigan. I hypothesized insectivorous and omnivorous passerines use VOC cues to choose which jack pines they will forage on. In a prior study of jack pines, the ratio of two specific VOCs – α -pinene to β -pinene – showed the most consistent change after insect damage (Wallin and Raffa 1999). I thus predicted the α -pinene to β -pinene ratio to be different in those trees on which birds preferred to forage on compared to those trees that birds did not prefer. I also predicted the preferred trees would have different overall VOC composition than those not preferred. To test these predictions, I recorded the amount of time insectivorous and omnivorous birds spent actively foraging on specific trees during four-hour surveys of jack pines. I collected the VOCs emitted from trees with foraging activity and from trees with no foraging activity. By calculating relative percentages of VOCs, I was able to compare the composition of the VOC signal between trees. I determined the ratio of the α -pinene to β -pinene signal to compare between preferred and non-preferred trees.

Methods

Insectivorous and Omnivorous Bird Surveys

I carried out this study in Marquette County, Michigan in a managed jack pine plantation (46.363085, -87.413933). I placed observational points randomly throughout the study area in an approximately 4-ha (10-acre) monotypic jack pine stand (Fig. 1) with points positioned no closer than 100m to one another.

I counted the number of trees visible from the center of the point and observed those trees for the duration of the count. I remained at the center of the plot for the duration of a 4-hour survey and recorded all foraging activity on trees visible from the center observation point. For each foraging event I recorded bird species, time foraging (seconds), and the individual tree used by the foraging bird. I defined avian foraging activity as behavior that involved active searching, probing and pecking. Flying out to catch aerial insects (“sallying” or “hawking”) was not relevant foraging due to my focus on the damage caused by the larval stage of phytophagous insects, however, I did not observe this behavior during this study.

VOC Collection

I collected VOCs from one or two of the trees that experienced the greatest foraging effort by birds during each observation period and from one or two trees that experienced no foraging. On each of the selected trees I attached a stable headspace apparatus to collect VOCs (Fig. 2). Due to the possibility that mechanical damage could change the signal, care was taken to minimize disturbance. Samples were paired, for each of the two preferred trees I selected for VOC sampling, I sampled two trees that did not receive foraging. This helped to minimize variation caused by weather, physical

damage, and date. The stable headspace apparatus was created by fastening two embroidery hoops to an oven bag (Reynolds, slow cooker size) and placing this around a branch of the tree. I attached it by gently fastening the opening of the bag around the branch with a cable tie. A solid state microextraction (SPME) fiber (divinylbenzene/carboxen/polydimethylsiloxane, 50/30um diameter) in a holder (custom made by S. Penglase, Department of Physics, NMU) was inserted into the bag and the carbon fiber was deployed. I sampled VOCs for 60 minutes after which I placed the fiber holders with the needles inserted into rubber-topped glass vials to prevent the possible contamination by other VOCs and transported them back to campus for compound identification. Prior to the start of sampling I conducted a trip blank, by placing the SPME fiber vial and transporting it for the same amount of time as it took to travel to and from the study site. I measured the VOCs accumulated during that time.

VOC Identification

I removed VOCs from the SPME fibers using a Shimadzu GC/MS 2010. The injection port temperature was set to 270° C and the column oven to 30°C. After inserting the fiber into the port, I immediately placed the capillary column (0.25mm) into liquid nitrogen to create a cold trap. I kept the column in the nitrogen for 1 minute then removed the column from the nitrogen and allowed the program to proceed. The temperature of the column oven was then raised to 280°C over 28 minutes, then held for 5 minutes.

To determine identity, I compared the retention time and mass spectra to reference standards (developed on site with common conifer VOCs using standards from Sigma Aldrich). I used automatic peak integration to determine peak area and manually verified the integration (Fig. 3). The compounds that were confidently identified are shown in table 1 with the average proportion of the total identified VOCs. I calculated the total area of the VOCs of interest and from that determined proportion of total

VOCs for each compound. Because of the importance in jack pines of the α -pinene: β -pinene ratio in response to insects and defoliation, this ratio was calculated (Wallin and Raffa 1999)

Statistical Analysis

I compared the α -pinene to β -pinene ratio between trees preferred for foraging and those not preferred. As the ratio data were non-normally distributed, I employed a two-tailed Mann-Whitney U test ($\alpha=0.05$; p-value calculated using the exact method). I compared VOC signal between trees with no foraging and trees with foraging using the non-parametric (VOC ratios were not normally distributed) Kruskal-Wallis test ($\alpha=0.05$). I used multiple Kendall's tau correlation analyses to examine whether any VOCs were correlated with sampling date. Date and temperature were used in subsequent principal component regression analyses.

Due to the explanatory variables (VOC proportions) being compositional, I was unable to simply model all VOCs against foraging using general linear model techniques as these data are considered multicollinear (Aitchison 1982). Hence, I performed a principal component logistic regression with the logit classification in the binomial family using XLSTAT-PLS (Escabias et al. 2005). As independent variables, I included all VOCs ratios, daily temperature at the start of the VOC sampling and Julian date without interactions to avoid overfitting. The binary response variable was the presence or absence of foraging. I used principal component analysis to transform the x variables, VOCs, temperature and Julian date, into principal components. I chose principal components with the highest correlation with the y value that explained a minimum of 70% of the variation. I then used the principal components as the independent variable in a logistic regression with the binary response of preferred for foraging or not preferred for foraging. A likelihood ratio test was used to calculate the significance of the model. After the logistic regression I used the eigenvalues of the original variables on the components to transfer

them back to the original space (XLSTAT-PLS built-in function). I then calculated a classification matrix to test the accuracy of the model.

Due to a large proportion of the foraging birds being Dark-eyed Juncos, I conducted a separate analysis just as described above, but using only the data from Dark-eyed Junco foraging, and then a third analysis that only used data from all bird species other than Dark-eyed Juncos.

Results

Survey Effort

I completed a total of 20 surveys across 20 days, accounting for 80 hours of observation. I conducted surveys between the hours of 0500 and 1100 and only surveyed if there was no rain, and wind less than 10 mph (to prevent damage to the sampling SPME fiber). VOC samples were not collected every day, as lack of foraging birds occurred frequently. I successfully measured VOCs from 17 trees that received foraging and 17 that did not. Total number of trees observed was 286, 260 of these received no foraging (Fig. 3).

Bird Species

Eight insectivorous and omnivorous bird species were observed during surveys. The most frequently observed species were Dark-eyed Juncos (*Junco hyemalis*). Juncos were often observed in foraging flocks, mean flock size was 2.5 +/- 1.05 SE. The another frequently observed species was Black-capped Chickadees, although only 2 individuals were observed foraging. Black-capped Chickadees were the most curious species and frequently came close to investigate the observer but did not forage in the

area. They were also seen in flocks, with a mean size 3 ± 1.4 . All other species, including the second most frequently observed species, Chipping Sparrow (*Spizella passerina*), were always seen feeding alone during the study. Most (87.5%) of social foraging flocks were composed of a single species. The time spent foraging by these birds ranged from 10 seconds to 90 seconds with a median time of 27.5 seconds and a mean of 32.8 seconds (Table 1).

VOCs Identified

I identified 7 VOCs that occurred in at least 33 of the 34 samples. The VOCs identified were α -pinene, β -pinene, β -myrcene, α -phellandrene, limonene, bornyl acetate and p-cymene. I found that the VOC with the greatest relative signal was most frequently either β -myrcene or α -pinene. Trees without foraging higher frequency of trees with β -myrcene as the highest relative signal while α -pinene signal was highest in trees with foraging (Fig. 4, Fig. 5). The trip blank showed no VOCs aside from those that were known artifacts of the machine column.

None of the VOCs were significantly different when compared individually between the foraged and non-foraged trees (Table 2, Fig. 6).

Trees that were foraged upon by birds showed significantly different α -pinene to β -pinene signal ratios than non-foraged trees (Fig. 7, $U=86$, $P<0.05$). Specifically, the ratio was significantly higher in the foraged trees than the non-foraged trees.

The date of observation was significantly associated with p-cymene ($P<0.01$, $\tau=0.3789$, $z=2.2921$), and bornyl acetate ($P<0.05$, $\tau=0.308$, $z=2.327$) signals (Fig. 8).

Principal Component Regression

The principal component analysis resulted in three components with eigenvalues >1.0. The three principle components explained a cumulative 67.35% of the variation, the fourth principal component was not much less than one and contributed to the variation explanation bringing the cumulative to 78.7% (Table 3).

I found a significant model for foraging activity (log-likelihood ratio $X^2 = 15.42$, $df=4$, $P=0.003$) when the presence/absence of foraging was regressed against the four principal components that were the most correlated with Y (y-aware selection), components 2,5,6 and 7 (Table 4).

Four of the seven VOCs weights were significant; α -pinene ($t=3.711$, $P=0.001$), β -pinene ($t=-2.575$, $P=0.015$), bornyl acetate ($t=2.570$, $P=0.016$), and p-cymene ($t=-2.094$, $P=0.045$) (Fig. 11).

Probability the tree received foraging = $-0.19 - 0.21 * \alpha\text{-phellandrene} + 0.85 * \alpha\text{-pinene} - 0.30 * \beta\text{-myrcene} - 2.02 * \beta\text{-pinene} + 11.15 * \text{bornyl acetate} - 0.02 * \text{limonene} - 5.15 * \text{p-cymene} - 1.08E-02 * \text{date} - 2.0 * \text{temperature}$. The model correctly predicted the presence/absence of foraging 82.35% of the time (Table 5).

The results from the Dark-eyed Junco showed one of the same positively significant drivers of foraging, α -pinene. Probability a tree received foraging by Dark-eyed Juncos = $4.02 - 0.22 * \alpha\text{-phellandrene} + 1.20 * \alpha\text{-pinene} - 0.92 * \beta\text{-myrcene} - 3.44 * \beta\text{-pinene} + 1.14 * \text{bornyl acetate} + 3.16 * \text{limonene} - 10.15 * \text{p-cymene} - 1.23E-02 * \text{date} - 4.31E-02 * \text{temperature}$. The other species principal component regression only contained one significant VOC, negatively weighted β -pinene. Probability a tree received foraging by other species = $1.15 - 0.52 * \alpha\text{-phellandrene} + 0.56 * \alpha\text{-pinene} + 4.37E-02 * \beta\text{-myrcene} - 3.46 * \beta\text{-pinene} + 9.71 * \text{bornyl acetate} + 0.67 * \text{limonene} - 5.07 * \text{p-cymene} - 1.22E-02 * \text{date} - 4.20E-02 * \text{temperature}$.

Discussion

VOCs Important to Foraging

I successfully developed a method to sample VOCs in the field and was able to obtain the VOC profiles of 34 jack pines (17 foraged/non-foraged pairs). The VOC profiles of these trees showed variability, including between trees that received foraging and those that did not. I found the proportional signal of certain VOCs predicted which trees birds chose to forage on. In total there were four VOCs that were significantly important to the model of tree preference. These VOCs were the compounds α -pinene, β -pinene, p-cymene and bornyl acetate. Alpha-pinene and bornyl acetate were weighted positively (indicating the relative signal was higher in preferred trees), β -pinene and p-cymene were significantly negatively weighted on the model. Prior studies of jack pine and other conifers indicates α -pinene increased in trees that have received defoliation by a phytophagous insect (Wallin and Raffa 1999; Lazebrnik 2012). Studies of jack pines infested by pine bark beetle (*Ips grandicollis*) generally show a decrease of α -pinene in the phloem after infestation, but defoliation by pine sawfly in results in an increase of α -pinene. β -pinene was found by Wallin and Raffa (1999) to be negatively correlated with defoliation levels one year of the study, consistent with the lower β -pinene in the preferred trees. The second year of the study showed the opposite pattern for β -pinene, and Lazebrink (2012) found no significant difference. Bornyl acetate was found to be higher in defoliated trees, and p-cymene was not found to be significantly different by Lazebrink (2012). Jack pines demonstrate chemotypes (i.e. VOC profiles) that vary according to climatic variables, management type, and possibly by genetics (Taft et al. 2015). Some chemotypes naturally contain higher percentages of α -pinene, β -

pinene or limonene. Because of the different chemotypes, trees could appear to react differently depending on what their baseline chemotype is. Even if the trees do respond differently, birds appear to learn to respond to VOCs as a foraging cue, suggesting specific VOCs used by foraging birds could vary between locations and habitats (Amo et al. 2016).

Responses to defoliation shows significant variation among studies indicating a complex phenomenon in need of more study. Previous studies of jack pines have indicated very different responses to stress than other species, even the closely related lodgepole pine (*Pinus contorta*). A prior study by Lazebrink et al. (2004), found the most common compound discovered in jack pines to be α -pinene, although β -myrcene concentration did increase above α -pinene when trees experienced drought stress. Alpha-pinene relative signal was the highest proportion of the VOC profiles in my study, with occasional larger amounts of α -phellandrene and β -myrcene (Figures 11 and 12). There are many possible reasons for these differences. Different chemotypes previously discussed is one possibility, another is signaling between trees. Tree communication is a yet poorly understood phenomenon, current evidence points to trees communicating regarding available water, insect damage, mechanical wounding and other sources of stress (Martin et al. 2003; Kivimäenpää et al. 2012; Taft et al. 2015; Lusebrink et al. 2016). The consistencies found between the VOC signal of trees that received foraging and trees that were damaged by insects indicated the possibility that birds are indeed choosing trees with higher insect abundance.

In modeling the VOCs of trees preferred by Dark-eyed Juncos, I found α -pinene was a significant predictor of Junco foraging. The model of all other species indicated avoidance in response to β -pinene and limonene. This indicates the Dark-eyed Juncos are likely the species that determined the α -pinene driver in the original logistic model. Future research should consider how different species use (or do not use) VOC signals.

Pinene Ratio

Birds foraged preferentially on some trees over others, and those jack pine trees that were preferred for foraging showed a significantly different ratio of α -pinene to β -pinene than those not foraged upon. Prior research on jack pine responses to jack pine budworm (*Choristoneura pinus*) found a high amount of variability in VOCs concentrated in the vascular tissue of trees that were responding to different levels of herbivore damage (Wallin and Raffa 1999). They found α -pinene to β -pinene ratio changed the most significantly in response to herbivore damage. This change decreased when light defoliation occurred and while it increased in the moderately and severely defoliated, the non-defoliated always had the highest ratio. While other cues may be still important, my study indicates that a difference in the VOC signal of trees used for foraging and those not, does exist. Future analysis should include a measurement of phytophagous insects, aviaries to attempt to minimize the interference caused by factors such as communication between trees, social interactions among birds, and tree physical features. Although these studies need to be done, I found that there are indeed differences in the VOC signal released by jack pines preferred for foraging by birds and trees they did not prefer. These differences were consistent with responses to herbivory, reinforcing the possibility that birds use trees with certain VOCs as more efficient foraging locations.

Figures

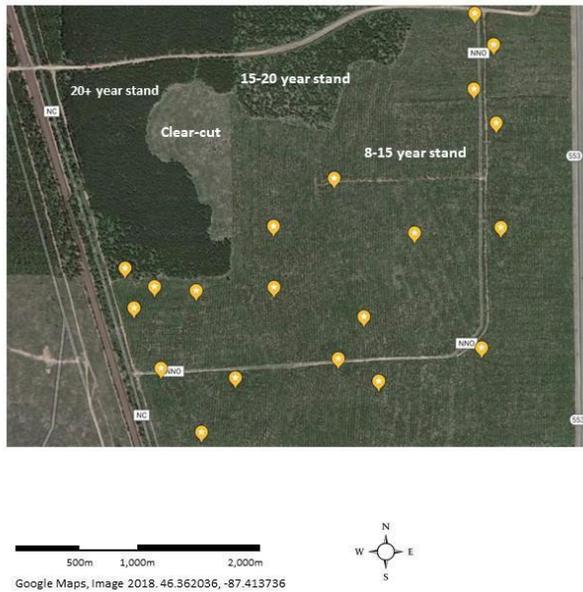


Fig 1 Map of project site with survey points, each survey point is located at least 100 meters from one another and points were randomly selected prior to survey start date



Fig 2 Stable headspace for VOC sampling using an oven bag (Reynolds, slow cooker liners 13inx21in), two embroidery hoops (one 8 inches in diameter and a second 6 inches) for stability, and zip-ties to fasten gently to the tree. Fiber holder is held by

clamps and the SPME fiber is inserted into the bag

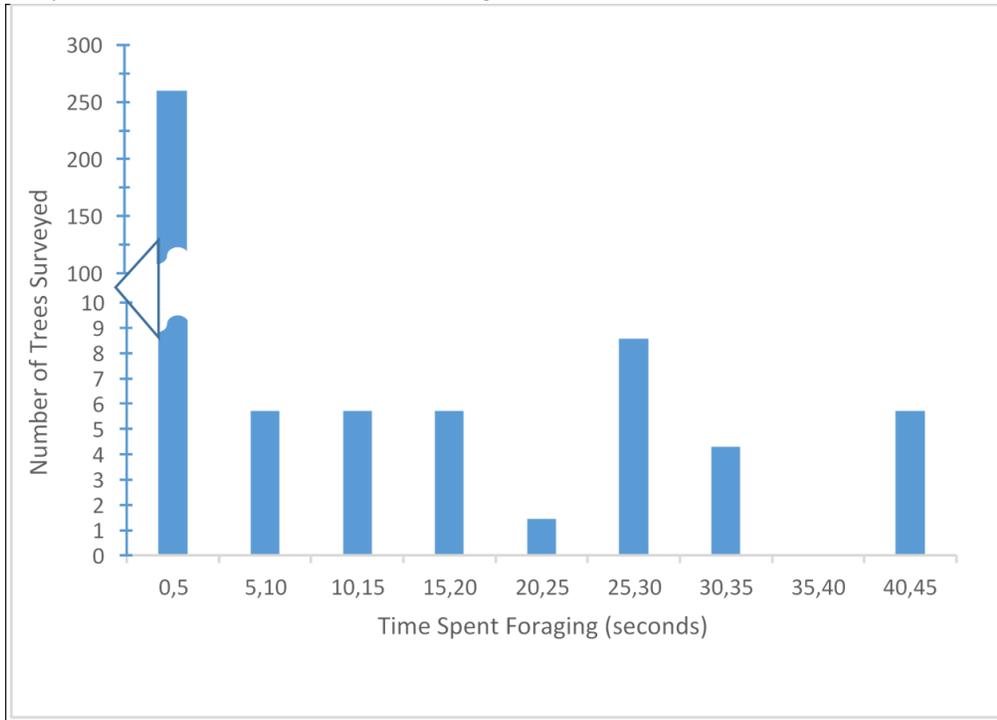


Fig 3 Of the 286 trees observed, 260 (y-axis) received no foraging time. The number of trees that did receive foraging time are split into five-second foraging time bins. VOC sampling was attempted on all 26 preferred trees and was successful on 17 as well as 17 non-preferred trees

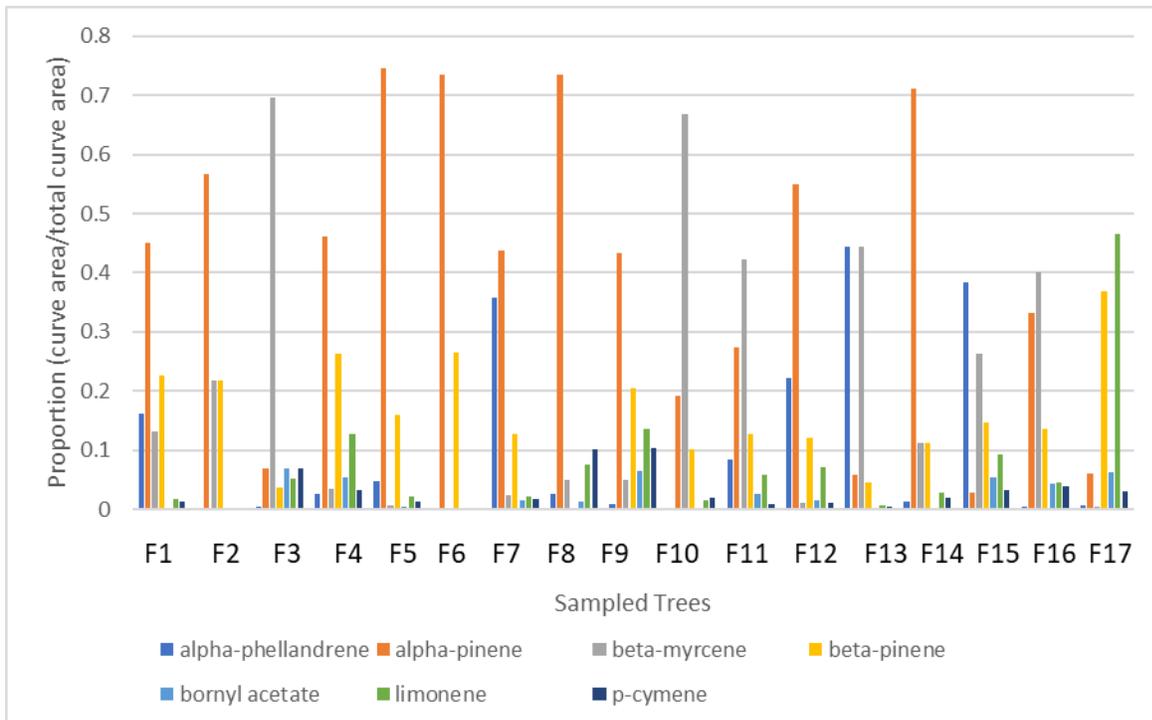


Fig 4 The VOC profile of samples from all trees that received foraging. A-pinene had the dominant VOC relative signal in most samples

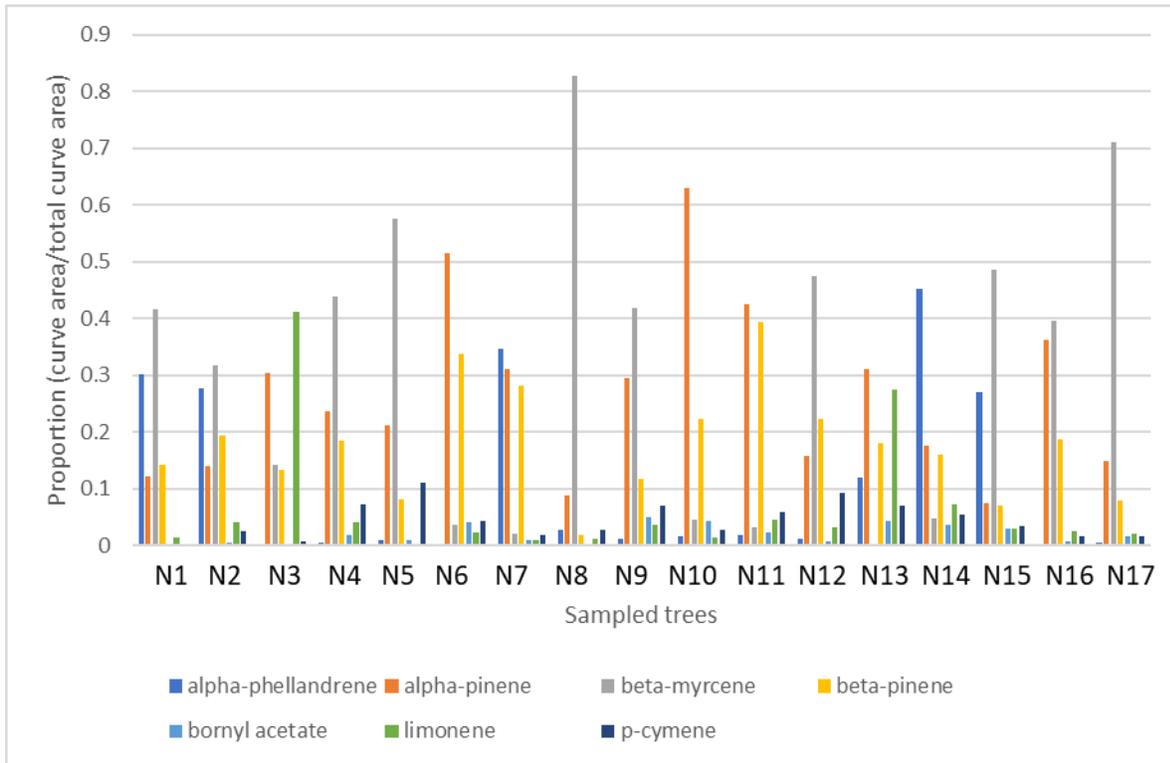


Fig 5 The VOC profile of samples from all trees that did not receive foraging. Unlike the preferred foraging trees, α -pinene is not the dominant VOC. Beta-myrcene is more frequently the highest VOC in the sample

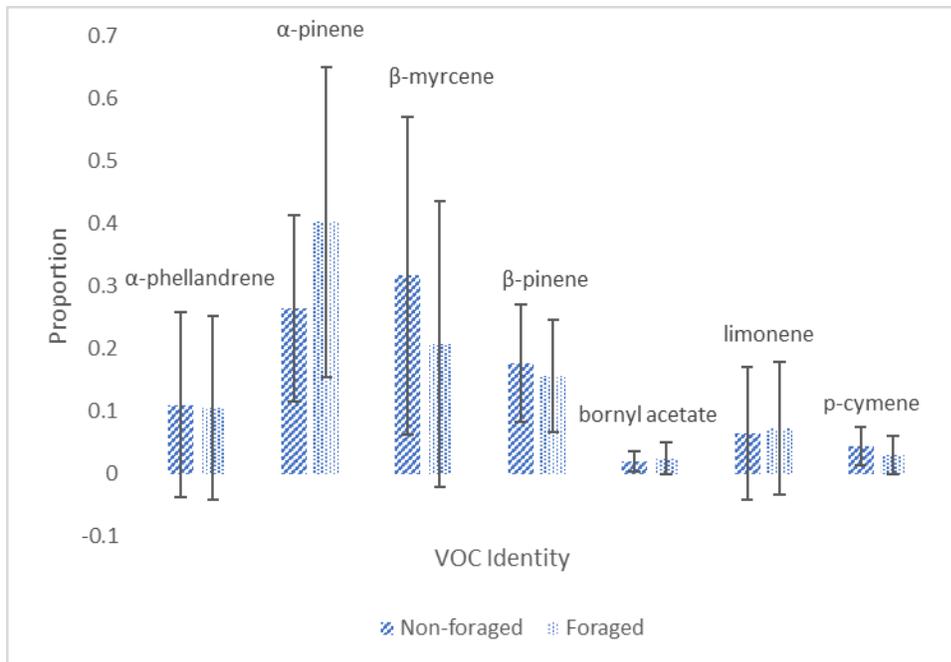


Fig 6 Mean (+se) proportions of each volatile organic compound (VOC) (proportions calculated as a percentage of the total amount of VOCs collected from each trees) from trees that received foraging (dots) and no foraging (lines) from birds. None of the mean proportions were significantly different between the preferred and non-preferred trees

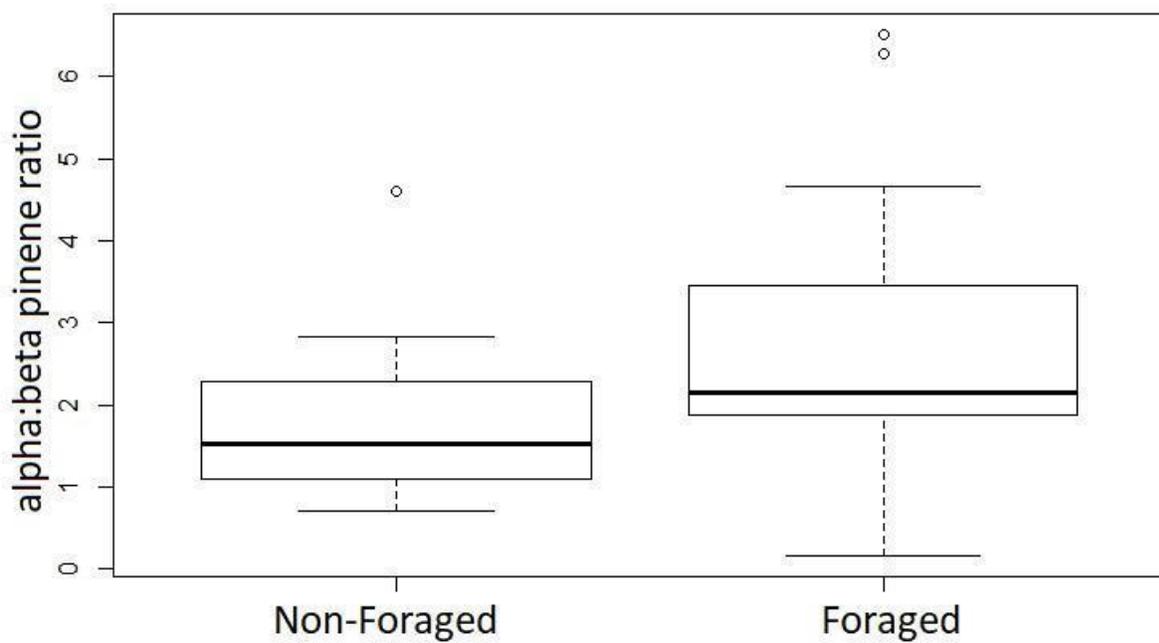


Fig 7 Box-plot of α -pinene to β -pinene ratio between trees with no foraging (0) and trees with foraging (1). Ratios measured on non-foraged and foraged trees were significantly different ($U=86, p<0.05$). As seen in the box-plot, the ratio is higher in the trees birds preferred for foraging

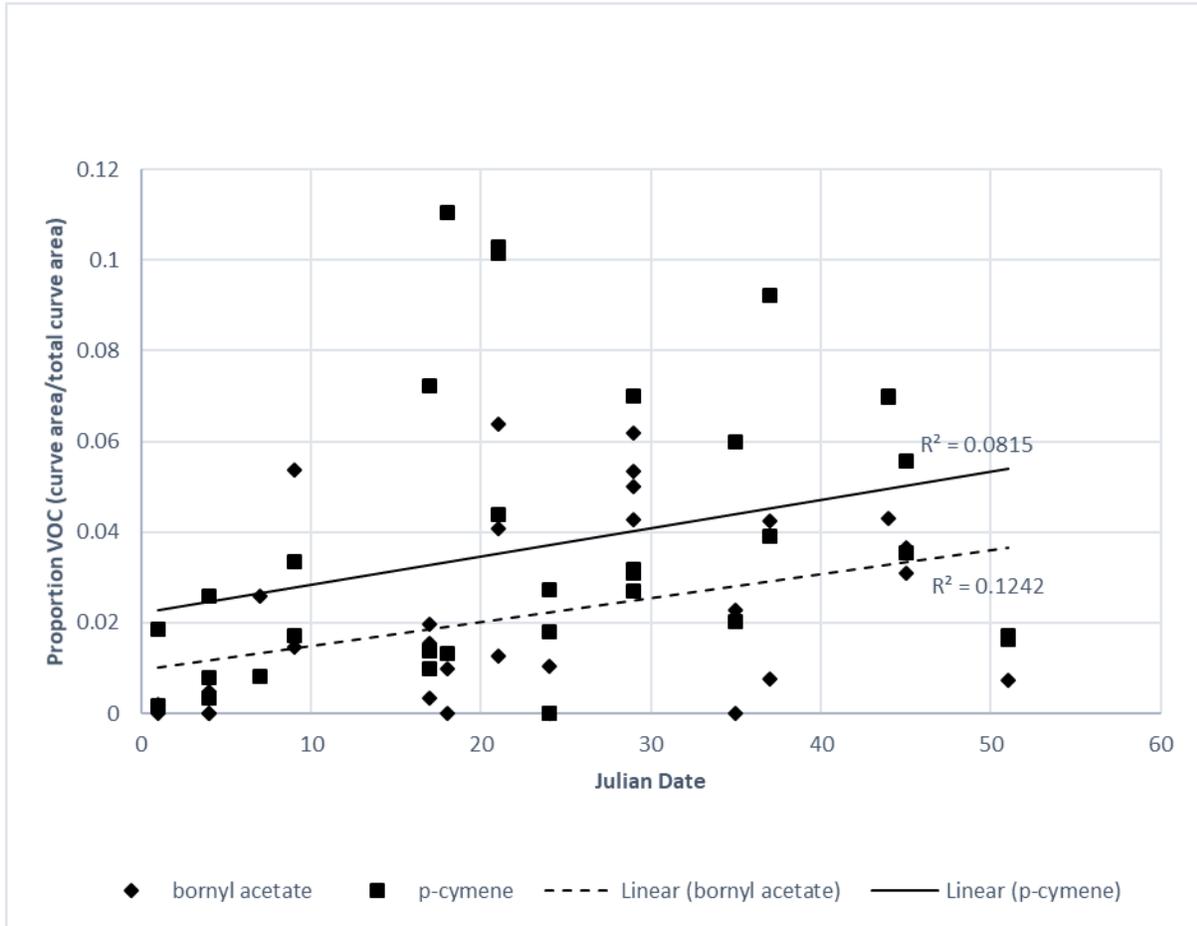


Fig 8 Proportion of VOCs plotted in increasing chronological order, from the initiation of observations on the study site (Day 0 = 5 June). Of all compounds measured, the proportions of p-cymene (orange squares) and bornyl acetate (blue diamonds) were the only two VOCs with significant correlations with date

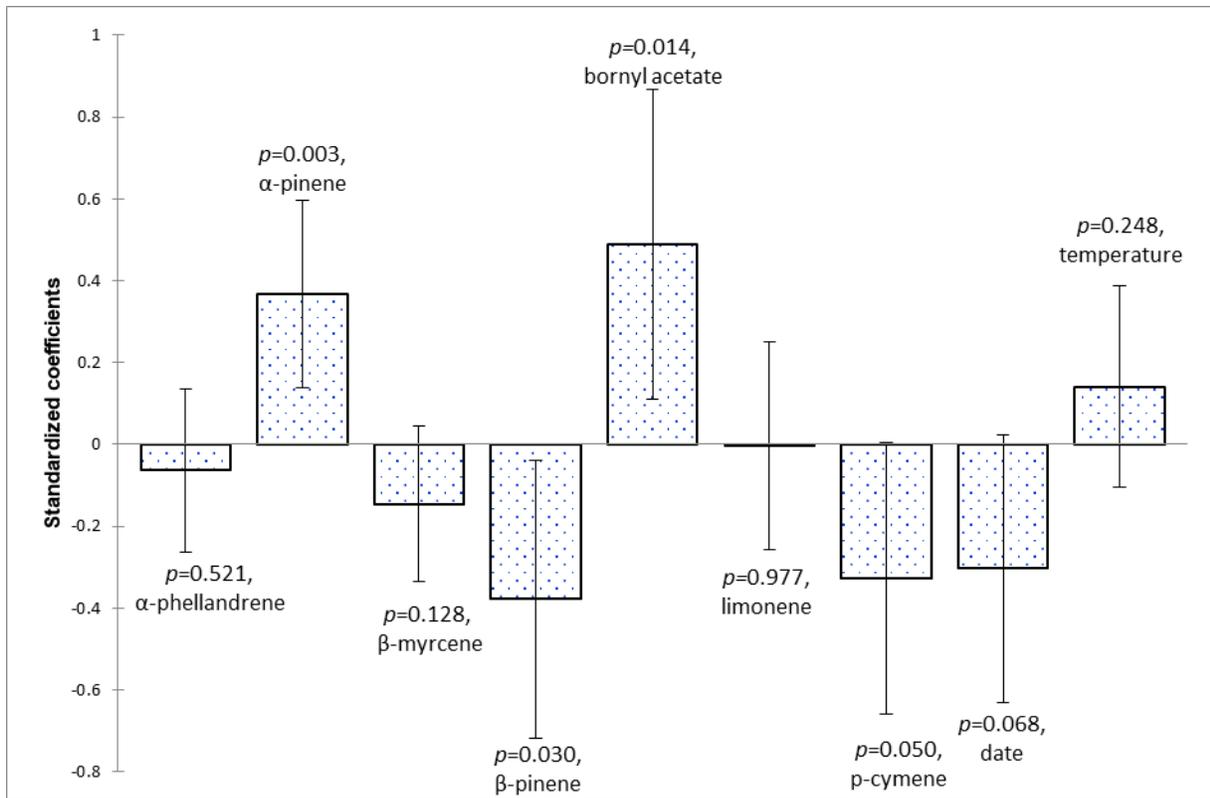


Fig 9 Standardized VOC weights (+/- SE) on the model predicting probability of avian foraging. Positive standardized coefficients (weights) indicate the VOC positively influenced avian selection whereas negative values indicate avoidance

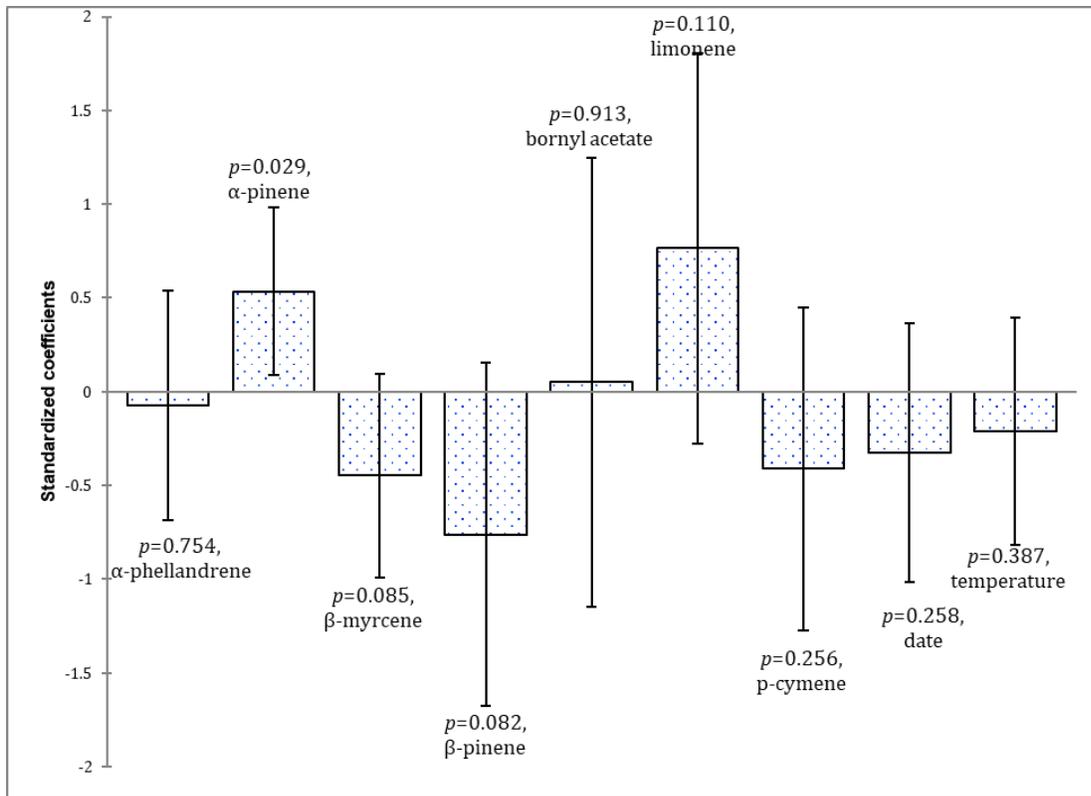


Fig 10 Standardized VOC weights (+/- SE) on the model predicting probability of Dark-Eyed Junco foraging. Positive standardized coefficients (weights) indicate the VOC positively influenced avian selection whereas negative values indicate avoidance

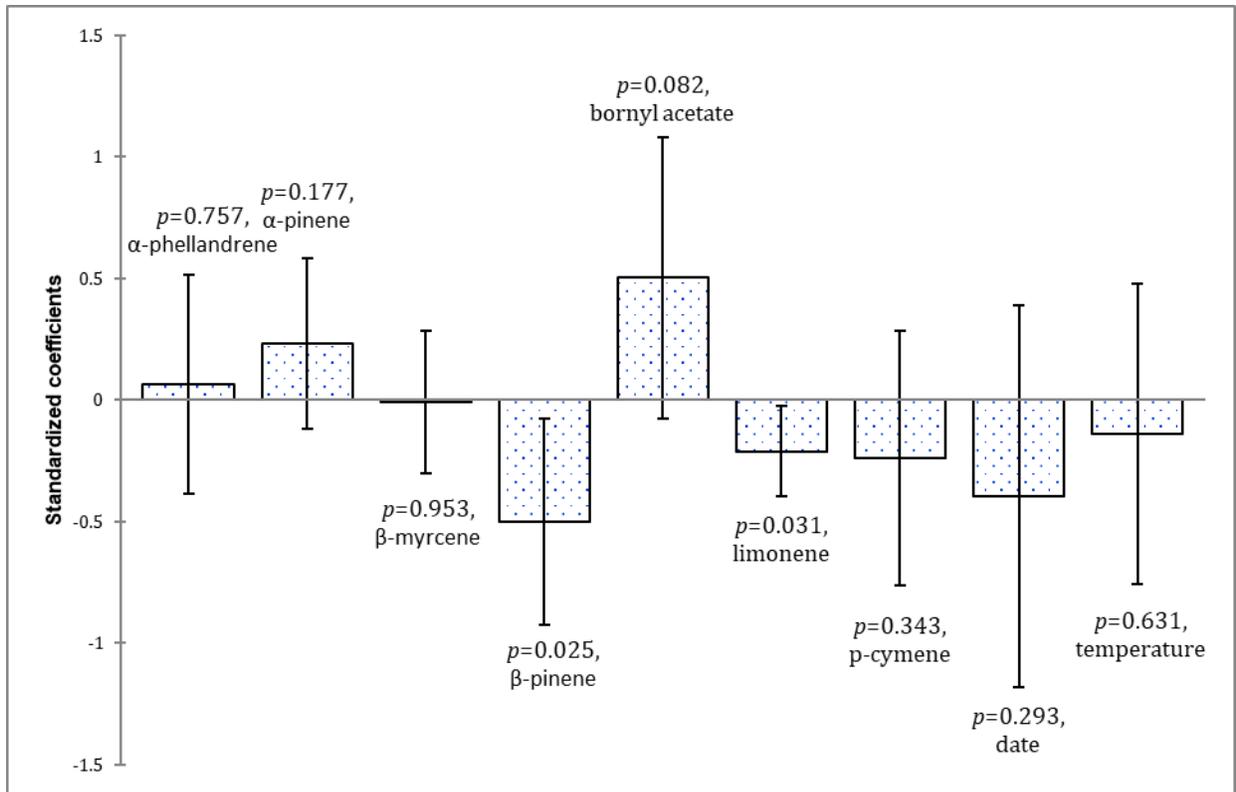


Fig 11 Standardized VOC weights (+/- SE) on the model predicting probability of species other than Dark-Eyed Junco foraging. Significant positive standardized coefficients (weights) indicate the VOC positively influenced avian selection whereas negative values indicate avoidance

Tables

Table 1 Species of insectivorous or omnivorous birds observed during survey times and the number of individuals observed foraging.

| Species | # Individuals |
|-------------------------|---------------|
| Dark-eyed Junco | 9 |
| Clay-colored Sparrow | 1 |
| Chipping Sparrow | 4 |
| Black-capped Chickadee | 2 |
| White-breasted Nuthatch | 1 |
| Nashville Warbler | 1 |
| White-throated Sparrow | 2 |
| Yellow-rumped Warbler | 3 |

Table 2 VOCs identified in this study and average proportions found in the trees that received avian foraging preference and those that did not. Standard error shown in parentheses

| VOC | Average Proportion Foraged Trees | Average Proportion Non-foraged Trees |
|------------------------|----------------------------------|--------------------------------------|
| α -pinene | 0.403 (0.154) | 0.266 (0.255) |
| β -pinene | 0.157 (0.097) | 0.177 (0.093) |
| β -myrcene | 0.208 (0.262) | 0.317 (0.235) |
| Limonene | 0.073 (0.109) | 0.065 (0.110) |
| Bornyl acetate | 0.025 (0.017) | 0.020 (0.027) |
| α -phellandrene | 0.106 (0.153) | 0.111 (0.152) |
| p-cymene | 0.030 (0.031) | 0.044 (0.032) |

Table 3 Eigenvalues of principal components. Variability % is the amount of the total variation described by the principal component

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 |
|-----------------|--------|--------|--------|--------|--------|--------|---------|
| Eigenvalue | 2.135 | 1.928 | 1.325 | 0.910 | 0.736 | 0.566 | 0.400 |
| Variability (%) | 26.686 | 24.104 | 16.558 | 11.380 | 9.201 | 7.077 | 4.995 |
| Cumulative % | 26.686 | 50.790 | 67.348 | 78.727 | 87.928 | 95.005 | 100.000 |

Table 4 Correlation between the components and foraging

| Variables | forage |
|-----------|--------|
| F1 | -0.124 |
| F2 | -0.242 |
| F3 | -0.004 |
| F4 | -0.094 |
| F5 | 0.201 |
| F6 | -0.218 |
| F7 | 0.464 |

Table 5 Classification table of the foraging model. 76.47% of the time the model correctly predicts non-foraging, and 88.24% of the time it correctly predicts foraging, for a total of 82.35 % correct classification

| From \ To | Non-foraged | Foraged | Total | % correct |
|-------------|-------------|---------|-------|-----------|
| Non-foraged | 13 | 4 | 17 | 76.47% |
| Foraged | 2 | 15 | 17 | 88.24% |
| Total | 15 | 19 | 34 | 82.35% |

Chapter Three

Introduction

As discussed in chapter one, the role of avian olfaction has been largely under-appreciated in birds, but in fact, responses to chemical cues are important for different species of birds for different reasons. Although many species of birds have a small number of functioning olfactory receptor genes, those species which have been studied more extensively show that their sense of smell can be highly developed, but only sensitive to a narrow range of niche-specific odors or chemicals (Steiger et al. 2008). In navigation, use of olfaction was first discovered in homing pigeons in 1971, but other species were largely ignored until 2009 when Holland et al (2009) found similar use of the olfactory system in migrating grey catbirds (*Dumetella carolinensis*) (Papi et al. 1973; Papi 1982; Holland et al. 2009; Gagliardo et al. 2011). Use of olfaction in kin-selection and individual recognition has been identified in several species, perhaps most notably in blue tits, as there is limited research on small passerines using olfaction for any function (Hagelin and Jones 2007; Rossi et al. 2017). The use of olfactory foraging is documented in several seabirds, and the limited existing research in small passerines is discussed in the previous two chapters (Hutchison and Wenzel 1980). My study focused on the olfactory cues used by insectivorous and omnivorous passerines when making foraging decisions in jack pines (*Pinus banksiana*). In the second chapter, I found a significant difference in the VOC profiles of trees that received foraging when compared to the VOC profiles of trees that did not receive foraging. Foraging birds chose trees with higher α -pinene/ β -pinene ratio, however I did not examine whether there were visual differences that were influencing the bird's decision or if it was the difference in VOCs driving the foraging decision. One way to determine if birds are responding to the VOCs is to take an experimental approach where VOCs are released near trees in the wild to see if there are differences in foraging behavior.

One prior study has attempted to attract birds by adding VOCs to trees, but with no significant results. That study (Koski et al. 2015) added combinations of VOCs to both live and dead mountain birch (*Betula pubescens*) adorned with plastic larvae mimics, and measured the accumulation of peck marks on the plastic mimics. They found no significant difference in the number of peck marks in the plastic larvae between trees with different treatments. The VOCs they used were a blend of linalool, α -ocimene and limonene which had been found to increase in insect damaged mountain birch (*Betula pubescens*) (Koski et al. 2015). Here I describe an experimental study aimed to determine if birds were responding to VOCs alone by manipulating the VOCs present on some trees and not others. To test the hypothesis that birds use VOC chemical cues to direct their foraging efforts, I treated some trees such that they had elevated levels of VOCs and compared the avian foraging response on those trees treated with ethanol. This study was different from Koski in the VOCs used, the survey technique, and tree type. The specific VOCs used by birds is still unknown, so using different VOCs than the previous study, will attempt to determine what compound birds may be using.

Methods

To experimentally create conditions where trees would present elevated levels of specific ambient VOCs, I attached vials of VOCs (α -pinene, β -pinene and limonene, Sigma Aldrich:cat# 147524,183164,112089, respectively) to three “treatment” trees and similar vials with ethanol to “ethanol” trees. I then compared the avian foraging response on VOC-treated trees to ethanol-treated trees. Surveys were performed on single days, where a VOC was presented on 3 treatment trees, and ethanol on 3 trees, then avian foraging effort was observed on all trees. I used 98% α -pinene, 99% β -pinene and 97% limonene, as three separate treatments on individual trees presented on rotating days. VOCs were aliquoted into uncapped 1ml tubes with wire hooks attached at the top. Each vial received 20ul of one VOC or 90% ethanol. All treatment and control trees were located in the same general observational area, but at least 5 meters from the center of the area and 5 meters from one another.

Using only one VOC per day, plus 90% ethanol, each tree received a total of 3 vials, once at the top branch of a tree, once at a center branch, and once on a lowest branch of the tree (Koski et al. 2015). I conducted surveys for 4 hours between 0530-1100. During the survey, I remained at the center of the point and recorded all visits, species, and activity types occurring on treated trees. Foraging activity was defined in the same way as in chapter 2.

Statistical Analysis

VOC treatments were combined, and the foraging time compared between the VOC treated trees and the 90% ethanol trees. The data were non-normal, so I chose the non-parametric Mann Whitney U test. The VOC treatments were one of the independent variables and 90% ethanol the other. The response variables was whether a tree received foraging or not.

Results

Seven survey days were completed, with a total of 28 survey hours (Table 1). VOCs α -pinene, and β -pinene were used three survey days each for four hours. Species identified foraging on trees during treatment periods were yellow-rumped warblers (*Setophaga coronata*), black-capped chickadees (*Poecile atricapillus*), chipping sparrows (*Spizella passerina*) and dark-eyed juncos (*Junco hyemalis*). Black-capped chickadees were identified the most frequently with four visits, all other species appeared twice. Each treatment received foraging from an insectivorous bird except for limonene, but this VOC received fewer surveys than the other VOCs. The Mann Whitney U test of the number of visits between groups treated with ethanol and treated with VOCs was not significant ($U=239.7$, $p=0.44$, $N=21$).

Discussion

I found birds foraged on trees treated with VOCs and ethanol, although visits did not significantly differ between the treatments. Ethanol was used as an alternative compound that was not documented to change in damaged jack pine trees. Black-capped Chickadees were the most frequent visitors, but this may be due to their curiosity regarding the shiny metal hooks. Twice a foraging Black-capped Chickadee got distracted by the hook and began pecking it. The other species were observed twice each. I did not have a large enough sample size to compare the visits between specific VOCs, but I was able to compare the number of visits birds made to trees treated with 90% ethanol to trees treated with a VOC. Sample size was limited by time as the breeding season is relatively short in the northern jack pine forests and the VOC sampling discussed in chapter 2 was also underway.

As demonstrated in chapter 2, the VOCs released by trees varied in their composition. Therefore, the background VOCs released from the trees were likely all different. This background VOC release of the trees used was unknown, the trees were in the previous chapters surveys and were individuals that were not preferred for foraging or sampled for VOCs. By adding vials of VOCs to these trees, I was “boosting” the trees’ normal VOC emission. In adding each VOC, I was increasing the proportion of that VOC to the other compounds already being released. While changing the composition to contain higher proportions of each of the VOC, should act as a similar attractant to the change from insect damage, the study needs a control to account for background variability. Also, the study should be repeated using dead or fake trees to control for the background VOC emission, or a baseline VOC sample taken prior to experimental addition.

Aside from α -pinene, the VOCs I used were not found to be significant drivers of foraging preference and β -pinene was found to be avoided. Therefore, future studies should focus on adding α -pinene and α -

phellandrene (but not limonene) to trees to determine if the birds are indeed sensing these VOCs and orienting their foraging attention to trees treated with these VOCs.

Two methods utilizing an RFID banded population could be used to determine if birds are responding to specific VOCs. One method could use multiple sets of three fake trees set up with RFID readers and treated with VOCs, one with α -phellandrene, one with α -pinene and one without VOCs as a control. These could be left to read for an extended period (3-7 days) to record hits by banded birds. The number of hits can be compared between the independent treatments (α -pinene, α -phellandrene and no VOC).

A second method to assess the possibility of VOC cue learning in birds could be conducted as well. Over a span of several weeks, the study could start with a RFID enabled feeder filled with seeds and treated with VOCs. After allowing the birds to forage for 3-7 days, the feeder can be switched from seeds to meal-worms and the feeder re-treated with VOCs. Meal-worms are a more desirable food source. After the birds have had the chance to potentially learn the association between VOCs and good food (3-7 days), the feeder should be switched back to seeds and retreated. After the same amount of time, the number of hits could be compared between the seed feeders before meal-worms and after meal-worms.

Figures

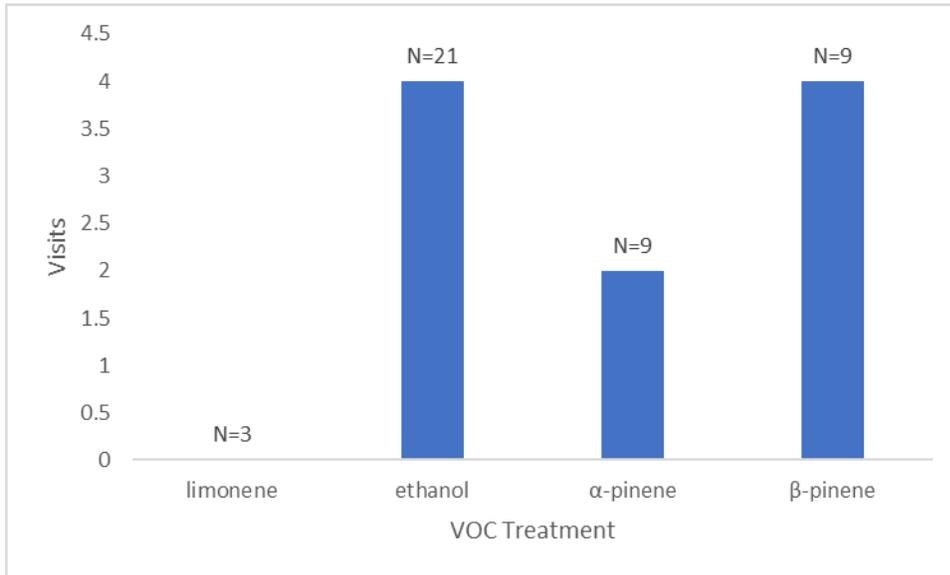


Fig 1 Number of visits to each of the treatment types, N= number of trees treated with the VOC over the course of the study

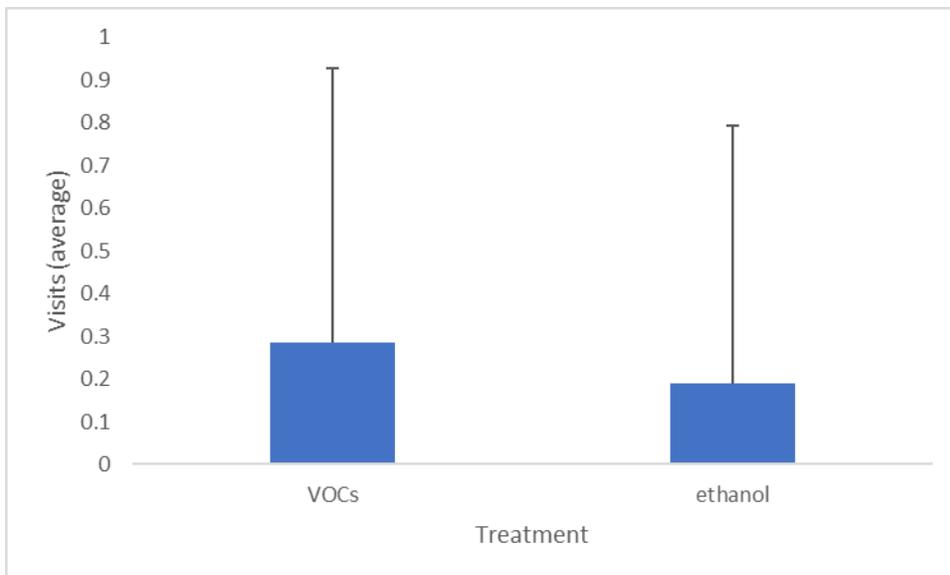


Fig 2 Average number of visits to trees treated with ethanol and trees treated with VOCs

Tables

Table 1 Completed survey time

| Survey Days | Survey Hours |
|-------------|--------------|
| 7 | 28 |

Table 2 Total number of trees treated with ethanol and VOCs

| Treatment | Total Number of Trees |
|----------------------|-----------------------|
| 90% ethanol | 21 |
| 97% limonene | 3 |
| 98% α -pinene | 9 |
| 99% β -pinene | 9 |

Table 3 Species identified and number of times during surveys

| Species | Number of Visits |
|---------|------------------|
|---------|------------------|

| | |
|--|---|
| Yellow-rumped Warbler (<i>Setophaga coronata</i>) | 2 |
| Chipping Sparrow (<i>Spizella passerina</i>) | 2 |
| Black-capped Chickadee (<i>Poecile atricapillus</i>) | 4 |
| Dark-eyed Junco (<i>Junco hyemalis</i>) | 2 |

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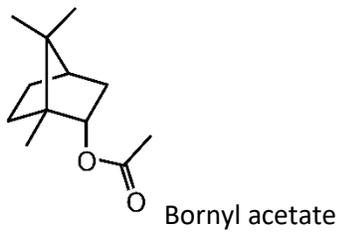
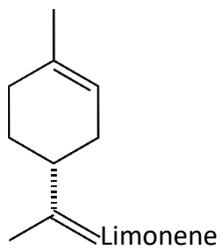
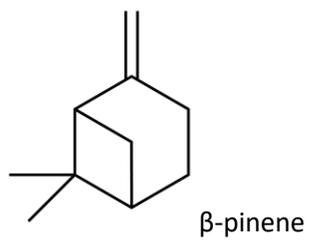
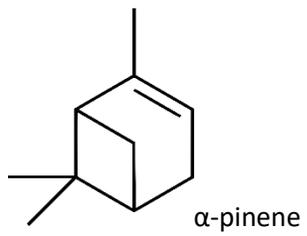
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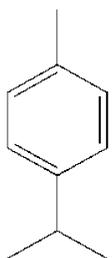
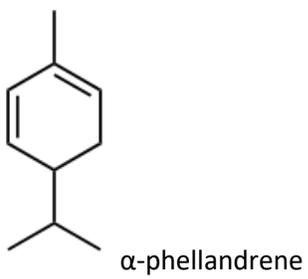
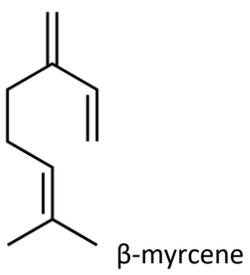
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Appendix I

Basic structure of VOCs identified in this study.





P-cymene