AliveSCENT: Evaluating the potential use of various essential oils as a mosquito repellent

Yesenia Collins, Emily Fugazzotto, Atena Jasim, and Dillon Tirado

Tyngsborough High School, Tyngsborough, Massachusetts, United States

Reviewed on 7 May 2022; Accepted on 25 June 2022; Published on 15 October 2022

Surprisingly, one of the most dangerous animals on the planet is also one of the smallest: the mosquito. These insects are responsible for more human deaths per year than the animals people fear the most —such as snakes, sharks, and bears—because they act as vectors for a number of diseases including malaria, West Nile virus, Zika virus, and dengue fever. AliveSCENT aims to combat this issue by engineering *Escherichia coli* to produce a lavender scent that repels mosquitoes. A bacterially produced insect repellent has potential advantages over currently available products. It would avoid dead insects from the use of 'bug zappers,' the fire hazard associated with candles, and the negative effects of using bug spray such as foul odor and possible breathing issues. This study tested the repellent properties of four monoterpene-containing essential oils that could potentially be produced in *E. coli*: jasmine, wintergreen, lavender, and limonene. While all of these were shown to be effective, the lavender scent was selected for subsequent development as success in engineering *E. coli* to produce this scent has been demonstrated in the past. In addition, we are already familiar with its biosynthesis pathway due to prior work with limonene. The ultimate goal of this project is to engineer *E. coli* to emit a lavender scent that would function as a mosquito deterrent. To achieve this, the native methylerythritol 4-phosphate (MEP) pathway in *E. coli* will be utilized to convert pyruvate into isopentenyl pyrophosphate (IPP). The pathway will be manipulated to produce (S)-linalool, a compound in lavender oil, through the addition of geranyl diphosphate synthase (GPPS) and (S)-linalool synthase (LINS), which will convert IPP to geranyl diphosphate (GPP) and then (S)-linalool in sequential steps. The pBbE5a-RFP plasmid (Addgene) will be used as the backbone for an AliveSCENT construct to introduce this functionality, due to its ampicillin resistance and red fluorescent protein marker. It will be used later to transform *E. coli* for further testing. We hope that a functional repellent containing the transformed *E. coli* could constitute an effective alternative to currently available insect deterrent products.

Keywords: Mosquito, *Aedes albopictus*, *Escherichia coli*, (S)-linalool

Mentors: *Rebekah Ravgiala and Michael Sheets*

Direct correspondence to rebekah.ravgiala@tyngsboroughps.org; michael.sheets4@gmail.com

Watch a video introduction by the authors at https://youtu.be/yTXHqE0VOJY

Background

Ask yourself, what is the most annoying part of outdoor activities on a hot summer night? It is hearing the dreaded sound of mosquitoes circling around in the air. What makes this situation worse is fear of the eventual development of an itchy bump on one's exposed skin. However unpleasant mosquito bites may be, these insects are capable of causing more than itching and irritation. Certain mosquito species serve as vectors that transmit life-threatening illnesses such as malaria, West Nile virus, Zika virus, and dengue fever (Centers for Disease Control and Prevention, 2019). According to the World Health Organization (2016), more than 50% of the world population is at risk of contracting a mosquito-borne illness (Keeffe, 2016). The number of people at risk of these illnesses is likely to increase substantially in the near future because of the planet's rising temperatures, since mosquitoes thrive in warmer climates (Rocklöv & Dubrow, 2020). Therefore, this insect is by far the most deadly animal on the entire planet, as every year up to one million people die from pathogens contracted through mosquito bites (Keeffe, 2016).

The goal of this project is to create a consumer product that utilizes *Escherichia coli*, modified with a plasmid that allows it to emit a lavender musk, to generate a safe and effective mosquito repellent. There are already numerous products on the market intended to repel, incapacitate, or kill mosquitoes, yet they all have disadvantages. For outdoor activities that require moving away from a single spot, aerosol insect repellent is the most commonly used product. However, these spray-on insect repellents have an undesirable odor and can potentially cause breathing problems, negatively impacting health. Such issues arise because most household bug sprays contain plant-derived chemicals called pyrethrins, which were originally isolated from the chrysanthemum flower, and excessive exposure can cause life-threatening breathing issues (*Insecticide Poisoning*, n.d.). However, if a consumer would like to repel insects from a fixed location, there are many more products available including bug nets, 'bug zappers,' and tiki torches. Unfortunately, these products also have their disadvantages. Bug nets are difficult to put around a large outdoor area and are not an appealing sight, and bug zappers create noise and can leave behind piles of dead mosquitoes. Additionally, tiki torches have limited functionality and are also a fire hazard. AliveSCENT aims to combat all of these issues by reprogramming *E. coli* to produce a mosquito-repelling lavender scent.

While essential oils have long been used in a wide array of fragrance products, including perfumes and candles, there is also scientific and anecdotal evidence suggesting that they have mosquito repellent properties (Aldulaimy et al., 2021). To test the validity of this theory, we conducted an experiment to test which essential oil, if any, is the most effective deterrent. Data collected from the experiment supported previous evidence, and further established the ability of essential oils to repel mosquitoes. The previously mentioned product using engineered *E. coli* could therefore address the problem of mosquito-spread diseases using synthetic biology.

Materials and methods

A colony of *Aedes albopictus* mosquitoes was raised for the purpose of this project. The team followed the same procedures outlined in our previous publication (Aldulaimy et al., 2021), including the established protocols for care and feeding.

Over the course of two weeks, four different investigations were carried out on alternate days to test the effectiveness of jasmine, wintergreen, lavender, and limonene as natural mosquito repellents. The experiments were conducted on different days to allow the mosquitoes time to recover between tests. This also ensured that any residual scent diffused out of the experimentation room, to avoid interference with the results of the next sample being tested. Prior to experimentation, *A. albopictus* mosquitoes were sorted by sex using aspirators, and 25 female mosquitoes were transferred into a separate designated experimentation bug dorm (Figure 1). Sexing mosquitoes without the use of a microscope is possible due to the observable differences between males and females. Two visually identifiable distinctions are females' larger size and thin antennae, whereas males are smaller in size and have feathery antennae (see Figure 2).

Females were used as opposed to males because they feed on human and animal blood, whereas males only eat nectar, water, and plant sap. Females use blood as a source of nutrients to lay eggs and reproduce (Abdo, 2021). Therefore, being able to repel female mosquitoes is particularly crucial when it comes to disease prevention. Once the 25 female mosquitoes were isolated from the rest of the mosquito colony, they were starved for 24 hours prior to the experiment. This was done to ensure that they would be hungry during the test.

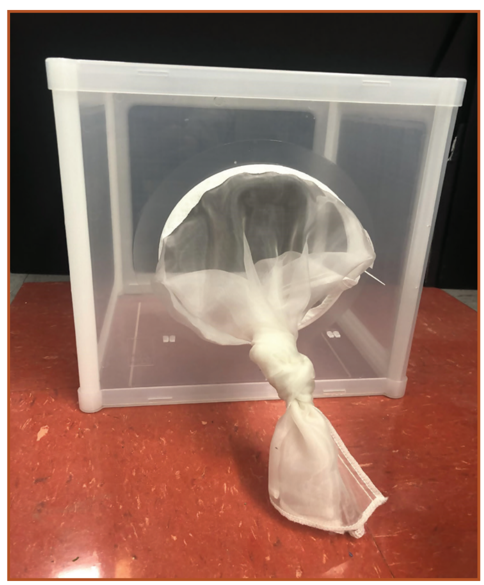

Figure 1. *One of the three 8″ × 8″ × 8″ bug dorms constructed and used to house and contain adult A. albopictus mosquitoes. The bug dorms also functioned as testing chambers for the experiments to evaluate four essential oils as potential mosquito repellents.*

Figure 2. *Image illustrating the differences between male and female A. albopictus mosquitoes. The female has a larger body and thin antennae, while the male is smaller with bushy antennae.*

On the day each essential oil was tested, a range of concentrations designated as 0% (control), 25%, 50%, and 100% solutions were prepared (as defined in Table 1). Each solution contained sucrose to entice mosquito landings, as this had previously been their primary food source. The control consisted only of sucrose solution, to compare the animals' hunger responses before and after each scent was added. A cotton ball was saturated in each essential oil concentration to be tested that day. Any avoidance of the cotton ball after the essential oil was added to the sugar solution would indicate that the mosquitoes are repelled by that scent. The solutions were separately placed in Petri dishes and then introduced to the bug dorm containing the 25 isolated and starved female mosquitoes, at different times. All experiments took place in a small 12′ × 8′ room with a closed door, where the mosquitoes were raised.

	100%	50%	25%	0% (control)
10% Sucrose	5 mL	5 mL	5 mL	10 mL
Essential oil	5 mL	2.5 mL	1.25 mL	---
Distilled water	---	2.5 mL	3.25 mL	---

Table 1. *Composition of test solutions prepared for each experiment. Each sample had a total volume of 10 mL, which was sufficient to completely saturate the cotton ball. A 10% sucrose solution was used in each sample to entice the mosquitoes to land on the cotton ball. For solutions other than the control, not landing on the cotton ball indicated that the sugar was less desirable after the scent was added. The sucrose solution comprised 50% of each test sample, aside from the control, which used sucrose solution alone. In the 100% solution, the essential oil was not diluted. The same preparations were used for each essential oil.*

This ensured that the environmental conditions they were accustomed to, including light and temperature, were maintained during the test. Before the experiment took place, a camera was positioned with a view of the mosquitoes, allowing the team to observe their activity without interfering with their behavior. In order to count the number of mosquitoes that landed on each cotton ball over each 10 min period, a stopwatch was used, and was placed in view of the camera to keep track of time. Every 30 s, the cumulative number of mosquitoes that had landed on the cotton ball was recorded. Once the experiment was over, the experimental room was aired out to clear any residual essential oil smell. Then, the mosquitoes were given a 10 min recovery period before the next concentration was tested. This process was repeated separately for jasmine, wintergreen, lavender, and limonene.

Throughout experimentation, personal protective equipment was worn—including protective goggles and non-latex gloves—to prevent contact of the essential oil with skin or eyes. The mosquitoes were raised and grown in a controlled environment, so there was zero risk of infection or transmittance of disease because they never came into direct contact with people, or animals with illnesses. The mosquitoes were acceptably taken care of throughout the investigation. They were raised in a suitable environment and were given 10% sucrose solution daily, added to a cotton ball, and allowed to feed ad libitum. After the experiment was complete, the mosquitoes were properly disposed of, safely and humanely. A 10% bleach solution was added to the container containing the larvae, while adult mosquitoes were transferred from the bug dorm into a container and were put into a freezer and euthanized.

Results

Jasmine

Jasmine was tested at 0%, 25%, 50%, and 100% concentrations to determine the effectiveness of this essential oil on mosquito repellency. For each concentration, the number of mosquitoes that landed on the cotton ball was recorded for 30 s intervals over a 10 min period, as outlined in the previous section. The results (Figure 3) showed that for the control sample (sucrose only), the cumulative number of mosquito landings was 17.

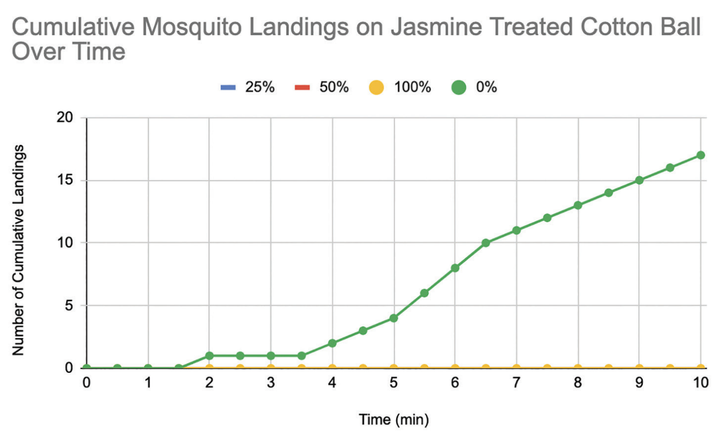

Figure 3. *Graph recording the cumulative number of A. albopictus landings on cotton balls treated with 0%, 25%, 50%, and 100% concentrations of jasmine essential oil. There is an upward trend for the cotton ball that was not treated with jasmine scent, but there were no landings for any of the test concentrations.*

This was most likely observed because the mosquitoes were starved for 24 h prior to the experiment and the cotton ball was drenched in their primary food source, an aqueous sucrose solution. In comparison, for all test samples (25%, 50%, and 100% essential oil concentrations), no mosquito landings on the cotton ball were observed. This suggests that jasmine would be a good candidate for expression by AliveSCENT.

Wintergreen

Wintergreen essential oil was also tested at 0%, 25%, 50%, and 100% concentrations to determine its effectiveness as a mosquito repellent. The results (Figure 4) showed that at 0% concentration, the cumulative number of mosquito landings was 12. Conversely, at the 25%, 50%, and 100% concentrations, there were no observed mosquito landings. This establishes that wintergreen essential oil is also a suitable candidate for expression by AliveSCENT.

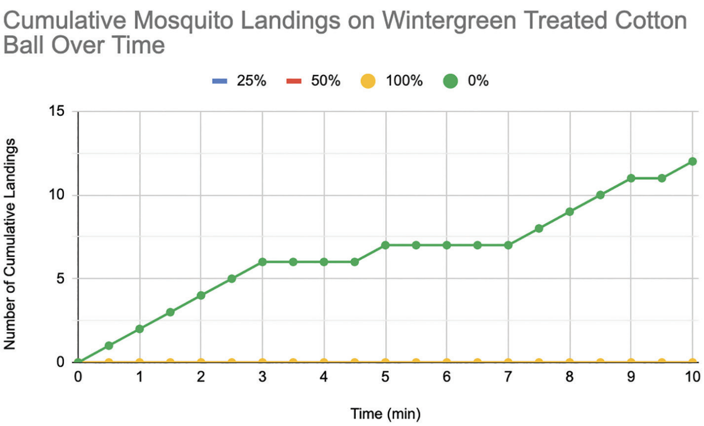

Figure 4. *Graph recording the cumulative number of A. albopictus landings on cotton balls treated with 0%, 25%, 50%, and 100% concentrations of the wintergreen essential oil. There is an upward trend for the cotton ball that was not treated with wintergreen scent, but there were no landings for any other concentrations.*

Lavender

Lavender essential oil was tested similarly, using 0%, 25%, 50%, and 100% concentrations to assess its suitability as a mosquito repellent.

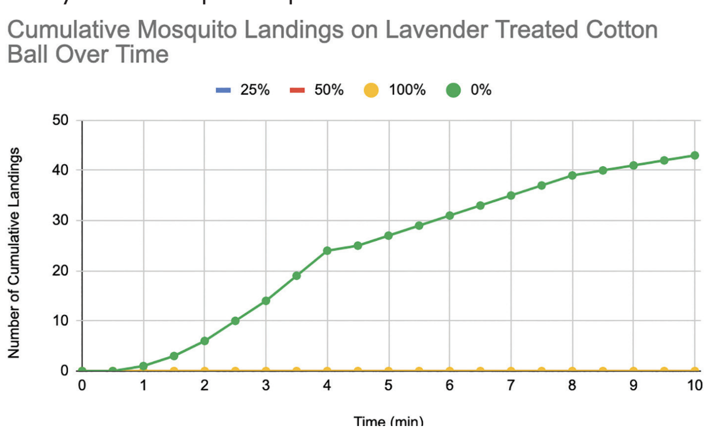

Figure 5. *Graph recording the cumulative number of A. albopictus landings on cotton balls treated with 0%, 25%, 50%, and 100% concentrations of lavender essential oil. Once again, an upward trend was observed for the untreated cotton ball, but there were no landings for any of the test concentrations.*

This time, 43 cumulative mosquito landings were seen with the control sample (0% concentration; Figure 5). In common with the previous cases, no mosquito landings were observed at the 25%, 50%, and 100% concentrations. Thus, lavender is also an appropriate candidate for use in AliveSCENT.

Limonene

Limonene was also tested for its mosquito repellent properties, using the 0%, 25%, 50%, and 100% concentrations as for previous experiments. At 0%, four cumulative mosquito landings were observed over the 10 min test period (Figure 6). Contrarily, at the 25%, 50%, and 100% concentrations, no mosquito landings were detected.

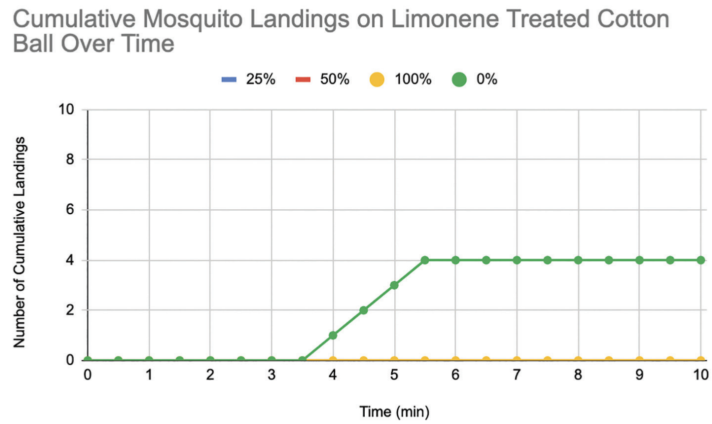

Figure 6. *Graph recording the cumulative number of A. albopictus landings on cotton balls treated with 0%, 25%, 50%, and 100% concentrations of limonene essential oil. There is an upward trend for the cotton ball that was not treated with limonene scent. There were no landings for any other concentrations.*

Discussions

The purpose of this study was to evaluate the effectiveness of various monoterpene-containing essential oils as mosquito repellents, with the intention that the scent determined to be the most effective would be used to construct AliveSCENT: a biologically produced mosquito deterrent. Such a product would have the potential to minimize the spread of mosquito-borne illnesses and prevent thousands of deaths. It is this team's hope, and our ultimate aim, that AliveSCENT could be made available commercially for use by the general public. In pursuit of this goal, we tested the effects of four scents: jasmine, wintergreen, lavender, and limonene.

This paper documents research on AliveSCENT over the past three years. Previous efforts to investigate the effectiveness of limonene yielded inconclusive results, since no mosquitoes landed on the control cotton ball (Aldulaimy et al., 2021). Thus, the control and test samples could not be meaningfully compared. Possible sources of error included the low volume of limonene used to prepare the treated cotton balls, which were not fully soaked. In addition, moving the mosquitoes from their nursery to a temporary testing location with bright lights and more people was problematic. This is because mosquitoes thrive in an environment that is dark and moist, and any change could result in altered behavior. We attempted to remedy these issues through improved experimental design, and also chose to expand the study to include other scents in case limonene was proven to be ineffective. More specifically, the experiment was adjusted so that the cotton balls were soaked in 10 mL of solution, as opposed to 2 mL, ensuring they were fully saturated. The mosquitoes were kept in the same location throughout the duration of the study. In addition, we used a webcam to record the movement of the mosquitoes in their nursery during each test. These recordings were then viewed retrospectively to ensure that the mosquitoes remained undisturbed, and could react as naturally as possible to each set of conditions in their bug dorms.

These modifications to the experimental protocols yielded far more success. Mosquitoes did land on the control cotton ball, in every case. Their avoidance of the sucrose-soaked cotton ball after the addition of each essential oil provides proof that these monoterpene-containing scents are able to deter the insects. However, the results did not assist in narrowing down the search for the most effective scent, as the mosquitoes avoided the cotton ball for every essential oil, regardless of concentration. We eventually chose the lavender scent, as it showed the most drastic change in mosquito landings between the control and essential oil conditions (Figure 7). Furthermore, our team is already familiar with the biosynthetic pathway for (*S*)-linalool, a major constituent of lavender oil, due to prior research on limonene (Figure 3). Both compounds are synthesized via the methylerythritol 4-phosphate (MEP) pathway, and are derived from the same precursor molecule, geranyl diphosphate (GPP). The present results are therefore encouraging, and mark the beginning of a new phase in the project: synthesizing (*S*)-linalool in *E. coli*. This development has paved the way for AliveSCENT to become a reality.

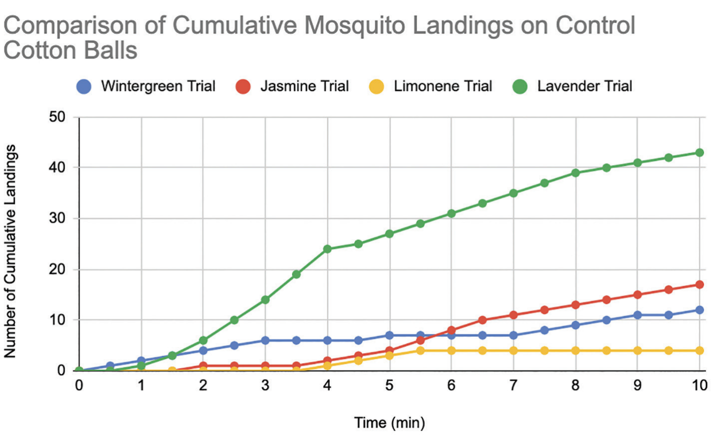

Figure 7. *Graph comparing mosquito landings on the control cotton balls (saturated with 10% sucrose solution only) that were used for each essential oil assessment. The cumulative number of landings was 17, 12, 43, and 4 for the jasmine, wintergreen, lavender, and limonene trials, respectively.*

Next steps

As mentioned above, the experimental results suggested that all four scents were equally effective at repelling mosquitoes. Having decided to use the lavender scent for AliveSCENT, and to facilitate the production of (*S*)-linalool in *E. coli*, a novel genetic device was designed for bacterial transformation. This device comprises five parts: a stationary phase promoter, a ribosomal binding site, a GPPS coding sequence, an additional ribosomal binding site, LINS, and a double terminator (Figure 9).

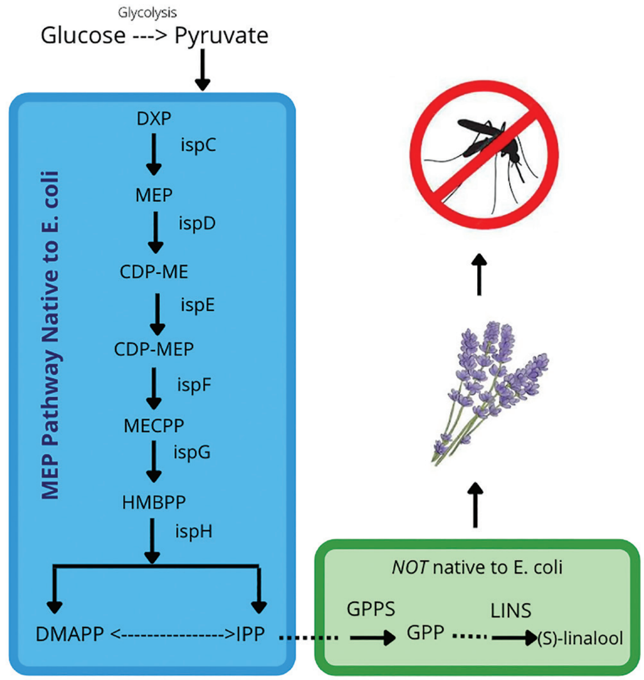

Figure 8. *General schematic of AliveSCENT. Pyruvate would feed into the native MEP pathway (shaded blue) of E. coli and eventually be converted to dimethylallyl pyrophosphate (DMAPP) and IPP. This would be transformed into (S)-linalool with the addition of GPPS and LINS (shaded green). (S)-Linalool would emit a lavender scent that would repel mosquitoes.*

The pBbE5a-RFP plasmid backbone will be used because it codes for ampicillin resistance and contains a red fluorescent protein (RFP) marker. Successfully transformed cells will be visually detected by the expression of this protein, because transformants will appear red under visible light (with excitation/emission maxima of 558 and 583 nm, respectively). The AliveSCENT plasmid will be constructed through Gibson Assembly Cloning, verified by sequencing, and then further transformed into *E. coli* (Figure 10). We will then extract the (*S*)-linalool from the bacteria and test the effectiveness of the bacterially produced compound as a mosquito repellent in the same manner reported in this paper.

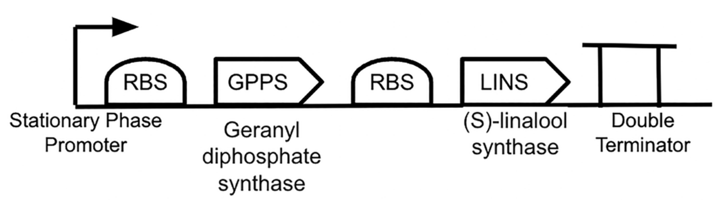

Figure 9. *Parts-level description of AliveSCENT. This genetic device is composed of a constitutive promoter (BBa_I14018), a ribosomal binding site (BBa_J01010), GPPS (BBa_K2753002), a second RBS, a LINS sequence (BBa_K3052003), and a double terminator (BBa_B0015).*

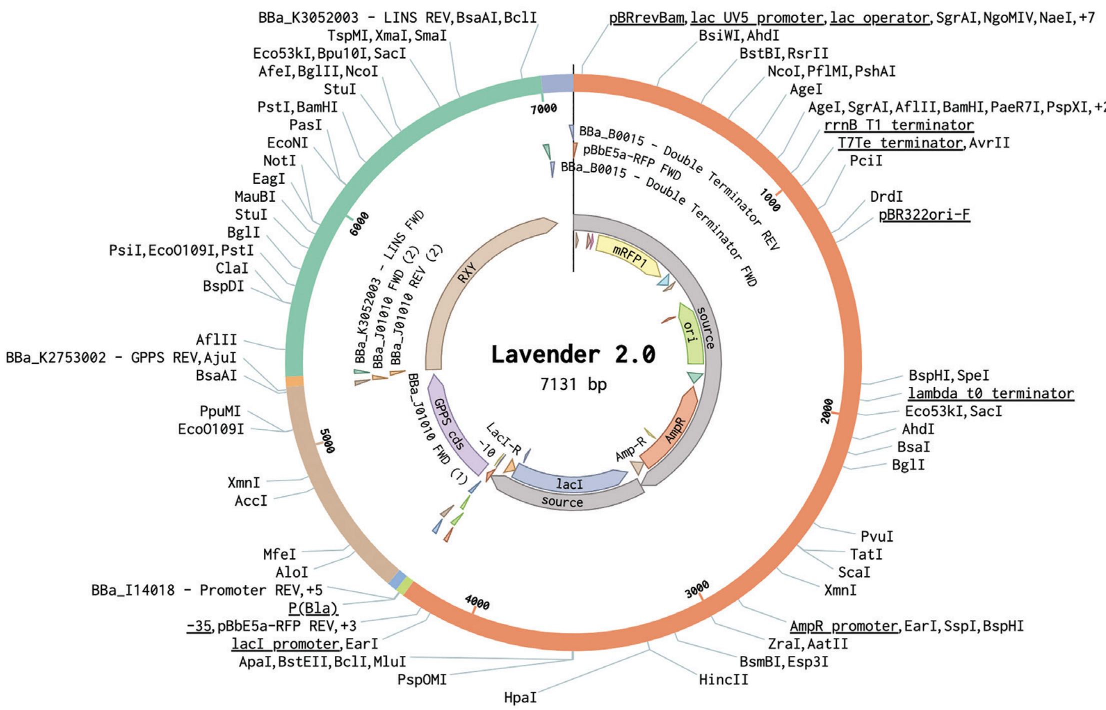

Figure 10. *The AliveSCENT plasmid contains a genetic device designed by the team (Figure 9) inserted into the pBbE5a-RFP plasmid from Addgene as a backbone. This backbone was chosen because it has both ampicillin resistance and an RFP marker. The RFP would allow for identification of transformed cells, as they would fluoresce red when placed under wavelengths that can excite RFP (e.g., 532 nm laser).*

While the present results demonstrate that lavender is an effective deterrent against mosquitoes, they do not indicate the concentration threshold below which the scent is no longer effective. Therefore, it is planned to further characterize (*S*)-linalool by incrementally testing at lower concentrations. As opposed to 100%, 50%, 25% and 0% lavender oil, the experiment will be done using concentrations of 25%, 12.5%, 6.25%, 3.125%, and 0%. This should allow us to determine the minimum effective concentration of (*S*)-linalool as an insect repellent.

Author contributions

Y.C., E.F., and D.T. conducted background research. Y.C. and E.F. designed the experimental protocols. Y.C. and E.F. conducted nursery assembly and mosquito care. Y.C., E.F., and D.T. performed the final experiment. Y.C., E.F., and D.T. wrote and edited the abstract. Y.C. created and developed the video. Y.C. and A.J. created the manuscript graphics. Y.C., E.F., and D.T. wrote the manuscript, which Y.C. and E.F. edited. Y.C. and D.T. designed the plasmid. Y.C. and E.F. compiled references.

Acknowledgements

The THS BioBuilder Club would not have been able to conduct the AliveSCENT project without the help of many amazing people. First and foremost, we would like to extend our thanks to Dr. Natalie Kuldell for catalyzing and fostering our scientific journeys. Through the creation of the BioBuilder platform, Dr. Kuldell has supported us in our scientific pursuits in real and meaningful ways and has paved the way for our communication and collaboration with real-world scientists. We would also like to extend our gratitude to our amazing mentor Michael Sheets from the Dunlop Lab at Boston University. He has been an invaluable resource crucial to the progression of our projects. He provided a multitude of creative ideas on how to execute our ideas and patiently led our team through the steps of developing a plasmid on Benchling. In addition, a special thanks go to Dr. Joseph Wagman for laying the foundation of our experiment through his advice, expertise, and donation of the *Aedes albopictus* eggs from Dr. Diana Ortiz's colony. We thank all of the Tyngsborough High School administration, who allowed our team to raise mosquitoes and perform our experiment within the school. We would also like to thank Sophia Lundberg for allowing the team to use her art from last year's publication for Figure 2. Last, but certainly not least, we would like to thank Dr. Rebekah Ravgiala, our club advisor, for her continued support and long hours spent helping us throughout our BioBuilderClub journey.

References

Abdo, L. B. (2021). Why do mosquitoes suck your blood? *Little Medical School*. https://littlemedicalschool.com/ottawa/blog/why-do-mosquitoes-suck-blood/

Aldulaimy, M., Angelo, E., Collins, Y., Cote, G., Fugazzotto, E., Lundberg, S., Patel, M., Puma, S., & Riley, C. (2021, October 25). AliveSCENT: Evaluating the potential use of limonene as a mosquito repellent. *BioTreks*. https://biotreks.org/e202115/

Centers for Disease Control and Prevention. (2019, August 15). *Fighting the world's deadliest animal*. https://www.cdc.gov/globalhealth/stories/2019/world-deadliest-animal.html

Insecticide poisoning. (n.d.). Mount Sinai. Retrieved April 29, 2022, from https://www.mountsinai.org/health-library/poison/insecticide-poisoning

Keeffe, P. (2016, February 23). *Mosquitoes: The most dangerous animal on earth?* Healthline. https://www.healthline.com/health-news/mosquitos-the-most-dangerous-animal-on-earth-021216

Rocklöv, J., & Dubrow, R. (2020). Climate change: An enduring challenge for vector-borne disease prevention and control. *Nature Immunology*, *21*, 479–483. https://doi.org/10.1038/s41590-020-0648-y