JOURNAL OF
EMERGING INVESTIGATORS

Article

# Impact of environmental stressors on ultrasonic acoustic emissions in different species of plants

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## SUMMARY

**As climate change continues to adversely impact our planet, there is a growing call to ensure plant growing methods are efficient, sustainable, minimize damage from pest attacks and make good use of our limited resources, such as supply of clean water. Current horticulture techniques also rely on the widespread use of pesticides, which result in environmental pollution. These limitations could be reduced by instead targeting individual plants. Optimizing growth at the individual plant level requires the ability to detect that plant's stress signals and apply the correct response. Prior research has shown that plants emit more using airborne ultrasonic sounds when under stress. We hypothesized that the rate, frequency and duration of acoustic emissions from plants would differ by species and that these characteristics would change when exposed to different environmental stressors. Using ultrasonic sound detectors in soundproofed boxes, we recorded plants under normal conditions and under a range of stressors. Under normal conditions, we found that there was species level variation with cabbage plants having the highest rate and duration of acoustic emissions, while garlic and almonds emitted fewest sounds and of shorter duration. We also found that the pattern of acoustic emission changed with the rate of emissions increasing by a statistically significant amount with four of the five stressors tested. Our findings may allow growers to interpret the sound emissions from the different species of plants to identify and sustainably address stressors.**

## INTRODUCTION

With climate change adversely impacting our planet, plant growing methods need to be efficient and sustainable. Current horticulture techniques waste significant quantities of scarce water and require widespread and excessive use of pesticides, because it is not possible to personalize their use at the plant level. For example, California's farmers use 80% of the state's water supply, about 1.1 trillion gallons, and nearly 20% of all US pesticide use, mainly on berries and nuts (1,2).

Delivering resources at an individual plant level would reduce the waste associated with the current methods. To achieve this, a horticulturist would require a method of detecting individual plant stress for crop species. In 2019, research found that tomato and tobacco plants emit airborne acoustic sounds when put under extreme levels of drought and pruning (3–5). Our research extends the prior research to use sound emissions to differentiate between a range of plant species as well as types and levels of stress.

We hypothesized that the rate, frequency and duration of acoustic emissions from plants would differ by species and that these characteristics would change when exposed to different environmental stressors. Specifically, we sought to understand (1) whether different plant species could be distinguished by acoustic emissions pattern, (2) whether an increase in stress correlated with increases in emissions, and (3) if the frequency (Hz) profile of acoustic emissions under different stressors could identify specific plant species from within a mix of plant species.

We completed 20 different experiments, recording over 250 hours of plant acoustic emissions. We tested five different stressors (drought, adverse sounds, pruning, contaminated water and a bug stressor on over 200 individual plants from five plant species (almond, strawberry, cabbage, sage and garlic). From this, we found evidence to support our hypothesis: our analysis of frequency, intensity, rate, and duration of sound emission found that plants do emit sounds, and these vary by species and that they emit louder and more frequent sounds at potentially different frequencies in stressful environments.

This understanding of the acoustic emissions of different species of plants may help growers respond more efficiently and sustainably to the needs of different plants and identify and manage plants under specific stressors within large fields. In the future, a system that uses this ultrasonic capability could revolutionize how growers plan for and manage plant growth.

## RESULTS

To determine whether acoustic emissions could be used to detect the presence of stress on individual plants, we recorded over 250 hours of plant acoustic emissions across 20 different experiments, including five plant species (almond, sage, garlic, strawberry and cabbage) and five stressors (**Table 1**). We chose these five species of plant to represent common crop species.

For each experimental configuration, we placed plant specimens in one of three sound-proofed boxes, each containing an ultrasonic sound detector to record emitted plant sounds (**Figure 1**). To ensure that plant sounds were isolated from any background noise, we analyzed the recordings to identify sounds uniquely detected by only one of the three detectors, which we interpreted as likely having been emitted by the plant in the same box.

| Stressor | Plant | Stressor levels |
|---|---|---|
| **Water Quantity** | **Almond** | Test 1: Maximum watering – 60mL<br>Test 2: 75% of maximum watering – 45mL<br>Test 3: 50% of maximum watering – 30mL<br>Test 4: 25% of maximum watering – 15mL |
| **Pruning** | **Sage** | Test 1: Light pruning at leaf<br>Test 2: Medium pruning at node<br>Test 3: Extensive pruning at stem |
| **Water Quality** | **Garlic** | Test 1: Stilled rainwater<br>Test 2: $CH_3COOH$ – Acidic at a concentration of 15g/L<br>Test 3: $NaHCO_3$– Alkaline at a concentration of 90g/L |
| **Adverse Sound** | **Strawberry** | Test 1: Bees buzzing<br>Test 2: Caterpillars munching<br>Test 3: Rock music (AC/DC) |
| **Bug presence** | **Cabbage** | Test 1: Caterpillar – small (*Vanessa cardui*)<br>Test 2: Caterpillar – large (*Danaus Plexippus*)<br>Test 3: Crickets (*Acheta domesticus*) |

**Table 1: Stressor level for each of five stressors applied to specified plant species:** The levels of each stressor is shown as well as the plant species used to test that stressor.

## Using acoustic emission patterns to distinguish different species

To determine if plants emit sounds and if these acoustic emissions differ by species, we analyzed the plant sounds identified from the recordings to determine if characteristics (rate, frequency, intensity, and duration) varied by species.

We detected ultrasonic acoustic emissions ranging from 20–50 kHz (the ultrasonic range). The pattern of acoustic emissions took the form of an intermittent short 'blip' at an average rate of 3 per hour and a duration ranging from less than one milliseconds (the smallest unit we could measure) to 2 milliseconds. Different plant species exhibited different 'profiles' of ultrasonic acoustic emissions, shown by variations in rate (number of signals per hour) and duration (milliseconds) (**Figure 2**).

Cabbage had the highest rate of acoustic emissions and the highest average duration of sound. Garlic and almond tended to emit fewest sounds with garlic emitting statistically significantly more intense sounds than strawberry, sage or cabbage ($p \leq 0.1$). Sage had the widest within-species variation in sound duration, and garlic showed the least.

Different plant species also had different 'profiles' of ultrasonic acoustic emissions when looking at frequency (hertz) and intensity (decibels) (**Figure 2**). Almond and sage had similar average frequencies, and while strawberry and garlic had statistically significantly lower average frequencies ($p \leq 0.1$). The frequency for garlic had a much wider within-species variation than the other species.

## Airborne sounds are positively correlated with the level of stress on the plant

To determine if airborne sounds are positively correlated with the level of stress on the plant, we tested increasing levels of stress on plants and monitored the changes in their emissions. We tested five stressors: drought, poor water quality, bug presence, adverse sounds and pruning (removing increasing amounts of foliage). We analyzed sound recordings of plants exposed to various stressors to determine if the presence of the stressors led to changes in plant acoustic emissions.

The stress comparisons we tested were: (1) Water quantity: comparing well-watered plants to those with increasingly less watering; (2) Water quality: comparing plants watered with pure water to plants watered with three types of contaminated water; (3) Bug presence: comparing plants with no bugs present to plants exposed to one of two types of caterpillar and crickets; (4) Adverse sounds: comparing plants exposed to no sounds to those exposed to one of recordings of bees, caterpillars or AC/DC music (5) Pruning: comparing plants with different levels of pruning from none too heavy.

Overall, we found increased acoustic emissions from plants when exposed to stressors (**Table 2**). On average, the rate of sound emissions statistically significantly increased from 2.51 per hour at baseline to 4.25 per hour when exposed to any stressor ($p \leq 0.1$).

Of the five stressors tested, four resulted in a statistically significant increase ($p \leq 0.1$) in the rate of sound emission overall (**Table 2**). The most impactful stressor was water quality (3.75 times as many sounds were emitted under the stressor than benchmark), followed by the bug presence stressor (1.94 times as many), the water quantity stressor (1.77 as many) and pruning levels (1.33 as many). Providing adverse sounds did not result in an increase in the rate of sound emission.

The water quantity stressor test confirms that reduced levels of watering is positively correlated with an increase in the rate of sound emissions (**Figure 3A**). Plants watered at maximum level (defined to be 60ml per daily watering) tended to show a reduced rate of sound emission compared with the benchmark rate prior to the application of the stressor. Plants with a watering level of 75% of the maximum amount (45ml per daily watering) showed an increased rate of sound emission, while plants watered at 50% (30ml per daily watering) showed a significantly increased sound emission rate. Finally, plants watered at 25% (15ml per daily watering) had a decreased level of sound emission. However, at this level, the plant was

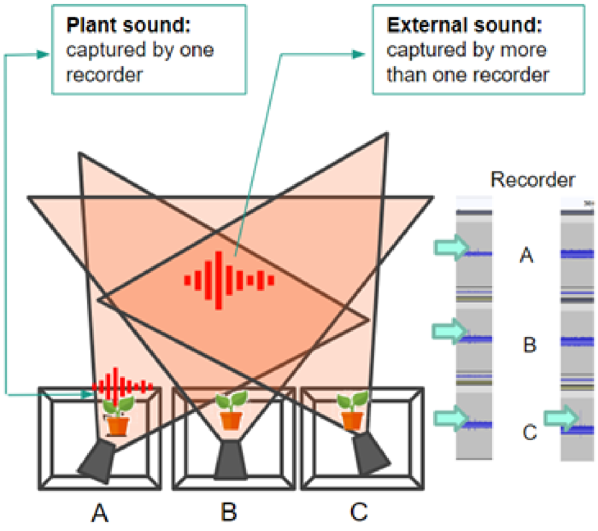

**Figure 1: Experimental set-up with three boxes and sound detectors/recorders.** Each experiment consisted of three boxes with the detectors oriented so that sounds from each plant were captured by only one recorder, while background sounds outside of the box would be captured by more than one recorder.

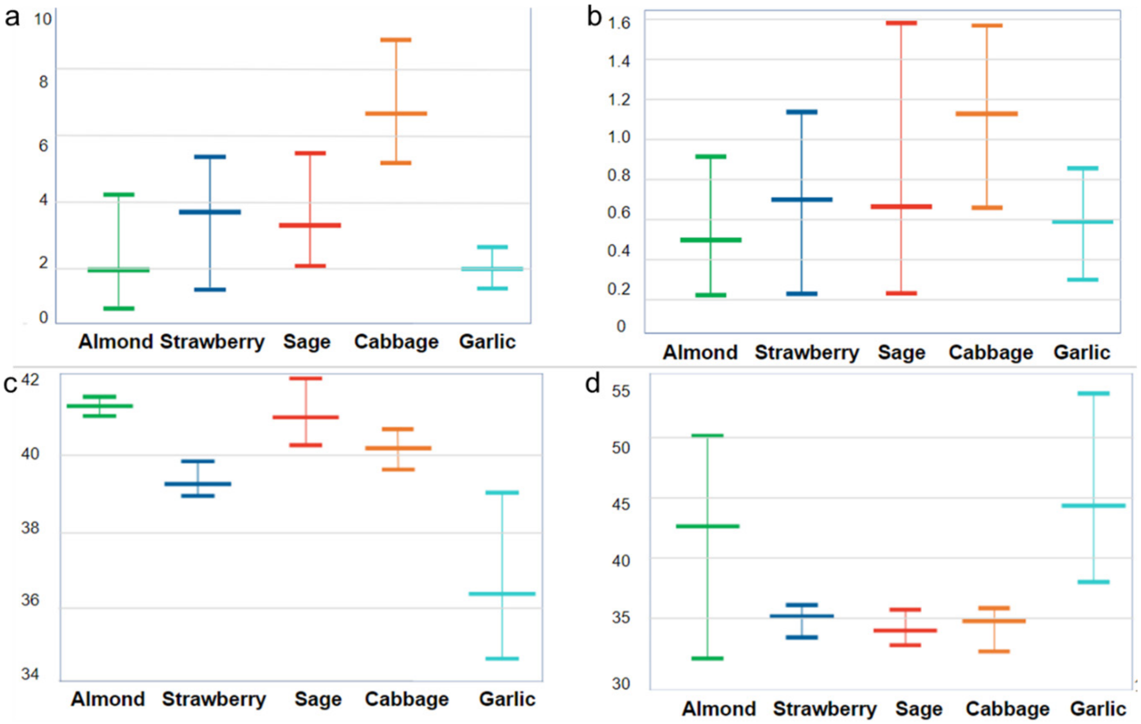

**Figure 2: Different species of plants demonstrated varied sound emission profiles.** Sound emissions were detected in the range between 20–50 kHz (the ultrasonic range). Figures a–d show different characteristics of the measured sounds: a) rate of sound emissions per hour; b) duration of each emission (in milliseconds); c) frequency of the emission (in kHz); d) intensity (in decibels). Error bars were evaluated at the 90% confidence interval (replicates by species: almond (n = 35); strawberry (n = 31); sage(n = 64); cabbage(n = 48); garlic(n = 39))

visibly wilting, and the reduced sound emission may reflect the extreme impact of reduced water on overall plant health.

The water quantity stressor test also confirmed that reduced levels of watering from the maximum daily of 60ml of water to one of three reduced levels (45ml, 30ml and 15ml per day) is positively correlated with an increase in the frequency and peak of sound emissions. Peak frequency of the sound emission tended to increase (+ 3,610 Hz) and there was also a recorded increase in the intensity of the sound between benchmark and test measurement (+2.9 dB). However, the average duration tended to decrease (-.08 ms) (**Table 3**).

The adverse sound stressor test showed the least impact in terms of overall change in the rate of sound emission and did not prove that plants respond to sounds corresponding to insects (for example caterpillars eating or bees buzzing) or at consistently high volume (for example AC/DC). We found no statistically significant difference in average duration of sound emissions (**Table 3**). However, we did record an increase in the frequency (+4,570 Hz in peak) and a decrease in intensity (-2.3DB in peak) of sound emissions.

The pruning stressor test also showed that as the intensity of the stressor increased the rate of sound emissions increased until the greatest level of pruning tested where we recorded a decrease in rate of sound emissions (**Figure 3B**). The recorded increase in the rate of sound emissions from plants exposed to light pruning (1.47x increase between the test and benchmark sound emission rates) was not statistically significant (p > 0.1). However, we did find a statistically significant increase (p ≤ 0.1) of 2.8x between the medium pruning stressor and benchmark sound emission rates. Finally, for the most extensive pruning stressor (with whole sections of stem, node and leaves removed), we recorded a decrease in the rate of sound emissions compared to the benchmark rate. Overall, the pruning test showed an increase in the intensity (+2.3Db in peak), and a decrease (-2,450 Hz) in the peak frequency of sounds emitted (**Table 3**). The bug presence stressor test overall showed an increase in the rate of sound emissions in response to a bug presence

| STRESSOR | Rate of Sound Emissions Benchmark (replicates) | Rate of Sound Emissions Test (replicates) | Ratio (Test/Benchmark) | z-score (*: Statistically significant at p=0.1) |
|---|---|---|---|---|
| **OVERALL** | **2.51** | **4.25** | **1.89** | **3.00*** |
| **Water Quantity-drought** | 0.69 (34) | 1.23 (74) | 1.77 | 1.79* |
| **Adverse Sounds** | 3.00 (31) | 1.95 (52) | 0.65 | 1.53 |
| **Pruning Levels** | 2.81 (64) | 3.73 (109) | 1.33 | 1.79* |
| **Bug Presence** | 4.63 (48) | 9.00 (110) | 1.94 | 3.88* |
| **Water Quality – contamination** | 1.42 (39) | 5.33 (90) | 3.75 | 6.01* |

**Table 2: Change in rate of sound emissions depending on stressor.** We calculated the ratios of sound emissions recorded during the benchmark versus when the stressor was applied. Ratios of more than 1 reflect an increase in the rate of sound emissions when exposed to a stressor. We then calculated Z-scores for each comparison. *: the rate of increase was statistically significant at p ≤ 0.1 (z-score ≥1.64).

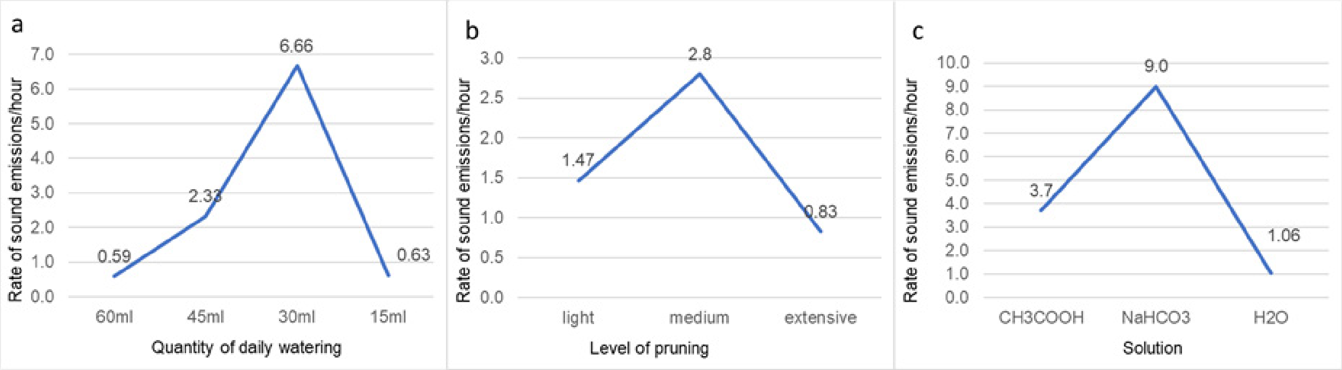

**Figure 3: Rate of sound emissions increased most in response to moderate reduction in water quantity stressor (a) and moderate pruning stressor (b) and with the NaHCO3 solution.** a) Sound emissions increased in response to decreases in daily watering levels up to 30ml daily watering. Sound emissions decreased at 15ml daily watering. b) Sound emissions increased with light pruning (+47%; index of 1.47) and medium pruning (+180%; index of 2.80) but decreased with extensive pruning. c) Sound emissions increased in response to watering with CH3COOH by 270% (index of 3.7) and by 800% (index of 9.0) with NaHCO3 with no change in rate of emissions with H2O (rainwater) as stressor.

(**Table 4**). However, there were differences by plant depending on the specific insect used for the test. The small caterpillar stressor showed a statistically significant increase ($p \leq 0.1$) in the rate of sound emissions for plants exposed to this stressor (1.92x), but there was no statistically significant change ($p > 0.1$) in rate of sound emission when plants were exposed to attack by large caterpillars. The increase in the rate of sound emission was most pronounced in the case of attack by crickets on plants(6x). The bug presence test showed an increase between the benchmark and test measurements in average peak frequency of the sound emission (+720 Hz) and a decrease in the average intensity of the sound (-1.3 dB) (**Table 3**).

The water quality stressor test showed an overall statistically significant ($p \leq 0.1$) increase in rate of sound emission of plants when exposed to different types of water quality. The stressors were water with acetic acid ($CH_3COOH$, at a concentration of 15g/L), water with sodium bicarbonate ($NaHCO_3$ at a concentration of 90g/L) and collected rainwater ($H_2O$ with unknown levels of environmental contamination) (**Table 3**). However, there was great variation depending on the individual stressor: The largest change was associated with sodium bicarbonate, with which there was a 9-fold increase in the rate, followed by acetic acid, which showed 3.7 times increase (**Figure 3**). There was no change associated with collected rainwater suggesting that it was not contaminated. The water quality test showed a considerable increase in the frequency peak of 5,020 Hz and an equally substantial decrease in the average intensity of 12 dB. The experiment again suggested that increases in frequency were a more generalizable measure of stress than intensity. However, as with the other tests, we were not able to test the statistical significance of these changes.

| Stressor (test plant species) | | Sound emissions (/hour) | Average duration (ms) | Peak Freq. (Hz) | Peak Intensity (dB) |
|---|---|---|---|---|---|
| Water quantity (almond) | Benchmark | 0.69 | 1.08 | 42,360 | 45.5 |
| | Test | 1.23* | 1.00 | 45,970 | 48.4 |
| Averse sounds (strawberry) | Benchmark | 3.00 | 1.20 | 39,960 | 35.6 |
| | Test | 1.95 | 1.10 | 44,530 | 33.3 |
| Pruning (sage) | Benchmark | 2.81 | 1.20 | 42,350 | 35.2 |
| | Test | 3.73* | 1.20 | 39,900 | 36.5 |
| Bug presence (cabbage) | Benchmark | 4.63 | 1.40 | 40,950 | 35.3 |
| | Test | 9.00* | 1.60 | 41,670 | 34.0 |
| Water quality (garlic) | Benchmark | 1.42 | 1.10 | 38,980 | 45.4 |
| | Test | 5.33* | 1.30 | 44,000 | 33.4 |

**Table 3: Difference in sound emission characteristics between benchmark plants and plants under each of the stressors.** For each stressor, the table shows the rate of sound emissions per hour, the average duration and the peak frequency and intensity which were calculated from analysis of the sound recordings. *: Statistically significant results at $p \leq 0.1$

It may be possible to determine which species is responding to a stressor by analyzing frequencies of emitted sounds in a mix of plants species.

Finally, we tested three species of plants (sage, strawberry and garlic) in combination with the water quality stressor. Emission frequencies detected varied by species, with sage having higher frequencies recorded, while strawberry and garlic had similar and lower frequencies (**Figure 4**). In this research, we were not able to complete sufficient replicates of the experiment in this study to reach statistically significant conclusions.

## DISCUSSION

The objective of this research was to make progress towards the development of a system to detect plant stress for commercial horticulture. Our research provides evidence that plants communicate acoustically, that the sound characteristics of this acoustic emission differ by species, and that airborne sounds are generally positively correlated with the level of stress. Our findings suggest that it may be possible to detect species-specific plant stress by monitoring increases in their acoustic emissions. However, we recognize that there remain many obstacles before such an application can be implemented. In the next phase of this research, the objectives would be (1) to assess whether it is possible to identify the plant sounds in the natural environment rather than in a soundproof box, which would require refinement of our methodology, and (2) to establish benchmarks for rate, frequency and intensity for each crop species through a much greater set of measurements in the natural environment,

| Cabbage Plant | Stressor: bug presence | Benchmark (rate/hour) | Test (rate/hour) | Ratio | Z-score (*: Statistically significant at p ≤ 0.1) |
|---|---|---|---|---|---|
| A | Small Caterpillar | 8.00 | 13.20 | 1.92 | 1.65* |
| B | Large Caterpillar | 7.50 | 4.80 | 0.64 | 1.31 |
| C | Crickets | 1.00 | 6.00 | 6.00 | 4.32* |
| Overall | | 4.63 | 9.00 | 1.33 | 1.95* |

**Table 4: Rate of sound emissions for each bug presence stressor and overall.** For the three individual stressors within the bug presence stressor category, the ratio is calculated and tested for statistical significance. The overall rate was calculated by summing the number of plant sounds across all stressors and averaging across the total number of hours recorded.
*Statistically significant results at $p \leq 0.1$.

which will likely require automation of the recording analysis. While we did extend the range of plant species and stressors reported on in prior research, we included only a relatively small number of individual plants across a relatively small number (five) of species and a small variety of stressors. Even within these restrictions, we were only able to test a limited number of permutations of plant and stressor and limited number of stressor levels. An extension of this research would be to test more combinations requiring a much larger study.

We found some stressors were more challenging to measure than others. For example, the bugs have to be physically placed on the plants, but it was not possible to ensure the intensity of the stress induced on the plant (some of the bugs might not feed on the plant at all).

The prior research generally used either sensors in physical contact with the plant or laboratory grade airborne ultrasound recorders to capture ambient sound and then used sophisticated machine learning (ML) based methods to distinguish between sounds emitted by plants and background noise (3,4). Placing sensors in physical contact with plants may not be scalable to a horticultural use case with large numbers of individual plants. In this research, we used commercially available low-cost airborne ultrasound recorders which did not require physical contact. If this could be proven effective outside of the laboratory setting (which required plants to be placed in sound proofed boxes), the low-cost required could provide a path to apply this work at horticulture scale. Using multiple recorders to isolate plant sound emissions from background noise, we were also able to remove the ML processing step.

To analyze the sound recordings, we used Audacity, which is primarily designed for music analysis and runs on a standard laptop computer (6). The ultrasonic sound detector converts the sounds captured into the range of human hearing and therefore within the range Audacity is designed to analyze. Due to the low rate of plant sound emissions, the recordings were made up of long periods of white noise followed by short sound blips which had to be manually identified and extracted. The smallest time unit in the Audacity software is one millisecond, which might not be granular enough to capture the precise start and end time of the sound emission. The measurement could be improved by using computer hardware and software designed specifically for waveform analysis.

## MATERIALS AND METHODS

### Experimental set-up

For each measurement, we placed a plant specimen into a soundproofed box. Each box contained an ultrasonic sound detector to record emitted plant sounds. To reduce vibrations from the ground, we placed the sound boxes on bean bags and suspended the sound detectors inside the box to isolate them from external vibrations. We used a commercially available ultra-sound detector (the BatBox Baton Ultrasound Bat Detector) to record and then convert ultrasonic frequencies into the human audible range by reducing the frequency by 10x (7).

Even with these precautions, we still detected some background sounds in test recordings using the same setup with no plants present. Therefore, to allow plant and these residual background sounds to be accurately distinguished, recordings were completed simultaneously in each of three boxes with the recorders oriented so that sounds from each plant were captured by only one recorder, while background sounds outside of the box would be captured by more than one recorder (**Figure 1**). If we detected a sound from only one recorder, it was classified as a plant acoustic emission. Otherwise, we assumed that it was a background sound. At least three hours of recordings were made for each experimental measurement starting with the first application of the stressors. In total, over 250 hours of recordings were analyzed.

### Experimental design and data collection

The first test suite was designed to measure plant acoustic emission profiles in non-stressed environments using individual plants. Plant species that we measured were almond (*Prunus amygdalus*), sage (*Salvia officinalis*), garlic (*Allium sativum*), strawberry (*Fragaria ananassa*) and cabbage (*Brassica oleracea*). We acquired the plants from local garden plant retailers in Menlo Park, California. For each plant species, we placed individual plants in each of the three boxes and ultra-sounds were recorded for a period of at least two hours. For each experiment, new plants which had not been exposed to any stressor were used to avoid cross-contamination between experiments.

We designed the second test suite to capture plant acoustic emission profiles for each stressor (**Table 1**). Experiments were repeated for each of the five plant species, first without any stressor to establish a benchmark for those specific plants and then with the stressors applied. For each of five stressors, we selected specific types and levels of stressor: (a) For the water quantity stressor, first we established a maximum/normal daily level for typical small garden plants suggested by local horticulturalists and defining 75%, 50% and 25% as additional levels (8). (b) For the water quality stressor, we used filtered tap water, water collected from a rainwater collection system, and dissolved $CH_3COOH$ and $NaHCO_3$ into filtered tap water to create those stressors with a concentration of 15g/L and 90g/L respectively. (d) For the adverse sound stressor, we selected bug sounds (a bee buzzing and a caterpillar eating a leaf) as well as loud rock music (AC/DC Back in Black). The bug sounds were found with google search on YouTube (9,10) (e) For the bug presence stressor, we selected two species of caterpillar (small: *Vanessa cardui* and large: *Danaus plexippus*)

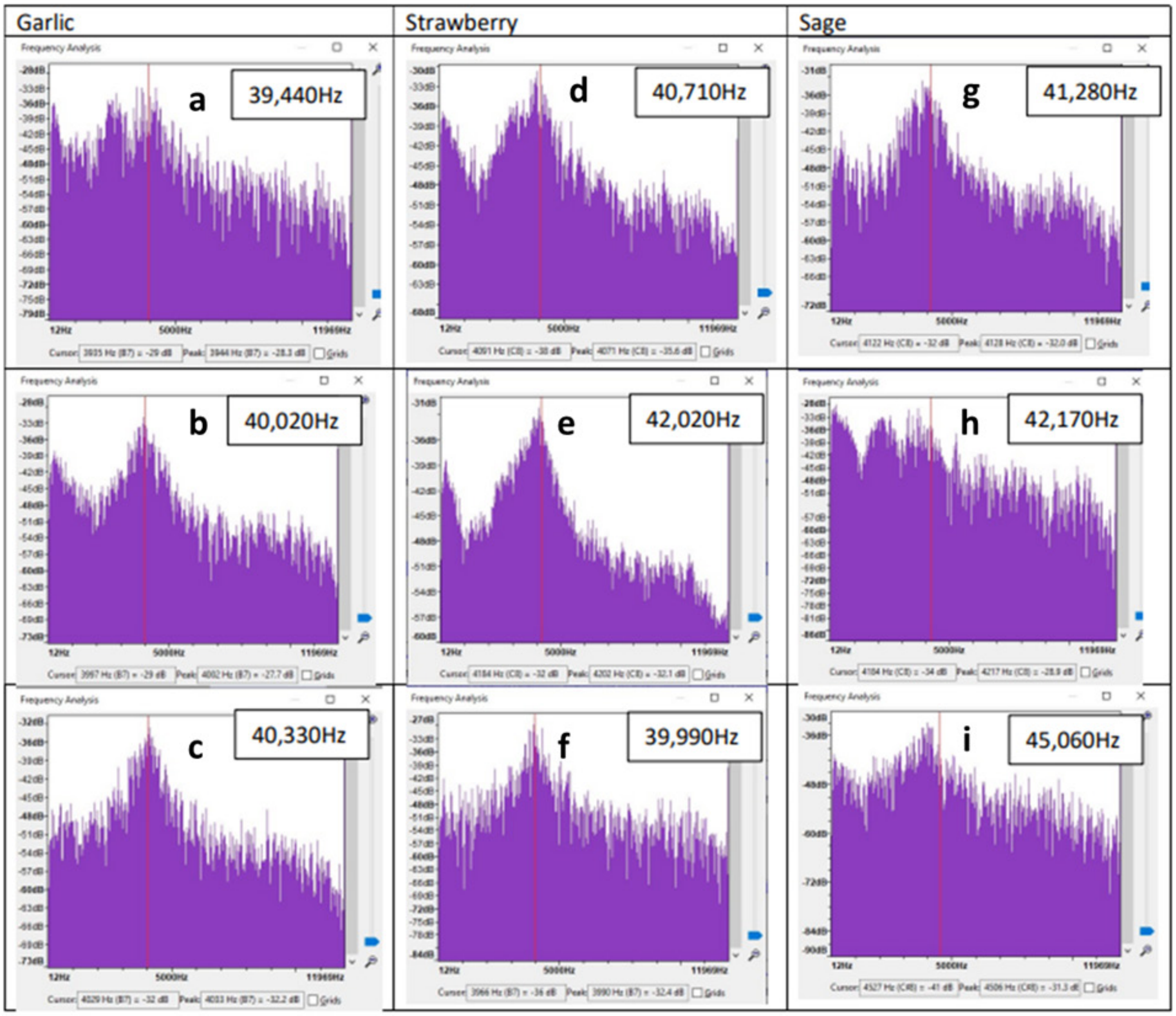

**Figure 4: Examples of sound emission characteristics from a combination of three plants (garlic, strawberry and sage) in the presence of a single stressor (water quality) with peak frequency labeled.** Each column of figures (e.g. 4a, 4d, 4g) shows the difference in peak frequency (labeled and shown as the red line) of a single sound emission from one specimen of the plant species for three separate measurements. Sage plants showed higher peak frequencies in each measurement (4g, 4h, 4i). Note that for 4h and 4i, the peak frequency is obscured by the red line.

and crickets (*Acheta domesticus*) which we purchased from educational suppliers.

For the adverse sound tests, each sound (a single bee buzzing, a caterpillar eating a leaf and AC/DC playing back in black) were played on a continuous loop on a small digital player which was suspended within the soundproof box close to but not in contact with the plant. This ensured that the plant was only exposed to sounds and not vibrations through physical contact. For the bug presence, we placed the bugs in physical contact onto the plant leaf surfaces at the start of the experiment.

The third test suite was designed to establish whether plants communicate acoustically with different frequencies under different stressors. We applied one of the stressors (the $CH_3COOH$ water quality stressor) to a set of three individual plants from different species to determine if it was possible to distinguish the sound emissions by species.

To see if there was a true difference between the measured rate of emissions in the benchmark and test recordings, we calculated a z-score statistic for each plant test. Scores greater than the standard value of 1.64 were assessed as statistically significant at a 90% confidence level (11).

**Received:** January 30, 2024
**Accepted:** June 18, 2024
**Published:** March 18, 2025

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