Do Terpenes Play a Role in the Stress-reducing Effects of a Forest Bathing Intervention?

NCT05316597

May 7, 2024

**1. Objectives**

*Aim 1.* Assess whether VOC inhalation regulates stress reduction and affective outcomes of the terpenes-on vs. terpenes-off sessions.

Primary physiological outcome: increases in the HF ($ms^2$) component of HRV.

Secondary outcomes: decreases in blood pressure, heart rate, self-reported stress, negative affect, and levels of inflammatory cytokines (IL-6, TNF-alpha, CRP) and cortisol in serum, and increases in positive affect, measured via mobile physiology equipment and blood using standard clinical methods.

Hypothesis: VOC inhalation will regulate increases in the HF ($ms^2$) component of HRV and decreases in blood pressure, heart rate, self-reported stress, and levels of inflammatory cytokines in serum as secondary outcomes.

*Aim 1a.* Assess the degree of association of absorbed dose of seven forest-derived VOCs in serum (i.e., α-pinene, β-pinene, β-myrcene, Δ 3- carene, limonene, β- carophyllene, α-humulene) with these outcomes.

Hypothesis: Absorbed dose of seven forest-derived VOCs in serum will be associated with stress reduction and affective outcomes.

**2. Design**

Potential participants were screened via a phone call and excluded if they were pregnant, smoking, and/or had a current or prior diagnosis of neurologic, hypertensive, psychiatric, respiratory disorder, or anosmia/hyposmia. Participants were also excluded if they were prescribed a short list of prescription medications known to influence terpene metabolic pathways and short-term inflammatory biomarkers including beta-blockers, antibiotics, statins, hypertension medications, steroid medications, and diabetes control medications. Eligible participants were asked to avoid certain foods, beverages, cleaning products, and supplements that contain terpene compounds in the 24 hours leading up to their forest sitting experience.

Additionally, the clinically validated University of Pennsylvania Smell Identification Test (UPSIT) (Sensonics International, Haddon Heights, NJ) was administered to determine whether participants had anosmia/hyposmia. The UPSIT is a 40-item, self-administered “scratch-and-sniff” test that uses microencapsulated odorants that are released by scratching designated spaces on a paper test booklet. Summed scores were used to evaluate olfactory function and identify/exclude participants with undiagnosed smell loss or total anosmia (summed UPSIT score ≤18).

Following enrollment, participants were randomly assigned to a sequence of two sessions using a computer-randomized order created by an investigator who was not a member of the field team. To control participants inhalation exposures to terpenes in the forest air, participants wore a powered air-purifying respirators (PAPRs; 3M™ Versaflo™ Powered Air Purifying Respirator, TR-800-PSK/94248; 3M, St. Paul, MN). In one session, the PAPR mask was fitted with a particle-only filter (3M™ Versaflo™ High-Efficiency Filter, TR-6710N-5) to allow for the inhalation of forest terpenes (“terpenes-on”). In the other session, the mask was fitted with a charcoal filter (3M™ Versaflo™ Organic Vapor/HEPA Cartridge, TR-6510N) that removed particles, terpenes, and other BVOCs from the breathing zone (“terpenes-off”). Equipment labels were covered and filters were labeled “A” (later revealed to be “terpenes-on”) or “B” (later revealed to be “terpenes-off”) to blind the study field team and participants to treatment assignment. A washout period of at least eight days between sessions was included to reduce carryover effects (Sibbald & Roberts, 1998).

Forest-sitting sessions took place at the UW Charles L. Pack Experimental Forest located near Eatonville, Washington (“Pack Forest”) during June to September 2022 and 2023 to avoid rain, snow, and cold weather. The sessions were 60 minutes each and took place between 10:00 AM and 4:00 PM in a stand of old- and second-growth Douglas Fir, Western Red Cedar, and other conifers accessible by a 10-minute drive on an unpaved single-lane road from the Pack Forest Conference Center. Participants were seated in a comfortable chair, but the session was otherwise unscripted.

## 3. Methods

We enrolled 43 adult participants between July 12, 2022, and September 21, 2023. Our target sample size of 40 participants was based on the estimated numbers needed to detect minimum differences in the study outcomes in the contrasting conditions for the same participant, based on the minimums in the ranges of Hedges' g values from prior studies and standard population-level intra-individual differences. Participants were recruited from the Tacoma and Seattle, WA area using physical flyers placed in community centers, libraries, and local universities, and electronic listings on Craigslist and the University of Washington (UW) Institute of Translational Health Sciences study recruitment site. Potential participants were screened via phone call and excluded if they were pregnant, smoking, not fluent in English, and/or had a current or prior diagnosis of neurologic, hypertensive, psychiatric, or respiratory disorder, or anosmia/hyposmia. Participants were also excluded if they were prescribed a short list of prescription medications known to influence terpene metabolic pathways and/or short-term inflammatory biomarkers including beta-blockers, antibiotics, statins, hypertension medications, steroid medications, and diabetes control medications or if they were unable to walk for 15-20 min on unsteady ground. Eligible participants were asked to avoid certain consumer products, foods, beverages, cleaning products, alcohol, marijuana, e-cigarettes, and supplements that contain terpenes in the 24 hours leading up to their forest-sitting experience.

Participants' usual nature contact was assessed using measures from Bratman et al. (2024) and Bratman et al. (2021), based on operationalizations from other nature exposure papers (Shanahan et al., 2016; White et al., 2019). Average nature contact frequency was assessed by presenting asking participants with the following question: "About how often do you usually visit or pass through outdoor natural areas for any reason? This includes, for example, walking, biking, or recreating outside in local, regional, or national parks, at the beach, beside or within lakes, creeks, or the ocean, gardening or tending to plants, camping, fishing, reading or walking outside next to trees, engaging in yard work with natural elements, etc…" Participants selected an answer from following options: "Never", "Once a year", "Once every three months", "Once a month", "2-3 times a month", "Once a week", "2-3 days a week", "4-5 days a week", or "6-7 days a week" (Bratman et al., 2024). Average nature contact duration was measured by presenting participants with the following question: "Over the last month, approximately how many HOURS PER WEEK do you consider yourself to have interacted with nature? This includes, for example, walking, biking, or recreating outside in local, regional, or national parks, at the beach, beside or within lakes, creeks, or the ocean, gardening or tending to plants, camping, fishing, reading or walking outside next to trees, engaging in yard work with natural elements, etc..." (Bratman et al., 2021). Participants entered their response in a provided text box.

Nature relatedness was measured during baseline measurements using the short-form nature relatedness scale (NR-6; Nisbet & Zelenski, 2013). The NR-6 consists of 6 items designed to capture how people view their relationship with nature (e.g., "My relationship to nature is an important part of who I am"). Each item is rated on a 5-point scale, ranging from 1 ("Disagree strongly") to 5 ("Agree strongly") with a total score calculated by averaging all six items. Cronbach's α was used to measure how closely correlated related scale items were as a group. In the current study, NR-6 showed good reliability (Cronbach's α = .76).

HF HRV was measured in milliseconds squared ($ms^2$) using a portable, continuous electrocardiogram sensor (EcgMove4; Movisens®, Karlsruhe, Germany) worn directly on the chest with adhesive electrodes. We applied a natural logarithmic transformation to 5-minute averages of HF HRV ('ln-HF HRV') following Shaffer & Ginsberg (2017). Additionally, HF HRV values were winsorized.

Systolic and diastolic blood pressure were measured with an automated cuff monitor (GE Healthcare CARESCAPE V100 Vital Signs Monitor; GE Healthcare, Chicago, IL) in millimeters of mercury (mmHg). HR was also measured with the automated cuff monitor in beats per minute (BPM).

SCLs were measured in microsiemens (µS) using a portable, continuous electrodermal sensor (EdaMove4; Movisens®, Karlsruhe, Germany) worn on a wristband and attached to the palm with adhesive electrodes.

General levels of positive and negative affect were assessed using the 20-item Positive and Negative Affect Schedule (PANAS; Watson et al., 1988) during baseline measurements and state levels were assessed during the forest-sitting sessions using the 10-item, shortened PANAS (I-PANAS-SF; Thompson, 2007). The PANAS is designed to assess affect over the past month and includes 10 items on positive affect (e.g., "Interested," "Active") and 10 on negative affect ("Distressed," "Irritable"). Each item is rated on a 5-point scale, ranging from 1 (“very slightly or not at all”) to 5 (“extremely”) with sum positive and negative affect scores calculated. In the current study, the PANAS showed good reliability for positive affect (α = .88) and negative affect (α = .85). On the I-PANAS-SF, 5 items assess positive affect (e.g., “Inspired”, “Alert”), and five assess negative affect (“Upset”, “Hostile”) at the present moment. Each item is rated on a five-point scale, ranging from 1 (“very slightly or not at all”) to 5 (“extremely”) from which sum positive and negative affect scores were calculated. In the current study, I-PANAS-SF showed good reliability for positive affect (Cronbach’s α = .86) and lower reliability for negative affect (Cronbach’s α = .40).

Baseline perceived stress was assessed using the four-item version of the Perceived Stress Scale (PSS-4) designed to assess perceptions of stress over the last month (e.g., “In the last month, how often have you felt that you were unable to control the important things in your life?”; Cohen et al., 1983). Each item was rated on a 5-point scale, ranging from 0 (“never”) to 4 (“very often”) and a sum score was calculated. In the current study, PSS-4 showed good reliability (Cronbach’s α = .78). State-level stress during the forest sessions was measured with one item. Participants were asked to rate to what extent they felt stressed at the moment on a 5-point scale, ranging from 1 (“very slightly or not at all”) to 5 (“extremely”).

Different aspects of the subjective experience of the forest setting were assessed following each session using a combination of questions about the sensory experience and the perceived restorativeness of the forest. Perceived restorativeness was assessed using the adapted 11-item Perceived Restorativeness Scale (PRS-11) which asks participants to rate the degree to which they agreed with statements such as “Places like that are fascinating” using an 11-point scale, ranging from 0 (“not at all”) to 10 (“completely”; Pasini et al., 2014), Additionally, participants were asked to rate the pleasantness of their sensory experience of the forest for sight, smell, and visual experience on a 10-point scale ranging from 1 (“not pleasant”) to 10 (“extremely pleasant”). Participants were also provided an open-ended text box to describe further thoughts or feelings they had about the sensory experience of the forest.

A trained phlebotomist or registered nurse collected approximately 5 mL of blood via venipuncture before and after each session using BD Vacutainer™ Push Button Blood Collection Sets into 6 ml BD Vacutainer™ Venous Blood Collection Tubes (BD, Franklin Lakes, NJ). On the day of collection, samples were stored at room temperature for 30 min to allow clotting, stored at 4°C, then centrifuged at 3,000 rpm for 10 min . Serum aliquots were separated into 2 mL Fisherbrand™ Externally and Internally Threaded Cryogenic Storage Vials (Thermo Fisher Scientific, Waltham, MA) and stored at -80°C before being transported on dry ice to the Centers for Disease Control and Prevention (CDC) Tobacco and Volatiles Branch (Atlanta, GA) and the UW Center for Studies in Demography and Ecology Biodemography Laboratory (Seattle, WA) for analysis.

Acute cytokine response was assessed using a custom three-plex enzyme immunoassay microarray (Quansys Biosciences, Logan, UT, part number 107749GR) to measure levels of CRP, TNF-α, IL-6, and serum cortisol. Chemiluminescence was quantified using a Quansys Q-view Imager LS. An 8-point, 5-parameter standard curve was used to estimate cytokine concentrations and assay limits of detection (Q-view software, Quansys Biosciences, Logan, UT).

A competitive microplate enzyme immunoassay, previously validated for use with plasma (Munro & Stabenfeldt, 1984), was adapted to measure cortisol in serum extracts using a purified polyclonal anti-cortisol antibody, R4866 (provided by C. Munro, UC Davis), and cortisol reference calibrators (Steraloids, catalog number Q3880). This assay has been used successfully with saliva, urine, hair, and dried blood spot samples (Doyle et al., 2019; Heller et al., 2018; Konishi et al., 2012; Trumble et al., 2010). The anti-cortisol antibody cross-reacts 100% with cortisol, 10% with prednisolone, 6% with prednisone, 6% with 11-deoxycortisol, 5% with cortisone, and less than 1% with all other steroids (data provided by C. Munro). Color reactions were quantified at 405 nm (test) and 570 nm (reference) using a Synergy HT microplate reader (Bio Tek Instruments, Inc., Winooski, VT). A 5-parameter standard curve was used to estimate cortisol concentrations (Gen5, Bio Tek Instruments Inc., Winooski, VT). For both acute cytokine and cortisol response analyses, all samples, standards, and controls were assayed in duplicate wells.

Concentrations of α-pinene, β-caryophyllene, β-myrcene, β-pinene, Δ-3-carene, and limonene were measured in serum using stable isotope dilution with headspace solid-phase microextraction (SPME) and gas chromatography-tandem mass spectrometry (HS-SPME GC-MS/MS) following Silva et al. (2020). Methods and results are detailed in Riederer et al. (*in preparation*). Briefly, samples were thawed, mixed using a hematology mixer (Fisher Scientific Inc., Pittsburgh, PA), and a 0.50 mL aliquot dispensed into a 10 mL solid phase microextraction vial. Purge-and-trap grade methanol was used to dilute pure chemicals into the primary standard and internal standard (ISTD) stock solutions. A 40 µL spike of the ISTD solution was added the sample, which was then crimp-sealed and mixed with a vortexer (S/P Multi-Tube Vortexer, Baxter Diagnostics Inc., Minster, OH) for 5 min. Vials were placed on a Peltier-cooled sample tray (15°C) on an Agilent 7890 GC (Agilent, Santa Clara, CA) coupled to an Agilent 7010 triple-quadrupole MS (Agilent, Santa Clara, CA) with a mounted CombiPAL autosampler and extra PAL autosampler arm (CTC Analytics AG, Zwingen, Switzerland). Samples were analyzed in positive ion electron-impact ionization with multiple reaction monitoring modes. Blank and quality control samples were also prepared with a 40 µL ISTD spike. Standards prepared following an identical method were included with each analytical run. Laboratory blanks consisted of VOC-free water in sealed ampoules opened on the day of analysis. We also collected 7 field blanks on random days comprised of VOC-free water drawn through serum tubes and handled identically to the serum samples.

Terpenes were measured in air at the forest site while participants were present using a method adapted from NIOSH Method 1552 (National Institute for Occupational Safety and Health, 1996) and detailed elsewhere (Riederer et al. *in preparation*). Briefly, air was drawn using a GilAir® Plus low-flow pump (Sensidyne®, St. Petersburg, FL, USA) via polypropylene tubing into a MilliporeSigma™Supelco™ CT420 proprietary glass thermal desorption tube optimized for terpene collection (MilliporeSigma, Burlington, MA, USA). The tube was mounted on a metal stand, near the participant, at breathing height (~ 0.5 m above the ground). One field blank, consisting of a tube taken to the site and end caps momentarily opened but air drawn through was collected for each day of sampling, while field duplicates were collected on alternating weeks. Primary samples, blanks and duplicates were analyzed for 22 terpenes using thermal desorption-gas chromatography–mass spectrometry (TD-GC/MS) at Eurofins Laboratory (Edmonton, Alberta, Canada).

Participants met research staff at a study site in Tacoma for informed consent, initial surveys, and measurements. Participants were then transported to Pack Forest (~one hr drive) in a vehicle fitted with a fan that blew filtered air into their seating area to reduce terpene exposure prior to their session. Upon arrival, they were outfitted with a PAPR with charcoal filter (‘zero filter’) to prevent terpenes inhalation during initial study protocols.

Participants were then outfitted with the physiology equipment in a clinic room, rested for 10 min, and completed a survey with affective assessments and the baseline blood draw before being transported to the forest site where the zero filter was changed to the randomly assigned filter. Blood pressure, HR, and self-reported affect and stress were assessed at the beginning (T1; 0-5 min) and end of the session (T4; 55-60 min). Self-reported affect and stress were also assessed at two additional points: T2 (17.5-22.5 min) and T3 (37.5-42.5 min). A second blood sample was collected at T4. HF HRV and SCL were assessed continuously. A full daily protocol is outlined in Figure 1.

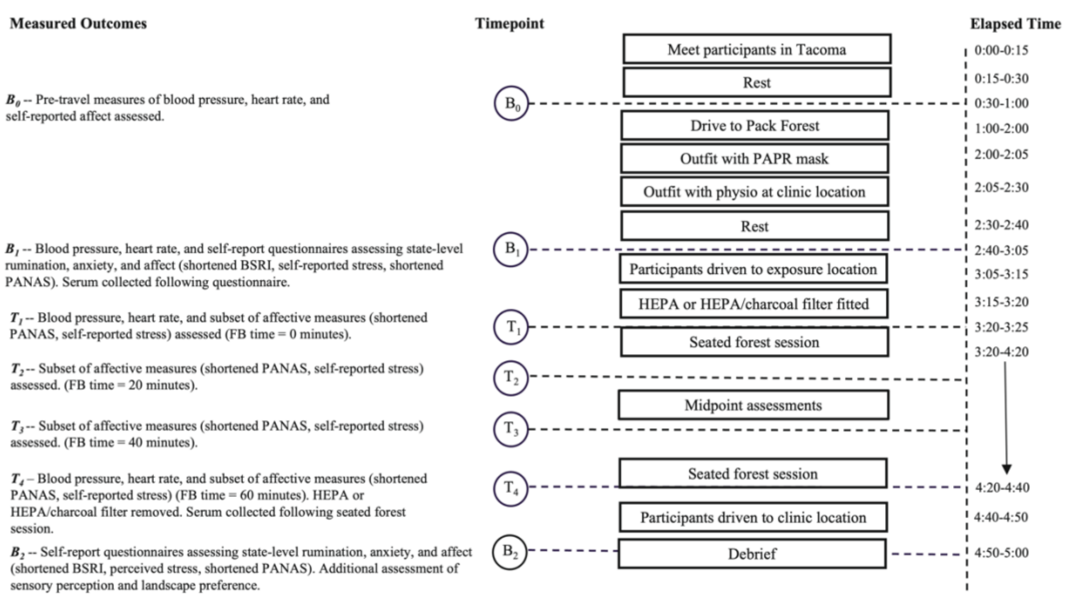


**Figure 1**. Overview of procedure sequence for a study session.

## 4. Relevant Scientific Background

Nature contact has been linked to psychological and physiological well-being in human beings (Bratman et al., 2019). As outlined by Frumkin et al. (2017), these benefits include stress reduction (assessed via decreased heart rate, blood pressure, cortisol levels, and self-report measures), decreased anxiety, negative affect, and depressive symptoms, and increased positive affect, prosocial behavior, and attentional capacity (Beil & Hanes, 2013; Berman et al., 2008; Bratman et al., 2015; Duncan et al., 2014; Ewert & Chang, 2018; Hong et al., 2018; Hunter et al., 2019; Jiang et al., 2014; Kaźmierczak, 2013; McAllister et al., 2017; Piff et al., 2015; Putra et al., 2020; Ward Thompson et al., 2016; Zhao et al., 2022). Additionally, nature contact has been associated with improved sleep, reduced mortality, and reduced risk of Type II diabetes (Astell-Burt et al., 2014; Astell-Burt & Feng, 2020; Bodicoat et al., 2014; Gascon et al., 2016; James et al., 2016; Johnson et al., 2018; Shin et al., 2020). Despite these demonstrated associations, the causal mechanisms that may be responsible for these effects are less well known. One possible pathway is reduction of stress and associated anti-inflammatory psychoneuroimmunological processes (Hartig et al., 2014; Kuo, 2015; Ulrich et al., 1991).

*Shinrin-yoku*, also known as "forest bathing", is a specific set of human-nature interactions that have been shown to improve mood, reduce anxiety and depressive symptoms, and have beneficial effects on HRV, blood pressure, HR, stress hormone levels, and inflammatory biomarkers (De Brito et al., 2020; Furuyashiki et al., 2019; Horiuchi et al., 2014; D.-S. Kim et al., 2015, 2015; Kobayashi et al., 2017, 2018; Lanki et al., 2017; J. Lee et al., 2015; Li et al., 2007, 2008; Mao, Cao, et al., 2012; Mao, Lan, et al., 2012; Ochiai et al., 2015; Oomen-Welke et al., 2022; B. J. Park et al., 2009; B.-J. Park et al., 2007; Song et al., 2015, 2017; Stigsdotter et al., 2017; Tsunetsugu et al., 2013; Yu et al., 2017). However, some studies have not observed significant changes or reported contrary observations when comparing outcomes following forest exposure to earlier baseline measurements or outcomes following control exposure, including no significant difference or a difference against the hypothesized direction in mood, stress hormone levels, blood pressure, and HRV (De Brito et al., 2020; Furuyashiki et al., 2019; Gidlow et al., 2016, 2016; Horiuchi et al., 2014; Kavanaugh et al., 2022; Y. Kim et al., 2022; Lanki et al., 2017; Mao, Cao, et al., 2012; Oomen-Welke et al., 2022; Stigsdotter et al., 2017; Yu et al., 2017).

One possible pathway for the psychophysiological benefits of forest bathing is through exposures to terpene compounds that contribute to the smell of the forest (Li et al., 2008). Terpenes are types of biogenic volatile organic compounds (BVOCs). Over 55,000 different chemically structured terpenes have been identified, all generally with a $(C_5H_8)_n$ chemical formula, and classified by differences in linked isoprene units (Kanwal et al., 2022). Emitted by plants, terpenes aid in plant reproduction, defense, inter- and intraspecific interactions, and abiotic stress response (Dudareva et al., 2006).

In animal and *in vitro* experiments, terpenes have demonstrated anti-inflammatory, anti-tumorigenic, and neuroprotective action (Cho et al., 2017). As summarized by De Cássia Da Silveira E Sá et al. (2013) and Eddin et al. (2021), rats, mice, and guinea-pigs exposed to terpenes displayed anxiolytic and anti-inflammatory responses via reduced plasma corticosterone levels, reduced inflammation in colonic tissue, and a reduction in bronchial resistance, paw edema, and leukocyte, neutrophil, IL-1β, TNF-α, and IL-6 levels (Bastos et al., 2011; De Almeida et al., 2017; Nascimento et al., 2009; Saiyudthong & Marsden, 2011; Santos, 2004; Zhang et al., 2018). Additionally, terpene exposure was shown to increase white blood cell count, IL-2, IL-10, T cell, and lymphocyte levels, and antibody cell production in rats and mice (Badr et al., 2011; Raphael & Kuttan, 2003; Trinh et al., 2011).

In human monocytes, terpenes can inhibit production of inflammatory cytokines, leukotrienes, and associated metabolites, including TNF-α, IL-1β, Il-4, Il-5, IL-6, IL-8, and leukotriene B4 (LTB4; De Cássia Da Silveira E Sá et al., 2013; Hart et al., 2000; Juergens et al., 1998, 2004). Additionally, terpene or essential oil exposure has been associated with anti-inflammatory effects in asthma patients, reduced anxiety and depressive symptoms, reduced stress, increased positive affect, and improved cognitive function and sleep (Chen et al., 2022; Fung et al., 2021; Goes et al., 2012; Juergens et al., 2003; Kerr et al., 2021; Koyama & Heinbockel, 2020; M. Lee et al., 2017; Moss & Oliver, 2012; Woo et al., 2023). These anti-inflammatory, anti-cancer, antioxidative, and neuroprotective properties of forest-derived terpenes may be mediated by signal transduction (i.e., a relay of signals within a cell) and changes in the production of transcription factor proteins that regulate gene transcription (i.e., NF-κB; Cui et al., 2022; D.-S. Kim et al., 2015; T. Kim et al., 2020; Latchman, 1993; Nair et al., 2019; Rufino et al., 2014).

Aoshima and Hamamoto (1999) proposed several pathways through which fragrant compounds might affect the brain: stimulation of the olfactory system and resulting psychological effects, absorption into the blood via the lungs by respiration, skin application, and gastrointestinal exposure via food. Similar pathways for essential oil inhalation were summarized by Fung et al. (2021). With respect to the olfactory system: essential oil molecules can bind to chemoreceptors in the nasal cavity and activate neural signaling, resulting in psychological changes (Cui et al., 2022; Faturi et al., 2010; Kagawa et al., 2003). Additionally, essential oil molecules may move through the olfactory system neuronal network via extracellular delivery or mucosa, arriving in the brain in a similar way to nasally delivered medications (Chioca et al., 2013; Cui et al., 2022; Fung et al., 2021; Hanson & Frey, 2008). From there, they may interact with transient receptor potential channels (TRP), gamma-aminobutyric acid, serotonin, and dopamine receptors (Hanson & Frey, 2008). Finally, essential oil molecules may also be transported via lung alveoli and blood to interact directly with the CNS (Chioca et al., 2013; Faturi et al., 2010; Fung et al., 2021; Kagawa et al., 2003).

In this study, participants were exposed to terpenes via inhalation of ambient forest air. Most inhaled air enters through the nose, and although human beings switch to oral breathing during exercise, approximately 30-35% of air is still inhaled via the nose in those cases as well (Koenig, 2000). Sense of smell, or olfaction, refers to the sensory process through which the olfactory system and brain interpret odors from the surrounding environment (Doty, 2001). The olfactory epithelium that lines the interior of the nose consists of olfactory receptor neurons (ORNs) that bind with odorants (Purves & Williams, 2001). ORN axons project sensory information to neurons in the olfactory bulb, which then project to the pyriform cortex in the temporal lobe, as well as the hypothalamus and the amygdala.

Olfaction and affective responses are linked in experimental studies, in part because odors can directly elicit individual affective responses following exposure (Kontaris et al., 2020; Retiveau et al., 2004; Seubert et al., 2008). Some odors may impair working memory, while others reduce anger and depressive symptoms, and improve mood (Komori et al., 1995; Martin & Chaudry, 2014; Retiveau et al., 2004; Schiffman et al., 1995). As outlined by Durrant et al. (2016), the olfactory bulb might also be the site of an early innate immune response, as experimental studies of mice found olfactory bulb expression of proinflammatory cytokines, including TNF-α, TNFR1, IL-1β, IL-6, and IκB kinase (Aniszewska et al., 2015; Leyva-Grado et al., 2009; Mori et al., 2005). In-vitro experiments have also shown that olfactory ensheathing cells (OECs) may activate NF-κB transcription factors, express Toll-like receptors (TLR) 2 and 4, and express inducible nitric oxide synthase (iNOS) mRNA and enzymes in response to bacteria and pathogen incubation (Harris et al., 2009; Vincent et al., 2007).

Here, we aim to fill a critical gap in knowledge by examining whether terpenes play a role in the health effects of nature contact in a real-world forest environment by assessing whether terpene inhalation, with a focus on the olfactory pathway, impacts stress reduction and affective outcomes using a randomized double-blind crossover trial design. The primary outcome was the HF component of HRV, a measure of parasympathetic nervous system (PNS) activity, or relaxation (add some citations to back up this claim). Secondary outcomes included measures of skin conductance levels (SCL), blood pressure, and HR (i.e., measures of sympathetic nervous system activity, or stress), self-reported stress and affect, and levels of inflammatory cytokines in serum. Terpene concentrations in serum were measured to assess the degree of association of absorbed dose with study outcomes. We predicted that exposure to forest terpenes would be associated with greater increases in HF HRV and positive affect, and greater decreases in SCL, blood pressure, HR, self-reported stress, negative affect, and levels of inflammatory cytokines in serum compared to the condition with no forest terpenes exposure. Additionally, we predicted that study outcomes would be associated with absorbed dose of forest terpenes.

To our knowledge, no study has been conducted that concurrently investigated real-world ambient terpene exposure in a forest, absorption of these terpenes, and impacts of experimental vs. control conditions of these terpene exposures on psychophysiological and immunological effects in human beings. Therefore, this study aims to further scientific understanding of the role that forest terpene inhalation exposures play in the multisensory pathways that link human well-being and forest exposure.

## 5. References


Aniszewska, A., Chłodzińska, N., Bartkowska, K., Winnicka, M. M., Turlejski, K., & Djavadian, R. L. (2015). The expression of interleukin-6 and its receptor in various brain regions and their roles in exploratory behavior and stress responses. *Journal of Neuroimmunology*, *284*, 1–9. https://doi.org/10.1016/j.jneuroim.2015.05.001

Aoshima, H., & Hamamoto, K. (1999). Potentiation of GABA Receptors Expressed in Xenopus Oocytes by Perfume and Phytoncid. *Bioscience, Biotechnology, and Biochemistry*, *63*(4), 743–748. https://doi.org/10.1271/bbb.63.743

Astell-Burt, T., & Feng, X. (2020). Does sleep grow on trees? A longitudinal study to investigate potential prevention of insufficient sleep with different types of urban green space. *SSM - Population Health*, *10*, 100497. https://doi.org/10.1016/j.ssmph.2019.100497

Astell-Burt, T., Feng, X., & Kolt, G. S. (2014). Is Neighborhood Green Space Associated With a Lower Risk of Type 2 Diabetes? Evidence From 267,072 Australians. *Diabetes Care*, *37*(1), 197–201. https://doi.org/10.2337/dc13-1325

Badr, G., Alwasel, S., Ebaid, H., Mohany, M., & Alhazza, I. (2011). Perinatal supplementation with thymoquinone improves diabetic complications and T cell immune responses in rat offspring. *Cellular Immunology*, *267*(2), 133–140. https://doi.org/10.1016/j.cellimm.2011.01.002

Bastos, V. P. D., Gomes, A. S., Lima, F. J. B., Brito, T. S., Soares, P. M. G., Pinho, J. P. M., Silva, C. S., Santos, A. A., Souza, M. H. L. P., & Magalhães, P. J. C. (2011). Inhaled 1,8-Cineole Reduces Inflammatory Parameters in Airways of Ovalbumin-Challenged Guinea Pigs: ANTI-INFLAMMATORY EFFECTS OF 1,8-CINEOLE ON GUINEA PIG AIRWAYS. *Basic & Clinical Pharmacology & Toxicology*, *108*(1), 34–39. https://doi.org/10.1111/j.1742-7843.2010.00622.x

Beil, K., & Hanes, D. (2013). The Influence of Urban Natural and Built Environments on Physiological and Psychological Measures of Stress—A Pilot Study. *International Journal of Environmental Research and Public Health*, *10*(4), 1250–1267. https://doi.org/10.3390/ijerph10041250

Berman, M. G., Jonides, J., & Kaplan, S. (2008). The Cognitive Benefits of Interacting With Nature. *Psychological Science*, *19*(12), 1207–1212. https://doi.org/10.1111/j.1467-9280.2008.02225.x

Bodicoat, D. H., O'Donovan, G., Dalton, A. M., Gray, L. J., Yates, T., Edwardson, C., Hill, S., Webb, D. R., Khunti, K., Davies, M. J., & Jones, A. P. (2014). The association between neighbourhood greenspace and type 2 diabetes in a large cross-sectional study. *BMJ Open*, *4*(12), e006076. https://doi.org/10.1136/bmjopen-2014-006076

Bratman, G. N., Anderson, C. B., Berman, M. G., Cochran, B., de Vries, S., Flanders, J., Folke, C., Frumkin, H., Gross, J. J., Hartig, T., Kahn, P. H., Kuo, M., Lawler, J. J., Levin, P. S., Lindahl, T., Meyer-Lindenberg, A., Mitchell, R., Ouyang, Z., Roe, J., … Daily, G. C. (2019). Nature and mental health: An ecosystem service perspective. *Science Advances*, *5*(7), eaax0903. https://doi.org/10.1126/sciadv.aax0903

Bratman, G. N., Daily, G. C., Levy, B. J., & Gross, J. J. (2015). The benefits of nature experience: Improved affect and cognition. *Landscape and Urban Planning*, *138*, 41–50. https://doi.org/10.1016/j.landurbplan.2015.02.005

Bratman, G. N., Mehta, A., Olvera-Alvarez, H., Spink, K. M., Levy, C., White, M. P., Kubzansky, L. D., & Gross, J. J. (2024). Associations of nature contact with emotional ill-being and well-being: The role of emotion regulation. *Cognition and Emotion*, 1–20. https://doi.org/10.1080/02699931.2024.2316199

Bratman, G. N., Young, G., Mehta, A., Lee Babineaux, I., Daily, G. C., & Gross, J. J. (2021). Affective Benefits of Nature Contact: The Role of Rumination. *Frontiers in Psychology*, *12*, 643866. https://doi.org/10.3389/fpsyg.2021.643866

Chen, M.-L., Chen, Y.-E., & Lee, H.-F. (2022). The Effect of Bergamot Essential Oil Aromatherapy on Improving Depressive Mood and Sleep Quality in Postpartum Women: A Randomized Controlled Trial. *Journal of Nursing Research*, *30*(2), e201. https://doi.org/10.1097/jnr.0000000000000459

Chioca, L. R., Antunes, V. D. C., Ferro, M. M., Losso, E. M., & Andreatini, R. (2013). Anosmia does not impair the anxiolytic-like effect of lavender essential oil inhalation in mice. *Life Sciences*, *92*(20–21), 971–975. https://doi.org/10.1016/j.lfs.2013.03.012

Cho, K. S., Lim, Y., Lee, K., Lee, J., Lee, J. H., & Lee, I.-S. (2017). Terpenes from Forests and Human Health. *Toxicological Research*, *33*(2), 97–106. https://doi.org/10.5487/TR.2017.33.2.097

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior*, *24*(4), 385–396.

Cui, J., Li, M., Wei, Y., Li, H., He, X., Yang, Q., Li, Z., Duan, J., Wu, Z., Chen, Q., Chen, B., Li, G., Ming, X., Xiong, L., & Qin, D. (2022). Inhalation Aromatherapy via Brain-Targeted Nasal Delivery: Natural Volatiles or Essential Oils on Mood Disorders. *Frontiers in Pharmacology*, *13*, 860043. https://doi.org/10.3389/fphar.2022.860043

De Almeida, A. A. C., Silva, R. O., Nicolau, L. A. D., De Brito, T. V., De Sousa, D. P., Barbosa, A. L. D. R., De Freitas, R. M., Lopes, L. D. S., Medeiros, J.-V. R., & Ferreira, P. M. P. (2017). Physio-pharmacological Investigations About the Anti-inflammatory and Antinociceptive Efficacy of (+)-Limonene Epoxide. *Inflammation*, *40*(2), 511–522. https://doi.org/10.1007/s10753-016-0496-y

De Brito, J. N., Pope, Z. C., Mitchell, N. R., Schneider, I. E., Larson, J. M., Horton, T. H., & Pereira, M. A. (2020). The effect of green walking on heart rate variability: A pilot crossover study. *Environmental Research*, *185*, 109408. https://doi.org/10.1016/j.envres.2020.109408

De Cássia Da Silveira E Sá, R., Andrade, L., & De Sousa, D. (2013). A Review on Anti-Inflammatory Activity of Monoterpenes. *Molecules*, *18*(1), 1227–1254. https://doi.org/10.3390/molecules18011227

Doty, R. L. (2001). Olfaction. *Annual Review of Psychology*, *52*(1), 423–452. https://doi.org/10.1146/annurev.psych.52.1.423

Doyle, J. A., Brindle, E., & Bolden, T. S. (2019). Development and validation of hair specimen collection methods among extremely short-length Afro-textured hair. *American Journal of Human Biology*, *31*(3), e23222. https://doi.org/10.1002/ajhb.23222

Dudareva, N., Negre, F., Nagegowda, D. A., & Orlova, I. (2006). Plant Volatiles: Recent Advances and Future Perspectives. *Critical Reviews in Plant Sciences*, *25*(5), 417–440. https://doi.org/10.1080/07352680600899973

Duncan, M. J., Clarke, N. D., Birch, S. L., Tallis, J., Hankey, J., Bryant, E., & Eyre, E. L. J. (2014). The effect of green exercise on blood pressure, heart rate and mood state in primary school children. *International Journal of Environmental Research and Public Health*, *11*(4), 3678–3688. https://doi.org/10.3390/ijerph110403678

Durrant, D. M., Ghosh, S., & Klein, R. S. (2016). The Olfactory Bulb: An Immunosensory Effector Organ during Neurotropic Viral Infections. *ACS Chemical Neuroscience*, *7*(4), 464–469. https://doi.org/10.1021/acschemneuro.6b00043

Eddin, L. B., Jha, N. K., Meeran, M. F. N., Kesari, K. K., Beiram, R., & Ojha, S. (2021). Neuroprotective Potential of Limonene and Limonene Containing Natural Products. *Molecules*, *26*(15), 4535. https://doi.org/10.3390/molecules26154535

Ewert, A., & Chang, Y. (2018). Levels of Nature and Stress Response. *Behavioral Sciences*, *8*(5), 49. https://doi.org/10.3390/bs8050049

Faturi, C. B., Leite, J. R., Alves, P. B., Canton, A. C., & Teixeira-Silva, F. (2010). Anxiolytic-like effect of sweet orange aroma in Wistar rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*(4), 605–609. https://doi.org/10.1016/j.pnpbp.2010.02.020

Frumkin, H., Bratman, G. N., Breslow, S. J., Cochran, B., Kahn Jr, P. H., Lawler, J. J., Levin, P. S., Tandon, P. S., Varanasi, U., Wolf, K. L., & Wood, S. A. (2017). Nature Contact and Human Health: A Research Agenda. *Environmental Health Perspectives*, *125*(7), 075001. https://doi.org/10.1289/EHP1663

Fung, T. K. H., Lau, B. W. M., Ngai, S. P. C., & Tsang, H. W. H. (2021). Therapeutic Effect and Mechanisms of Essential Oils in Mood Disorders: Interaction between the Nervous and Respiratory Systems. *International Journal of Molecular Sciences*, *22*(9), 4844. https://doi.org/10.3390/ijms22094844

Furuyashiki, A., Tabuchi, K., Norikoshi, K., Kobayashi, T., & Oriyama, S. (2019). A comparative study of the physiological and psychological effects of forest bathing (Shinrin-yoku) on working age people with and without depressive tendencies. *Environmental Health and Preventive Medicine*, *24*(1), 46. https://doi.org/10.1186/s12199-019-0800-1

Gascon, M., Triguero-Mas, M., Martínez, D., Dadvand, P., Rojas-Rueda, D., Plasència, A., & Nieuwenhuijsen, M. J. (2016). Residential green spaces and mortality: A systematic review. *Environment International*, *86*, 60–67. https://doi.org/10.1016/j.envint.2015.10.013

Gidlow, C. J., Jones, M. V., Hurst, G., Masterson, D., Clark-Carter, D., Tarvainen, M. P., Smith, G., & Nieuwenhuijsen, M. (2016). Where to put your best foot forward: Psycho-physiological responses to walking in natural and urban environments. *Journal of Environmental Psychology*, *45*, 22–29. https://doi.org/10.1016/j.jenvp.2015.11.003

Goes, T. C., Antunes, F. D., Alves, P. B., & Teixeira-Silva, F. (2012). Effect of Sweet Orange Aroma on Experimental Anxiety in Humans. *The Journal of Alternative and Complementary Medicine*, *18*(8), 798–804. https://doi.org/10.1089/acm.2011.0551

Hanson, L. R., & Frey, W. H. (2008). Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease. *BMC Neuroscience*, *9*(S3), S5. https://doi.org/10.1186/1471-2202-9-S3-S5

Harris, J. A., West, A. K., & Chuah, M. I. (2009). Olfactory ensheathing cells: Nitric oxide production and innate immunity. *Glia*, *57*(16), 1848–1857. https://doi.org/10.1002/glia.20899

Hart, P. H., Brand, C., Carson, C. F., Riley, T. V., Prager, R. H., & Finlay-Jones, J. J. (2000). Terpinen-4-ol, the main component of the essential oil of Melaleuca alternifolia (tea tree oil), suppresses inflammatory mediator production by activated human monocytes. *Inflammation Research*, *49*(11), 619–626. https://doi.org/10.1007/s000110050639

Hartig, T., Mitchell, R., De Vries, S., & Frumkin, H. (2014). Nature and Health. *Annual Review of Public Health*, *35*(1), 207–228. https://doi.org/10.1146/annurev-publhealth-032013-182443

Heller, M., Roberts, S. T., Masese, L., Ngina, J., Chohan, N., Chohan, V., Shafi, J., McClelland, R. S., Brindle, E., & Graham, S. M. (2018). Gender-Based Violence, Physiological Stress, and Inflammation: A Cross-Sectional Study. *Journal of Women's Health*, *27*(9), 1152–1161. https://doi.org/10.1089/jwh.2017.6743

Hong, A., Sallis, J. F., King, A. C., Conway, T. L., Saelens, B., Cain, K. L., Fox, E. H., & Frank, L. D. (2018). Linking green space to neighborhood social capital in older adults: The role of perceived safety. *Social Science & Medicine*, *207*, 38–45. https://doi.org/10.1016/j.socscimed.2018.04.051

Horiuchi, M., Endo, J., Takayama, N., Murase, K., Nishiyama, N., Saito, H., & Fujiwara, A. (2014). Impact of Viewing vs. Not Viewing a Real Forest on Physiological and Psychological Responses in the Same Setting. *International Journal of Environmental Research and Public Health*, *11*(10), 10883–10901. https://doi.org/10.3390/ijerph111010883

Hunter, M. R., Gillespie, B. W., & Chen, S. Y.-P. (2019). Urban Nature Experiences Reduce Stress in the Context of Daily Life Based on Salivary Biomarkers. *Frontiers in Psychology*, *10*, 722. https://doi.org/10.3389/fpsyg.2019.00722

James, P., Hart, J. E., Banay, R. F., & Laden, F. (2016). Exposure to Greenness and Mortality in a Nationwide Prospective Cohort Study of Women. *Environmental Health Perspectives*, *124*(9), 1344–1352. https://doi.org/10.1289/ehp.1510363

Jiang, B., Chang, C.-Y., & Sullivan, W. C. (2014). A dose of nature: Tree cover, stress reduction, and gender differences. *Landscape and Urban Planning*, *132*, 26–36. https://doi.org/10.1016/j.landurbplan.2014.08.005

Johnson, B. S., Malecki, K. M., Peppard, P. E., & Beyer, K. M. M. (2018). Exposure to neighborhood green space and sleep: Evidence from the Survey of the Health of Wisconsin. *Sleep Health*, *4*(5), 413–419. https://doi.org/10.1016/j.sleh.2018.08.001

Juergens, U. R., Dethlefsen, U., Steinkamp, G., Gillissen, A., Repges, R., & Vetter, H. (2003). Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: A double-blind placebo-controlled trial. *Respiratory Medicine*, *97*(3), 250–256. https://doi.org/10.1053/rmed.2003.1432

Juergens, U. R., Engelen, T., Racké, K., Stöber, M., Gillissen, A., & Vetter, H. (2004). Inhibitory activity of 1,8-cineol (eucalyptol) on cytokine production in cultured human lymphocytes and monocytes. *Pulmonary Pharmacology & Therapeutics*, *17*(5), 281–287. https://doi.org/10.1016/j.pupt.2004.06.002

Juergens, U. R., Stöber, M., & Vetter, H. (1998). The anti-inflammatory activity of L-menthol compared to mint oil in human monocytes in vitro: A novel perspective for its therapeutic use in inflammatory diseases. *European Journal of Medical Research*, *3*(12), 539–545.

Kagawa, D., Jokura, H., Ochiai, R., Tokimitsu, I., & Tsubone, H. (2003). The sedative effects and mechanism of action of cedrol inhalation with behavioral pharmacological evaluation. *Planta Medica*, *69*(7), 637–641. https://doi.org/10.1055/s-2003-41114

Kanwal, A., Bilal, M., Rasool, N., Zubair, M., Shah, S. A. A., & Zakaria, Z. A. (2022). Total Synthesis of Terpenes and Their Biological Significance: A Critical Review. *Pharmaceuticals*, *15*(11), 1392. https://doi.org/10.3390/ph15111392

Kavanaugh, J., Hardison, M. E., Rogers, H. H., White, C., & Gross, J. (2022). Assessing the Impact of a Shinrin-Yoku (Forest Bathing) Intervention on Physician/Healthcare Professional Burnout: A Randomized, Controlled Trial. *International Journal of Environmental Research and Public Health*, *19*(21), 14505. https://doi.org/10.3390/ijerph192114505

Kaźmierczak, A. (2013). The contribution of local parks to neighbourhood social ties. *Landscape and Urban Planning*, *109*(1), 31–44. https://doi.org/10.1016/j.landurbplan.2012.05.007

Kerr, D., Hegg, M., & Mohebbi, M. (2021). Effects of diffused essential oils for reducing stress and improving mood for clinical nurses: An interventional time series study. *Nursing Forum*, *56*(2), 305–312. https://doi.org/10.1111/nuf.12548

Kim, D.-S., Lee, H.-J., Jeon, Y.-D., Han, Y.-H., Kee, J.-Y., Kim, H.-J., Shin, H.-J., Kang, J., Lee, B. S., Kim, S.-H., Kim, S.-J., Park, S.-H., Choi, B.-M., Park, S.-J., Um, J.-Y., & Hong, S.-H. (2015). Alpha-Pinene Exhibits Anti-Inflammatory Activity Through the Suppression of MAPKs and the NF-κB Pathway in Mouse Peritoneal Macrophages. *The American Journal of Chinese Medicine*, *43*(04), 731–742. https://doi.org/10.1142/S0192415X15500457

Kim, T., Song, B., Cho, K. S., & Lee, I.-S. (2020). Therapeutic Potential of Volatile Terpenes and Terpenoids from Forests for Inflammatory Diseases. *International Journal of Molecular Sciences*, *21*(6). https://doi.org/10.3390/ijms21062187

Kim, Y., Choi, Y., & Kim, H. (2022). Positive Effects on Emotional Stress and Sleep Quality of Forest Healing Program for Exhausted Medical Workers during the COVID-19 Outbreak. *International Journal of Environmental Research and Public Health*, *19*(5), 3130. https://doi.org/10.3390/ijerph19053130

Kobayashi, H., Song, C., Ikei, H., Park, B.-J., Lee, J., Kagawa, T., & Miyazaki, Y. (2017). Population-Based Study on the Effect of a Forest Environment on Salivary Cortisol Concentration. *International Journal of Environmental Research and Public Health*, *14*(8), 931. https://doi.org/10.3390/ijerph14080931

Kobayashi, H., Song, C., Ikei, H., Park, B.-J., Lee, J., Kagawa, T., & Miyazaki, Y. (2018). Forest Walking Affects Autonomic Nervous Activity: A Population-Based Study. *Frontiers in Public Health*, *6*, 278. https://doi.org/10.3389/fpubh.2018.00278

Koenig, J. Q. (2000). *Health Effects of Ambient Air Pollution: How safe is the air we breathe?* Springer US : Imprint : Springer.

Komori, T., Fujiwara, R., Tanida, M., Nomura, J., & Yokoyama, M. M. (1995). Effects of Citrus Fragrance on Immune Function and Depressive States. *Neuroimmunomodulation*, *2*(3), 174–180. https://doi.org/10.1159/000096889

Konishi, S., Brindle, E., Guyton, A., & O’Connor, K. A. (2012). Salivary concentration of progesterone and cortisol significantly differs across individuals after correcting for blood hormone values. *American Journal of Physical Anthropology*, *149*(2), 231–241. https://doi.org/10.1002/ajpa.22114

Kontaris, I., East, B. S., & Wilson, D. A. (2020). Behavioral and Neurobiological Convergence of Odor, Mood and Emotion: A Review. *Frontiers in Behavioral Neuroscience*, *14*, 35. https://doi.org/10.3389/fnbeh.2020.00035

Koyama, S., & Heinbockel, T. (2020). The Effects of Essential Oils and Terpenes in Relation to Their Routes of Intake and Application. *International Journal of Molecular Sciences*, *21*(5), 1558. https://doi.org/10.3390/ijms21051558

Kuo, M. (2015). How might contact with nature promote human health? Promising mechanisms and a possible central pathway. *Frontiers in Psychology*, *6*, 1093. https://doi.org/10.3389/fpsyg.2015.01093

Lanki, T., Siponen, T., Ojala, A., Korpela, K., Pennanen, A., Tiittanen, P., Tsunetsugu, Y., Kagawa, T., & Tyrväinen, L. (2017). Acute effects of visits to urban green environments on cardiovascular physiology in women: A field experiment. *Environmental Research*, *159*, 176–185. https://doi.org/10.1016/j.envres.2017.07.039

Latchman, D. S. (1993). Transcription factors: An overview. *International Journal of Experimental Pathology*, *74*(5), 417–422.

Lee, J., Park, B.-J., Ohira, T., Kagawa, T., & Miyazaki, Y. (2015). Acute Effects of Exposure to a Traditional Rural Environment on Urban Dwellers: A Crossover Field Study in Terraced Farmland. *International Journal of Environmental Research and Public Health*, *12*(2), 1874–1893. https://doi.org/10.3390/ijerph120201874

Lee, M., Lim, S., Song, J.-A., Kim, M.-E., & Hur, M.-H. (2017). The effects of aromatherapy essential oil inhalation on stress, sleep quality and immunity in healthy adults: Randomized controlled trial. *European Journal of Integrative Medicine*, *12*, 79–86. https://doi.org/10.1016/j.eujim.2017.04.009

Leyva-Grado, V. H., Churchill, L., Wu, M., Williams, T. J., Taishi, P., Majde, J. A., & Krueger, J. M. (2009). Influenza virus- and cytokine-immunoreactive cells in the murine olfactory and central autonomic nervous systems before and after illness onset. *Journal of Neuroimmunology*, *211*(1–2), 73–83. https://doi.org/10.1016/j.jneuroim.2009.03.016

Li, Q., Morimoto, K., Kobayashi, M., Inagaki, H., Katsumata, M., Hirata, Y., Hirata, K., Suzuki, H., Li, Y. J., Wakayama, Y., Kawada, T., Park, B. J., Ohira, T., Matsui, N., Kagawa, T., Miyazaki, Y., & Krensky, A. M. (2008). Visiting a Forest, but Not a City, Increases Human Natural Killer Activity and Expression of Anti-Cancer Proteins. *International Journal of Immunopathology and Pharmacology*, *21*(1), 117–127. https://doi.org/10.1177/039463200802100113

Li, Q., Morimoto, K., Nakadai, A., Inagaki, H., Katsumata, M., Shimizu, T., Hirata, Y., Hirata, K., Suzuki, H., Miyazaki, Y., Kagawa, T., Koyama, Y., Ohira, T., Takayama, N., Krensky, A. M., & Kawada, T. (2007). Forest Bathing Enhances Human Natural Killer Activity and Expression of Anti-Cancer Proteins. *International Journal of Immunopathology and Pharmacology*, *20*(2_suppl), 3–8. https://doi.org/10.1177/03946320070200S202

Mao, G.-X., Cao, Y.-B., Lan, X.-G., He, Z.-H., Chen, Z.-M., Wang, Y.-Z., Hu, X.-L., Lv, Y.-D., Wang, G.-F., & Yan, J. (2012). Therapeutic effect of forest bathing on human hypertension in the elderly. *Journal of Cardiology*, *60*(6), 495–502. https://doi.org/10.1016/j.jjcc.2012.08.003

Mao, G.-X., Lan, X.-G., Cao, Y. B., Chen, Z.-M., He, Z.-H., Lv, Y.-D., Wang, Y.-Z., Hu, X.-L., Wang, G. F., & Yan, J. (2012). Effects of Short-Term Forest Bathing on Human Health in a Broad-Leaved Evergreen Forest in Zhejiang Province, China. In *Biomedical and Environmental Sciences* (Vol. 25, Issue 3, p. 317).

Martin, G. N., & Chaudry, A. (2014). Working memory performance and exposure to pleasant and unpleasant ambient odor: Is spatial span special? *International Journal of Neuroscience*, *124*(11), 806–811. https://doi.org/10.3109/00207454.2014.890619

McAllister, E., Bhullar, N., & Schutte, N. S. (2017). Into the Woods or a Stroll in the Park: How Virtual Contact with Nature Impacts Positive and Negative Affect. *International Journal of Environmental Research and Public Health*, *14*(7), 786. https://doi.org/10.3390/ijerph14070786

Mori, K., Kaneko, Y. S., Nakashima, A., Nagatsu, I., Takahashi, H., & Ota, A. (2005). Peripheral lipopolysaccharide induces apoptosis in the murine olfactory bulb. *Brain Research*, *1039*(1–2), 116–129. https://doi.org/10.1016/j.brainres.2005.01.078

Moss, M., & Oliver, L. (2012). Plasma 1,8-cineole correlates with cognitive performance following exposure to rosemary essential oil aroma. *Therapeutic Advances in Psychopharmacology*, *2*(3), 103–113. https://doi.org/10.1177/2045125312436573

Munro, C., & Stabenfeldt, G. (1984). Development of a microtitre plate enzyme immunoassay for the determination of progesterone. *Journal of Endocrinology*, *101*(1), 41–49. https://doi.org/10.1677/joe.0.1010041

Nair, A., Chauhan, P., Saha, B., & Kubatzky, K. F. (2019). Conceptual Evolution of Cell Signaling. *International Journal of Molecular Sciences*, *20*(13), 3292. https://doi.org/10.3390/ijms20133292

Nascimento, N. R. F., Refosco, R. M. D. C., Vasconcelos, E. C. F., Kerntopf, M. R., Santos, C. F., Batista, F. J. A., De Sousa, C. M., & Fonteles, M. C. (2009). 1,8-Cineole induces relaxation in rat and guinea-pig airway smooth muscle. *Journal of Pharmacy and Pharmacology*, *61*(3), 361–366. https://doi.org/10.1211/jpp.61.03.0011

National Institute for Occupational Safety and Health. (1996). *NIOSH Manual of Analytic Methods (NMAM) 1552: Terpenes*. National Institute for Occupational Safety and Health.

Nisbet, E. K., & Zelenski, J. M. (2013). The NR-6: A new brief measure of nature relatedness. *Frontiers in Psychology*, *4*. https://doi.org/10.3389/fpsyg.2013.00813

Ochiai, H., Ikei, H., Song, C., Kobayashi, M., Miura, T., Kagawa, T., Li, Q., Kumeda, S., Imai, M., & Miyazaki, Y. (2015). Physiological and Psychological Effects of a Forest Therapy Program on Middle-

Aged Females. *International Journal of Environmental Research and Public Health*, *12*(12), 15222–15232. https://doi.org/10.3390/ijerph121214984

Oomen-Welke, K., Schlachter, E., Hilbich, T., Naumann, J., Müller, A., Hinterberger, T., & Huber, R. (2022). Spending Time in the Forest or the Field: Investigations on Stress Perception and Psychological Well-Being—A Randomized Cross-Over Trial in Highly Sensitive Persons. *International Journal of Environmental Research and Public Health*, *19*(22), 15322. https://doi.org/10.3390/ijerph192215322

Park, B. J., Tsunetsugu, Y., Kasetani, T., Kagawa, T., & Miyazaki, Y. (2009). The physiological effects of Shinrin-yoku (taking in the forest atmosphere or forest bathing): Evidence from field experiments in 24 forests across Japan. *Environmental Health and Preventive Medicine*, *15*(1), 18. https://doi.org/10.1007/s12199-009-0086-9

Park, B.-J., Tsunetsugu, Y., Kasetani, T., Hirano, H., Kagawa, T., Sato, M., & Miyazaki, Y. (2007). Physiological Effects of Shinrin-yoku (Taking in the Atmosphere of the Forest)—Using Salivary Cortisol and Cerebral Activity as Indicators—. *Journal of PHYSIOLOGICAL ANTHROPOLOGY*, *26*(2), 123–128. https://doi.org/10.2114/jpa2.26.123

Pasini, M., Berto, R., Brondino, M., Hall, R., & Ortner, C. (2014). How to Measure the Restorative Quality of Environments: The PRS-11. *Procedia - Social and Behavioral Sciences*, *159*, 293–297. https://doi.org/10.1016/j.sbspro.2014.12.375

Piff, P. K., Dietze, P., Feinberg, M., Stancato, D. M., & Keltner, D. (2015). Awe, the small self, and prosocial behavior. *Journal of Personality and Social Psychology*, *108*(6), 883–899. https://doi.org/10.1037/pspi0000018

Purves, D., & Williams, S. M. (Eds.). (2001). *Neuroscience* (2nd ed). Sinauer Associates.

Putra, I. G. N. E., Astell-Burt, T., Cliff, D. P., Vella, S. A., John, E. E., & Feng, X. (2020). The Relationship Between Green Space and Prosocial Behaviour Among Children and Adolescents: A Systematic Review. *Frontiers in Psychology*, *11*, 859. https://doi.org/10.3389/fpsyg.2020.00859

Raphael, T. J., & Kuttan*, G. (2003). Immunomodulatory Activity of Naturally Occurring Monoterpenes Carvone, Limonene, and Perillic Acid. *Immunopharmacology and Immunotoxicology*, *25*(2), 285–294. https://doi.org/10.1081/IPH-120020476

Retiveau, A. N., Iv, E. C., & Milliken, G. A. (2004). COMMON AND SPECIFIC EFFECTS OF FINE FRAGRANCES ON THE MOOD OF WOMEN. *Journal of Sensory Studies*, *19*(5), 373–394. https://doi.org/10.1111/j.1745-459x.2004.102803.x

Rufino, A. T., Ribeiro, M., Judas, F., Salgueiro, L., Lopes, M. C., Cavaleiro, C., & Mendes, A. F. (2014). Anti-inflammatory and Chondroprotective Activity of (+)-α-Pinene: Structural and Enantiomeric Selectivity. *Journal of Natural Products*, *77*(2), 264–269. https://doi.org/10.1021/np400828x

Saiyudthong, S., & Marsden, C. A. (2011). Acute effects of bergamot oil on anxiety-related behaviour and corticosterone level in rats. *Phytotherapy Research*, *25*(6), 858–862. https://doi.org/10.1002/ptr.3325

Santos, F. (2004). 1,8-cineole (eucalyptol), a monoterpene oxide attenuates the colonic damage in rats on acute TNBS-colitis. *Food and Chemical Toxicology*, *42*(4), 579–584. https://doi.org/10.1016/j.fct.2003.11.001

Schiffman, S. S., Suggs, M. S., & Sattely-Miller, E. A. (1995). Effect of pleasant odors on mood of males at midlife: Comparison of African-American and European-American men. *Brain Research Bulletin*, *36*(1), 31–37. https://doi.org/10.1016/0361-9230(94)00134-M

Seubert, J., Rea, A. F., Loughead, J., & Habel, U. (2008). Mood Induction with Olfactory Stimuli Reveals Differential Affective Responses in Males and Females. *Chemical Senses*, *34*(1), 77–84. https://doi.org/10.1093/chemse/bjn054

Shaffer, F., & Ginsberg, J. P. (2017). An Overview of Heart Rate Variability Metrics and Norms. *Frontiers in Public Health*, *5*, 258. https://doi.org/10.3389/fpubh.2017.00258

Shanahan, D. F., Bush, R., Gaston, K. J., Lin, B. B., Dean, J., Barber, E., & Fuller, R. A. (2016). Health Benefits from Nature Experiences Depend on Dose. *Scientific Reports*, *6*(1), 28551. https://doi.org/10.1038/srep28551

Shin, J. C., Parab, K. V., An, R., & Grigsby-Toussaint, D. S. (2020). Greenspace exposure and sleep: A systematic review. *Environmental Research*, *182*, 109081. https://doi.org/10.1016/j.envres.2019.109081

Sibbald, B., & Roberts, C. (1998). Understanding controlled trials: Crossover trials. *BMJ*, *316*(7146), 1719–1720. https://doi.org/10.1136/bmj.316.7146.1719

Silva, L. K., Espenship, M. F., Newman, C. A., Blount, B. C., & De Jesús, V. R. (2020). Quantification of Seven Terpenes in Human Serum by Headspace Solid-Phase Microextraction–Gas Chromatography–Tandem Mass Spectrometry. *Environmental Science & Technology*, *54*(21), 13861–13867. https://doi.org/10.1021/acs.est.0c03269

Song, C., Ikei, H., Kobayashi, M., Miura, T., Li, Q., Kagawa, T., Kumeda, S., Imai, M., & Miyazaki, Y. (2017). Effects of viewing forest landscape on middle-aged hypertensive men. *Urban Forestry & Urban Greening*, *21*, 247–252. https://doi.org/10.1016/j.ufug.2016.12.010

Song, C., Ikei, H., Kobayashi, M., Miura, T., Taue, M., Kagawa, T., Li, Q., Kumeda, S., Imai, M., & Miyazaki, Y. (2015). Effect of Forest Walking on Autonomic Nervous System Activity in Middle-Aged

Hypertensive Individuals: A Pilot Study. *International Journal of Environmental Research and Public Health*, *12*(3), 2687–2699. https://doi.org/10.3390/ijerph120302687

Stigsdotter, U. K., Corazon, S. S., Sidenius, U., Kristiansen, J., & Grahn, P. (2017). It is not all bad for the grey city – A crossover study on physiological and psychological restoration in a forest and an urban environment. *Health & Place*, *46*, 145–154. https://doi.org/10.1016/j.healthplace.2017.05.007

Thompson, E. R. (2007). Development and Validation of an Internationally Reliable Short-Form of the Positive and Negative Affect Schedule (PANAS). *Journal of Cross-Cultural Psychology*, *38*(2), 227–242. https://doi.org/10.1177/0022022106297301

Trinh, H.-T., Lee, I.-A., Hyun, Y.-J., & Kim, D.-H. (2011). *Artemisia princeps* Pamp. Essential Oil and Its Constituents Eucalyptol and *α* -terpineol Ameliorate Bacterial Vaginosis and Vulvovaginal Candidiasis in Mice by Inhibiting Bacterial Growth and NF- *κ* B Activation. *Planta Medica*, *77*(18), 1996–2002. https://doi.org/10.1055/s-0031-1280094

Trumble, B. C., Brindle, E., Kupsik, M., & O'Connor, K. A. (2010). Responsiveness of the reproductive axis to a single missed evening meal in young adult males. *American Journal of Human Biology*, *22*(6), 775–781. https://doi.org/10.1002/ajhb.21079

Tsunetsugu, Y., Lee, J., Park, B.-J., Tyrväinen, L., Kagawa, T., & Miyazaki, Y. (2013). Physiological and psychological effects of viewing urban forest landscapes assessed by multiple measurements. *Landscape and Urban Planning*, *113*, 90–93. https://doi.org/10.1016/j.landurbplan.2013.01.014

Ulrich, R. S., Simons, R. F., Losito, B. D., Fiorito, E., Miles, M. A., & Zelson, M. (1991). Stress recovery during exposure to natural and urban environments. *Journal of Environmental Psychology*, *11*(3), 201–230. https://doi.org/10.1016/S0272-4944(05)80184-7

Vincent, A. J., Choi-Lundberg, D. L., Harris, J. A., West, A. K., & Chuah, M. I. (2007). Bacteria and PAMPs activate nuclear factor κB and Gro production in a subset of olfactory ensheathing cells and astrocytes but not in Schwann cells. *Glia*, *55*(9), 905–916. https://doi.org/10.1002/glia.20512

Ward Thompson, C., Aspinall, P., Roe, J., Robertson, L., & Miller, D. (2016). Mitigating Stress and Supporting Health in Deprived Urban Communities: The Importance of Green Space and the Social Environment. *International Journal of Environmental Research and Public Health*, *13*(4), 440. https://doi.org/10.3390/ijerph13040440

Watson, A. Y., Bates, R. R., & Kennedy, D. (Eds.). (1988). *Air Pollution, the Automobile, and Public Health* (p. 1033). National Academies Press. https://doi.org/10.17226/1033

White, M. P., Alcock, I., Grellier, J., Wheeler, B. W., Hartig, T., Warber, S. L., Bone, A., Depledge, M. H., & Fleming, L. E. (2019). Spending at least 120 minutes a week in nature is associated with good health and wellbeing. *Scientific Reports*, *9*(1), 7730. https://doi.org/10.1038/s41598-019-44097-3

Woo, C. C., Miranda, B., Sathishkumar, M., Dehkordi-Vakil, F., Yassa, M. A., & Leon, M. (2023). Overnight olfactory enrichment using an odorant diffuser improves memory and modifies the uncinate fasciculus in older adults. *Frontiers in Neuroscience*, *17*, 1200448. https://doi.org/10.3389/fnins.2023.1200448

Yu, C.-P., Lin, C.-M., Tsai, M.-J., Tsai, Y.-C., & Chen, C.-Y. (2017). Effects of Short Forest Bathing Program on Autonomic Nervous System Activity and Mood States in Middle-Aged and Elderly Individuals. *International Journal of Environmental Research and Public Health*, *14*(8), 897. https://doi.org/10.3390/ijerph14080897

Zhang, N., Zhang, L., Feng, L., & Yao, L. (2018). Cananga odorata essential oil reverses the anxiety induced by 1-(3-chlorophenyl) piperazine through regulating the MAPK pathway and serotonin system in mice. *Journal of Ethnopharmacology*, *219*, 23–30. https://doi.org/10.1016/j.jep.2018.03.013

Zhao, Y., Bao, W.-W., Yang, B.-Y., Liang, J.-H., Gui, Z.-H., Huang, S., Chen, Y.-C., Dong, G.-H., & Chen, Y.-J. (2022). Association between greenspace and blood pressure: A systematic review and meta-analysis. *Science of The Total Environment*, *817*, 152513. https://doi.org/10.1016/j.scitotenv.2021.152513