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Article

Redefining and advancing tree disease diagnosis through VOC emission measurements

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SUMMARY

In the United States, 25% of tree loss is due to infestations and disease. Pesticidal treatments can save trees during early stages of infestation, which is a challenge because symptoms appear late. Previous studies evaluated the potential of volatile organic compound (VOC) emissions as an early infestation detection method, currently limited by expensive technology that cannot be used on a widespread scale. We hypothesized that an affordable and accessible Adafruit SGP40 gas sensor can detect differences between VOC emissions of diseased and non-diseased trees for ash and beech trees, but not for maple trees, since the maple tar spot disease does not harm the health of the tree. Bark VOCs of 19 non-infested ash and 15 infested ash were measured, revealing statistically significant differences between those categories ($p < 0.05$). Leaf VOC emissions of 40 American beech and 40 Norway maple were studied in the field. We compared the emissions of symptomatic leaves from diseased trees with asymptomatic leaves from diseased trees and leaves from non-diseased trees. For both beech and maple, we found statistically significant differences between the VOC emissions of leaves from diseased and non-diseased trees. These promising results indicate this affordable gas sensor could be used for early detection of multiple tree diseases. Future research should validate the results using a larger sample size, include a larger diversity of locations, and apply the sensor to other tree diseases.

INTRODUCTION

Every year, the overall tree population in the United States decreases. From 2002 to 2022, the loss is estimated at 46.5 million hectares of tree cover. This is due not only to droughts, wildfires, and logging, but also to tree diseases and infestations (1). For example, ash trees (*Fraxinus spp.*) are endangered due to infestation by the emerald ash borer (*Agrilus planipennis*) (2). Once infested, ash trees have only a one percent chance of survival without human intervention (2). The American beech (*Fagus grandifolia*) is facing a similar threat. The beech leaf disease was first observed in 2012 in Ohio, and its spread is outpacing our understanding of its pathology (3). Shown to be caused by a nematode (*Litylenchus crenatae mccannii*), the disease creates "bands" on leaves that impede overall health (3). Maple tar spot disease is another prevalent disease that has affected maple trees (*Acer platanoides*). Although the tar spots do not threaten short-term tree health, they have the potential to interfere with photosynthesis and weaken maple health long-term (4). The presence of maple tar spot disease has been recently amplified by increased pollution (4). While many of these illnesses can be prevented or treated with pesticides, treatments must be implemented at early stages of sickness for success (5). Early detection is a challenge because visible outer signs often appear during late stages of infestation (5).

Recent research has studied the variability between the volatile organic compound (VOC) emissions of infested and non-infested trees as a potential infestation detection technology (6). VOC is an umbrella term referring to a large category of organic molecules with high vapor pressures at standard atmospheric conditions (7). VOCs have been discovered to play crucial roles in plant life. All plant tissues produce VOCs (8). There are two categories of VOCs: constitutive VOCs (regularly produced volatiles) and inducible VOCs (volatiles that are released after outside stresses) (8). Plants have developed sophisticated VOC emission mechanisms that provide them with survival advantages (9). For instance, VOCs aid in attracting pollinators, defending against herbivores by stimulating feeding on insect eggs and leading to decreased oviposition, and facilitating plant-to-plant communication (6,9,10).

Research has been conducted to study the potential of VOC emissions as a non-invasive infestation detection method. Apple trees infested by phytophagous mites were found to have increased volatile emission levels (11). Volatile emissions have been used in the detection of the striped rice stem borer (*Chilo suppressalis*) in infested rice fields (12). Another study compared different infestation-induced health decline classes of green ash trees using gas chromatography and an electronic olfactory device (e-nose) (13). It was found that there were no meaningful differences in volatiles in outer bark samples, but differences in patterns, quantities, and kinds of molecules were observed in sapwood samples (13).

The American ash tree population is decimated by the emerald ash borer, an invasive beetle that damages trees by destroying their phloem and xylem, which becomes fatal after long infestation periods (2). Because symptoms of emerald ash borer infestation (canopy dieback, epicormic shoots, d-shaped exit holes, cracks in bark) appear when the tree has declined beyond repair, it is imperative that a reliable early infestation detection method is developed.

The beech leaf disease has diffused throughout the East Coast (14). It is characterized by crinkled or "banded" leaves, linked to a nematode that originates in the leaf buds of the tree (15). The banding interferes with leaves' abilities to photosynthesize, and sufficient nematode presence can cause canopy reduction, killing the tree in 6-10 years (14).

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Early disease detection is crucial to treatment application. A previous study looked towards machine learning and spectral measurements as a potential beech leaf disease detection method, but no techniques are in common use yet (16).

A disease caused by the fungus family Rhytisma, the maple tar spot disease, originated in the 1800s in Europe (4). A direct correlation has been shown between pollution and increased tarred maple leaves (4). Ever since the disease was first spotted in the United States in 1940, it has spread across the country (4). With increased pollution in the modern era, the disease has grown more abundant. The tar spots could potentially interfere with photosynthesis and weaken maple health long-term (4). Learning more about the effect of the tar spots on maple leaf VOC emissions could provide valuable information about this disease.

In previous research, VOC measurements were mainly performed using mass spectrometry and gas chromatography techniques, which identify the types and quantities of volatile compounds (9, 10, 11). These procedures require heating plant samples to release the volatiles from within in a laboratory setting with specialized equipment. While these methods have led to the identification of significant variations in volatile patterns between infested and non-infested trees, they cannot be used as a widespread diagnosis tool due to their complexity and high cost.

There are currently no established tree disease detection tools available that are practical, affordable, and usable on a widespread scale. This study focused on evaluating the potential of a gas sensor in measuring VOC emission differences between diseased and non-diseased trees in the field. We studied ash infestation, beech leaf disease, and the maple tar spot disease to show a broad application of the sensor among various tree diseases. We hypothesized that the gas sensor would detect a difference between bark VOC emissions from infested ash and non-infested ash, as well as between the VOC leaf emissions of diseased and non-diseased beech. However, we did not expect to measure a significant difference between emissions of tarred and non-tarred maple leaves because the tar spot disease is not known to affect tree health, so VOC emissions should not be altered. This study demonstrated that diseased ash, beech, and maple trees consistently emit lower VOC levels compared to healthy trees, with statistically significant differences across all species. These findings suggest that VOC emissions could serve as a reliable, non-invasive indicator for early disease detection in trees, offering potential for improved forest health monitoring and management.

RESULTS

We sought to assess an Arduino-connected Adafruit SGP40 gas sensor's ability to measure VOC emission differences between diseased and non-diseased trees. We tested ash infestation (bark samples), beech leaf disease (two leaves per non-diseased tree, two banded leaves and two non-banded leaves per diseased tree), and the maple tar spot disease (two leaves per non-diseased tree, two tarred leaves and two non-tarred leaves per non-diseased tree) in-field at multiple different locations in the Westchester County area.

For ash trees, the mean (112 for infested trees, 227 for non-infested trees), medians (78 for infested trees, 234 for non-infested trees), and the 95% confidence intervals (66-158 for infested trees, 176-278 for non-infested trees) of VOC emission measurements were higher for non-infested trees than for infested trees (**Figure 1**). There was more variability of VOC values for the non-infested trees group, as well as overall higher VOC values than for the infested tree group. This suggests that a healthy ash tree has a more dynamic VOC emission profile. The VOC emissions from ash trees showed a statistically significant difference between infested and non-infested trees (two-tailed p-value = 0.00065). There was no overlap between the 95% confidence intervals for infested and non-infested trees, and VOC emissions were significantly different between the two groups (**Figure 2**).

For beech trees, the mean VOC emission value was smallest for banded-diseased, larger for non-banded diseased, and largest for non-banded non-diseased. The medians are in accordance with the same trend (**Figure 3**). The inter-quartile range of non-banded non-diseased was

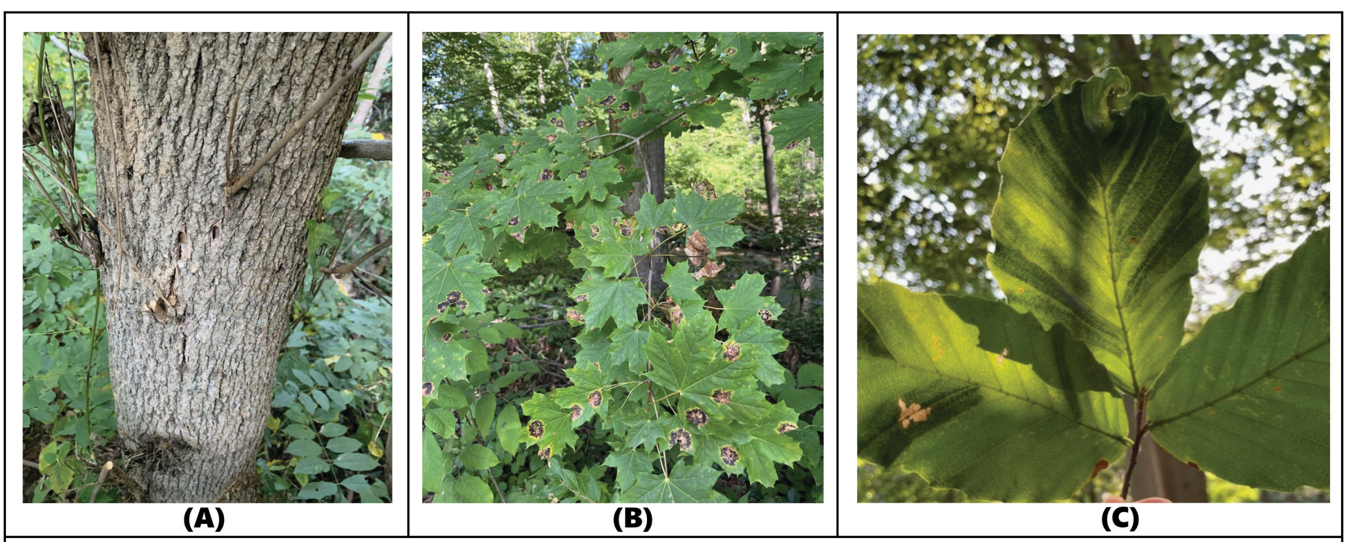

Figure 1: Symptoms of different tree diseases. A: Bark of infested ash (Fraxinus) shows cracked bark (red arrow), epicormic shoots (white arrow), and d-shaped exit holes (yellow dotted arrow). B: A Norway maple tree (*Acer platanoides*) containing tarred leaves (black arrow). C: Image displaying banded beech (*Fagus grandifolia*) leaves. Bands (yellow arrow) were often visible only when leaves were held in sunlight.

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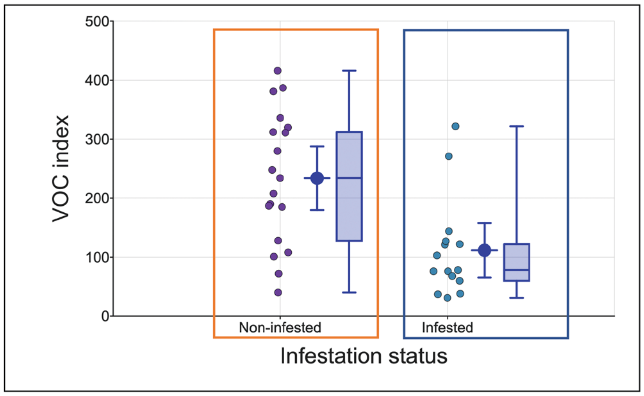

Figure 2: Distribution of VOC Emissions for Infested and Non-Infested Ash Trees. This figure shows the bark VOCs of 19 non-infested ash and 15 infested ash that were measured. When a tree was selected to be tested, the device was turned on, and the program was allowed time to run (for generally five minutes), letting the VOC emissions stabilize. The VOC index was calculated by subtracting the stabilization VOC emissions from the bark measured VOC emissions. Figure reveals a statistically significant difference between those categories ($p < 0.05$). The large dot and corresponding vertical lines show the mean and standard deviation, and the box-and-whiskers plot contains the median and the inter-quartile range. The orange rectangle includes all the VOC indexes from the non-infested trees. The blue rectangle includes all the VOC indexes from the infested trees.

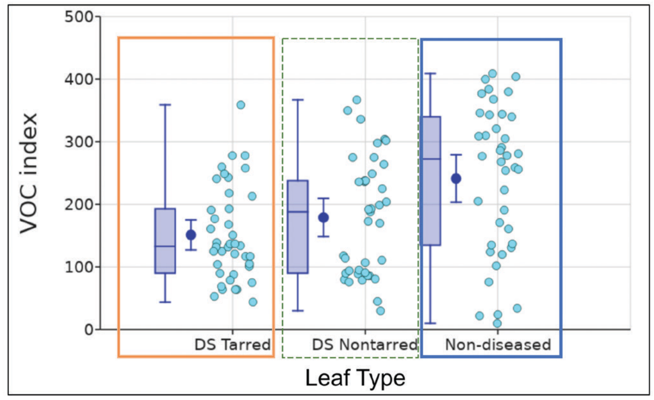

Figure 4: Distribution of VOC Emissions for Three Categories of Maple. This figure shows the VOC emissions of three categories of maple leaves. The orange rectangle includes all the VOC emissions from the tarred leaves of diseased trees. The green-dotted rectangle includes all the VOC emissions from the not-tarred leaves of diseased trees. The blue rectangle includes all the VOC emissions from the non-banded leaves of non-diseased trees. DS is an abbreviation for disease. There were significant differences, as measured through a two-tailed *t*-test ($p < 0.01$) between non-diseased maple and diseased leaves from both diseased categories (tarred diseased and non-tarred diseased) whereas the comparison between tarred diseased and non-tarred diseased revealed insignificant differences. The large dot and corresponding vertical lines show the mean and standard deviation, and the box-and-whiskers plot contains the median and the inter-quartile range.

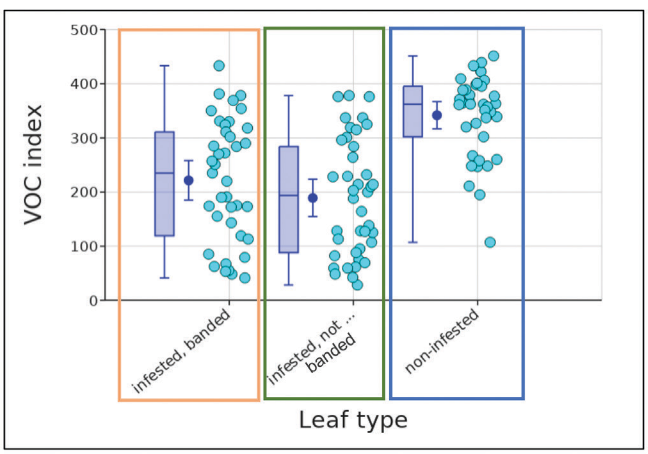

Figure 3: Distribution of VOC Emissions for Each Category of Beech Leaves. This figure shows the VOC emissions of three categories of beech leaves: infested and banded, infested tree but non-banded leaves, and from non-infested trees. Each light blue dot represents the calculated emission value of a beech leaf from a unique tree. There were significant differences, as measured through a two-tailed *t*-test ($p < 0.01$) between non-banded non-diseased and both diseased categories (banded-diseased and non-banded diseased) whereas the comparison between banded-diseased and non-banded diseased revealed insignificant differences. The large dot and corresponding vertical lines show the mean and standard deviation, and the box-and-whiskers plot contains the median and the inter-quartile range. The orange rectangle includes all the VOC emissions from the infested-banded leaves. The green rectangle includes all the VOC emissions from the infested trees but not banded leaves. The blue rectangle includes all the VOC emissions from the non-banded leaves from non-infested trees.

smaller than those of non-banded diseased and banded diseased.

There were significant differences ($p < 0.01$) between non-banded non-diseased and both diseased categories (banded diseased and non-banded diseased) whereas the comparison between banded diseased and non-banded diseased revealed insignificant differences. Non-banded non-diseased leaves had higher VOC emissions than banded diseased and non-banded diseased leaves (**Figure 3**).

For maple, the results were similar. Leaves from non-diseased trees exhibited the highest mean VOC emissions, whereas tarred-diseased and non-tarred diseased leaves recorded generally lower values. Similar to ash trees, there was minimal overlap between the 95% confidence intervals of non-tarred non-diseased and both diseased categories (Figure 4). There were significant differences between the VOC emissions ($p < 0.05$) of non-diseased maple leaves and those from diseased leaves in both categories (tarred diseased and non-tarred diseased), whereas the comparison between tarred diseased and non-tarred diseased leaves showed no significant difference (**Figure 4**).

We compared various VOC emission indexes (numerical values that positively correlate with VOC concentration) to determine which index serves as the best threshold for disease diagnosis, for which values falling below are considered diseased and above are non-diseased. The VOC emission index of 300 had the highest beech diagnosis accuracy of 77% with a sensitivity of 78% and a specificity of 76% (**Table 1, Figure 5**).

DISCUSSION

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	Diseased Nonbanded	Non Diseased	
Tested Positive	31	9	Positive Predictive Value (Precision) 0.78
Tested Negative	9	29	Negative Predictive Value 0.76
	Sensitivity 0.78	Specificity 0.76	Accuracy 0.77

Table 1: Confusion Matrix for Optimal Beech Leaf Disease Threshold Value of 300. This table shows the calculation of the sensitivity and specificity, positive predictive value, negative predictive value and accuracy for a VOC index of 300.

Overall, we showed a significant difference between VOC emissions of diseased and non-diseased trees, in which the latter generally had higher overall emissions. This is consistent with previous research that studied tree VOC variations, such as a prior study that measured ash tree VOC emissions using gas chromatography (13). My hypothesis that we could measure significant differences between diseased and non-diseased beech and ash tree emissions was supported. However, we did not find significant differences between diseased and non-diseased maple emissions. Possible explanations for the generally lower VOC emissions of diseased trees across all three species could be either an adaptive lowering of emissions to communicate distress or a compromised VOC emission capacity among diseased trees (6).

We also found a trend, though not statistically significant, between the two categories of leaves from diseased beech trees (banded diseased and non-banded diseased), with banded leaves having overall higher VOC emissions. This could suggest a spike in emissions in banded leaves as a distress signal that non-banded leaves do not exhibit. Additionally, the nematodes in the banded leaves could have a VOC contribution of their own, though further research is required to explain these results. This is a plausible explanation because the sensor detects multiple types of VOCs. Conversely, non-tarred leaves from diseased maples had higher overall emissions than tarred leaves. It can be hypothesized that the maple tarred leaf is not emitting as high a concentration of VOCs because the tar itself blocks the VOC release or alternatively, the fungus itself produces minimal VOC emissions. Nevertheless, the differences in VOC emissions between diseased and non-diseased maple suggest that the tar spot disease may have an effect not yet understood.

The consistent finding that non-diseased trees have overall higher leaf VOC emissions could provide insight on the way plants alter their emissions in response to nematode or fungus infestation, suggesting there is a deliberate or coincidental lowering of emissions upon infestation. It is possible that different tree species facing different diseases may alter their VOC emissions in different ways, both qualitatively (kinds of VOCs emitted) and quantitatively (VOC emission rates). We have shown that the Adafruit SGP40 gas sensor is capable of detecting both bark infestations and leaf diseases. Although fully asymptomatic diseased trees could not be tested, the fact that asymptomatic leaves from diseased trees had VOC differences is promising and can suggest that fully asymptomatic diseased trees may also have a VOC difference.

There are some potential limitations to the study – for example, we did not confirm nematode absence in non-diseased trees by PCR or direct leaf extraction. It might

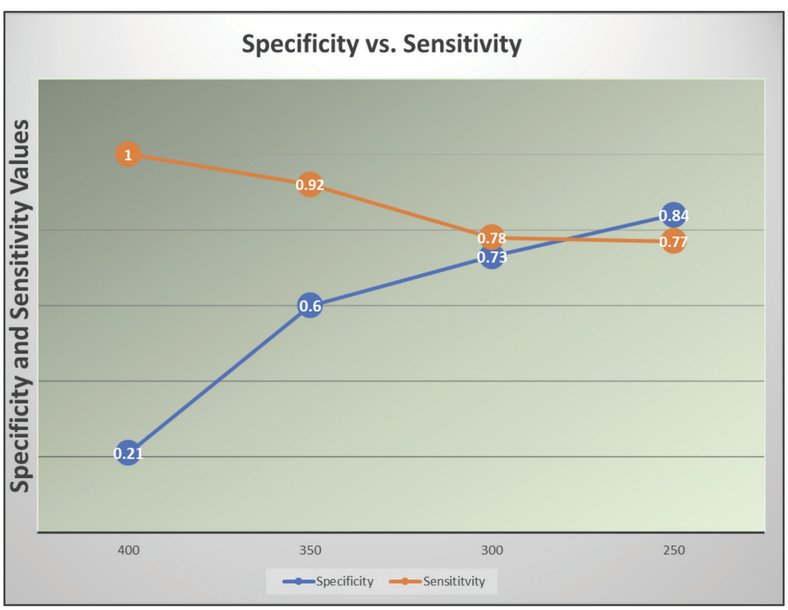

Figure 5: Specificity and Sensitivity for Multiple Thresholds. The chart shows the specificity (blue line) and the sensitivity (orange line) represented on Y axis of different VOC thresholds represented on X axis for ash diagnosis. The VOC threshold of 300 is the optimal accuracy with the specificity of 0.73 and a sensitivity of 0.78.

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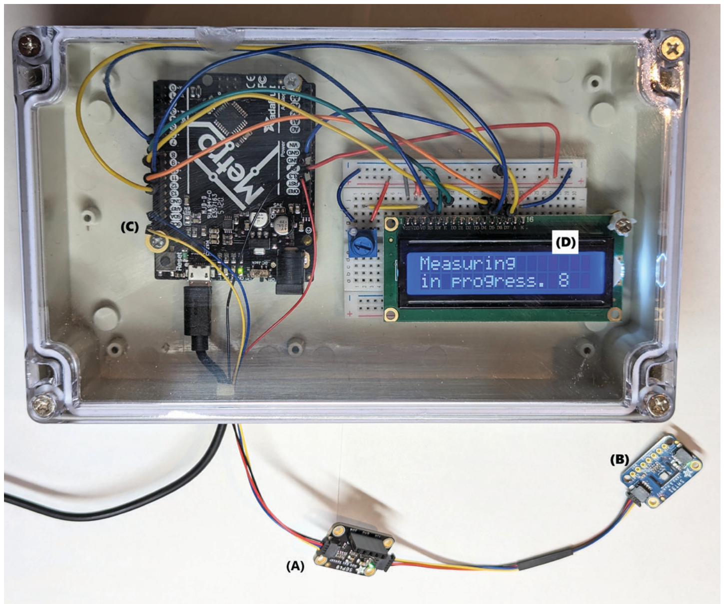

Figure 6: Testing System with LCD Screen Displaying Disease Verdict. This figure shows the measuring device during a measuring cycle. It consists of the SGP40 gas sensor (A), SHT31 humidity and temperature sensor (B), and LCD screen (D) which are all connected to the microcontroller running the application (C).

have been possible that some of the trees classified as non-diseased could've been in very early stages of infestation, too early to affect tree health or VOC emission. Another limitation was a limited location diversity, as all trees were tested in one county. This could mean that the results cannot be generalized to areas outside of the testing region. Lastly, the ability of the sensor to only measure quantitatively and its inability to differentiate among types of VOCs was a limitation. There may be variations in the kinds of VOCs emitted, which could make diagnosis more specific and accurate (7).

Future research should test fully asymptomatic trees that are confirmed to be infested by nematode detection. It would also be useful to further investigate the reasons for why variations in VOC emissions between diseased and non-diseased trees occur.

The significant difference between emissions of diseased asymptomatic leaves and non-diseased beech leaves suggests a promising potential of VOC measurements as an early disease infestation method, because these leaves appear identical but vary in their emissions. In the future, this technique should be standardized and made accessible to the public.

Based on the significant differences in VOC emissions between diseased and non-diseased trees, a portable, affordable, and easy-to-use gas sensor can be implemented on a large scale to detect beech, ash, and maple diseases. Moreover, after further testing, it may have the potential to be used by arborists and homeowners in the detection of other diseases as well. The device, if effectively applied, can be used as a tool to optimize pesticide treatment, helping to save species affected by disease from endangerment or even regional extinction.

MATERIALS AND METHODS

To measure VOCs from tree bark and leaves, we used a testing device consisting of an Adafruit SGP40 gas sensor, SHT31 humidity and temperature sensor, Arduino microcontroller, and an LCD screen (**Figure 6**). The gas sensor provides information about the total VOC emission levels. As described by the Adafruit manufacturer, changes in the sensor's electrical resistivity due to the presence of VOCs can be converted through a logarithmic algorithm into numerical VOC index values on a scale of 0-500, where a larger VOC index corresponds to a higher concentration (18). This conversion was automatically done through a computer program found in an Arduino library for the SGP40 sensor. The Arduino SGP40 program was modified so that it would display the measured VOC indexes on a laptop, then stored in Excel worksheets for analysis. Because the SGP40 sensor's readings are affected by humidity and temperature, an Adafruit SHT31 sensor was wired to an Arduino so those variables (humidity and temperature) could be measured and controlled for by the program. An app for the Adafruit microcontroller was created in the Arduino programming language to automate calculations for disease diagnosis. It provides instructions and makes the VOC measurement process user-friendly, displaying the disease verdict on the LCD screen.

The monitoring and managing ash (MaMA) website was used to find the location of ash trees along the Bronx River Pathway, Greenburgh Nature Center, Weinberg Nature Center, Bartlett Preserve, and the New York Botanical Garden (NYBG) (17). Trees were identified as ash by diamond-patterned bark and elliptical, odd-compounded leaf patterns. MaMA guidelines were used for determining if a tree was infested: the presence of epicormic shoots, canopy dieback, d-shaped exit holes, and larva galleries all indicated infestation (Figure 1A). Ash trees were considered infested if they had at least two of the symptoms. Non-infested ash had none of the symptoms. The VOCs of 19 non-infested trees and 15 infested trees were measured in total.

American beech trees were selected randomly at multiple locations at the NYBG and Bronx River Pathway. They were identified by their characteristic smooth gray bark and elliptic, jagged leaves (Figure 1C). Beech trees containing no banded leaves were diagnosed as non-diseased, and diseased beech were identified by the presence of any banded leaves. Beech containing the beech bark disease, which causes warts on tree trunks, were excluded. The VOCs of 20 diseased and 20 non-diseased beech trees were measured in total.

Norway maple trees were tested at the NYBG and Bronx River Pathway. Maple trees with no tarred leaves were considered non-diseased, and vice versa (Figure 1B). 20 diseased and 20 non-diseased maple samples were tested.

When arriving at a tree, the device was turned on, VOC indexes were allowed to stabilize, and the stabilization index (atmospheric baseline reading) was recorded to exclude ambient atmospheric VOCs. To measure VOC emissions from ash trees, inner bark was analyzed because prior research concluded there was no difference between outer bark samples of infested trees and non-infested trees (13). A superficial incision of 1 cm^2 area and varying depth needed to reach inner bark was cut. The incision was made at diameter breast height (DBH), 4.5 ft above the ground. After the values stabilized, the SGP40 sensor was inserted in the incision and covered with a plastic bag to contain emitted VOCs. The position was maintained until a new stabilized value was reached, typically around two minutes.

The testing procedure was identical for both beech and maple trees. The SGP40 sensor was placed in an unsealed Ziploc bag to allow stabilization to both the VOCs of the plastic and the environment. After the readings stabilized, a leaf from the tree was immediately placed into the bag to be tested. The Ziploc bag was replaced, and the procedure was repeated for each sample group, I tested two leaves of each category per tree tested.

The VOC emission value for every tree was calculated by subtracting the initial stabilization index from the final stabilization. For each species, each sample group was statistically compared to the other groups from the same species using a two-tailed *t*-test. The significance threshold was $p < 0.05$. Data Classroom was used for graphing and analysis.

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