JOURNAL OF
EMERGING INVESTIGATORS

Article

# The external presence of running water influences the root growth of pea plants (*Phaselous vulgaris*)

**Jonathan Shu, Philip Allen**
Interlake Senior High School, Bellevue, Washington

## SUMMARY
**Each year, invasive tree roots cause large amounts of damage to underground pipes. While this is usually due to leaks and cracks, tree roots can also invade pipes that are structurally sound. We are interested in investigating whether plant roots have an affinity towards flowing water, measured through mass, even when the running water is not in direct contact with soil. We tested this by creating a choice chamber with water running under one end and no stimulus on the other end. We grew the plants in their chambers until their stems reached 10 inches before removing the plants to measure the mass of the roots growing towards either side of the choice chamber. Data from other scientists as well as a previously conducted experiment seemed to show that flowing water attracted plant roots. Therefore, we hypothesized that there would be greater root mass on the side of the chamber exposed to flowing water. Overall, the masses of the roots growing towards flowing water were greater than the masses of the roots growing towards the end with no stimulus, showing that plant roots did have an affinity towards flowing water. The remarkable sensitivities of plants to sound are still being discovered, but the delicacy already shown by experiments such as ours leaves concerns regarding a new facet of the impact of sound pollution on our ecosystems.**

## INTRODUCTION
Tree roots are notorious for growing towards and clogging sewer pipes, causing serious amounts of damage each year (1). This issue is usually treated through heavy use of strong herbicides. What makes this notable is that the direction of root growth does not seem random, but instead very intentional (1).

Plants have long been known to show clear responses to environmental stimuli, known as tropisms. These tropisms, such as gravitropism and phototropism, are what cause roots to follow the force of gravity, as well as leaves and stalks to bend towards the light (2). The growth hormone auxin primarily controls these responses (2). Contrary to the typical notion of plants as stationary and unresponsive, plants have developed surprisingly sensitive reactions to external influences. In addition to these well-known tropisms, there is also growing evidence showing that plants are sensitive towards more minor stimuli like sound vibrations (3).

A previous study looked the response of plants towards external sources of water, finding that roots grew towards flowing water rather than stagnant water despite neither water source directly contacting the soil (4). In a preliminary experiment, we replicated this study's setup, and similarly found that roots were longer and more numerous towards the water flowing outside the soil. Root responses to water are often due to hydrotropism, which is thought to be linked to membrane proteins as well as water uptake through the root. As water potential decreases, it affects the activity of proteins in the plasma membrane such as aquaporins, in turn affecting the ease in which water could travel through the root, known as hydraulic conductivity (5). This conductivity could change root direction through cell elongation or through changing concentrations of abscisic acid, a growth inhibitor. There is a "set point" at which plasma membrane protein behavior changes could cause a signal cascade resulting in directional changes (5). However, hydrotropism requires direct contact between the root and water. The fact that plant root growth was still concentrated towards flowing water in the study and our preliminary experiment even without direct contact raises the possibility of lesser-known mechanisms, such as sound vibrations.

We therefore hypothesized that the roots of the garden bean (*Phaselous vulgaris*) would show increased growth in the direction of externally flowing water, possibly responding to sound to compensate for the lack of contact. Through these studies, we were able to show that plants have evolved remarkably accurate responses to stimuli.

## RESULTS
In our preliminary experiment, we measured the length of the longest root growing down either side of the chamber, as well as the number of roots longer than two thirds the length of an arm of the chamber (3.33 inches). We found some signs of a relationship between flowing water and greater root growth. In six of nine trials, we saw a greater percentage of roots longer than 3.33 inches growing towards the flowing water. However, these percentages of increase varied widely, from 50% to 100% (Figure 1). Additionally, one trial had a majority of growth towards the stagnant water. However, both flow and stagnant control chambers experienced equal growth on either side, suggesting an influence of flowing water.

The data was similar in regard to the length of the longest

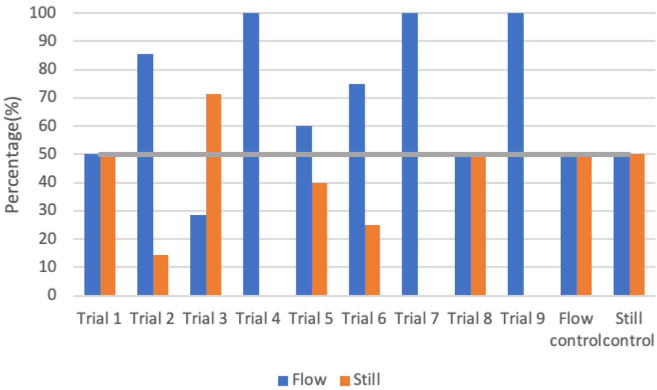


**Figure 1: The percentage of roots longer than 3.33 inches growing down either side of each chamber.** This graph shows the number of roots that grew to at least two-thirds the length of the choice chamber; the data is represented as a percentage of the total amount of roots longer than 3.33 inches. There is a horizontal line marking 50 percent, the height of the bar if growth was even This graph shows a general trend of more numerous roots towards flowing water, as well as even growth in the control chambers.

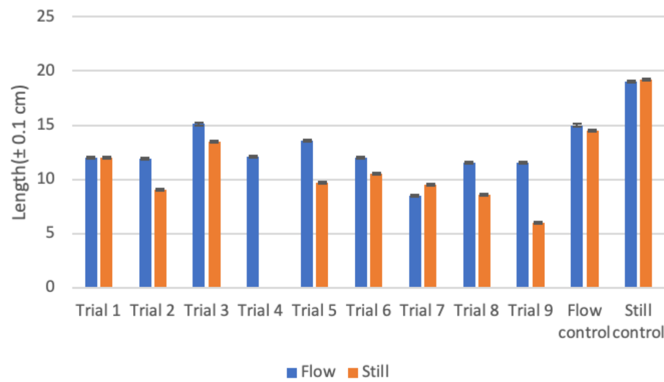


**Figure 2: The maximum lengths of roots growing down either side of each chamber.** The graph shows the longest root growing down either end of each trial with error bars of 0.1 cm to account for measurement error. This graph shows greater maximum root length towards flowing water, as well as even growth in the control chambers.

root growing down either end of the chamber. Seven of nine trials saw a longer maximum root length growing towards flowing water. As with the previous data, there was much variance between trials. Some trials experiencing complete one-sided growth towards flowing water while others were even or saw more growth towards stagnant water. The maximum length of the roots down either end of both control tubes was even, showing differences of 0.5 centimeters or less (Figure 2). Viewing both forms of measurement as a whole, we came to a tentative conclusion that flowing water will attract roots over stagnant water.

In our final experiment, we measured the mass of the roots growing down either side of the chamber. We found that the roots growing down the ends exposed to flowing water were on average 0.35 grams heavier than ends exposed to no water, making the roots exposed to flowing water about 51% heavier than those exposed to no stimulus (Figure 3).

We used the Wilcoxon signed-rank test on our raw data to determine if there was a statistical correlation that could further strengthen our conclusions. The null hypothesis was that the medians of the "flow" and "still" data groups were equal, while the alternative hypothesis was that the two medians differed. Since the sample size was smaller, we used the W value to evaluate the hypothesis. The critical value for W ($p<0.05$) at a sample size of 10 is 10; the W value of our data set was zero. The Wilcoxon signed-rank test therefore showed that our results were statistically significant.

In addition, each individual trial saw a greater percentage of total root mass growing towards the running water (Figure 4). In the control tubes, plants grew exposed to the same conditions (flowing water or no water) on both ends of the choice chamber, there was little change in growth. Instead, both sides saw an even distribution of root mass with each end of the chamber containing 49-51% of the total root mass (Figure 4). There appeared to be no specific side that the roots favored in the control tubes, showing that the experiment experienced minimal effects from influences like gravitropism. An additional observation was that there was only root branching on the roots exposed to flowing water (Figure 5). Overall, the resulting data and statistical analysis shows a correlation between greater root growth and the presence of flowing water.

## DISCUSSION

The data collected supports the hypothesis of greater growth in the direction of the flowing water. There was an evident trend of greater root mass down the end of the chamber exposed to flowing water. This larger mass signifies greater growth and implies that plants are "attracted" to running water, concentrating their growth in that direction. The observation that only roots growing towards flowing water showed branching further supports this. Root branching facilitates water uptake and nutrient extraction (6), suggesting that roots on the "flow" side of the chamber were more developed and used. In both control scenarios, the plants showed little change in growth, growing down both ends without distinction. This shows that specifically changing the stimulus did, in fact, result in a change in growth. These results are supported by the findings of our preliminary experiment, which saw longer and more numerous root growth towards externally flowing water rather than stagnant water. This distinction between flowing water and stagnant water justifies the hypothesis of sound detection, rather than simply the presence of water. The results of our experiment were also consistent with that of the study done by M. Gagliano, where seedling roots showed a greater proportion of growth towards flowing water enclosed in a pipe as opposed to stagnant water (4).

Throughout these experiments, we identified several sources of error including a potential lack of variable control. Other influences, such as gravitropism or hydrotropism, will overpower a plant's response to external flowing water.

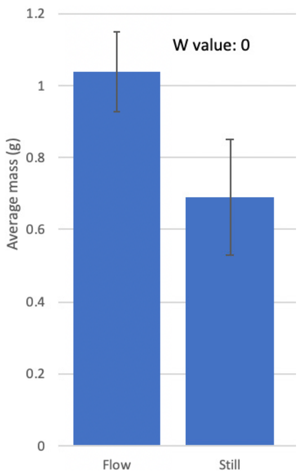


**Figure 3: The average mass of roots growing down either side of each chamber.** This graph shows the average mass of the roots growing down either side of the chambers of each trial, with error bars representing one standard deviation. This graph also shows the W value of zero obtained from the Wilcoxon signed-rank test.

Failing to control for these aspects in an early test trial resulted in roots that followed the tilt of the chamber, away from the running water. Rerunning the trial with a leveled tube saw growth in the opposite direction, towards flowing water. Despite this, we believe it is unlikely our experiments were impacted by other tropisms, as the soil was level and evenly moistened. There also could have been error in measuring the differences in root growth, as root activity involves many factors. In our preliminary experiments, this source of error was especially prevalent as we came to see that root length or number did not accurately represent root growth some of the time. We attempted to correct for this error in the final experiment, and measuring mass seemed to be a comprehensive and effective method. We identified a related, more potent source of error in maintaining root configuration while removing the plants from their choice chambers. Once the roots lost the support of the surrounding soil, some of the original directionality and positioning was inevitably lost, which could have led to inaccurate masses on either side.

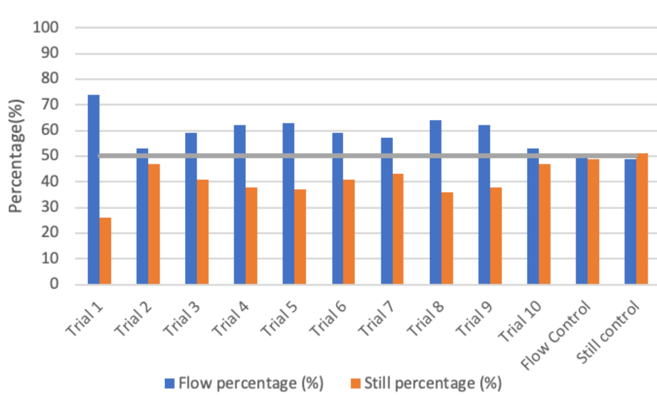


**Figure 4: The percentage of root mass of each trial growing down either side of each chamber.** The graph shows the root mass growing towards either end of the chamber as a percentage of the total root mass. There is a horizontal line marking 50 percent, the height of the bar if growth was even. This graph shows a greater percentage of mass growing towards flowing water for each trial, as well as the minimal difference in mass distribution when there was no difference in stimuli.

This issue could be resolved in the future by using clear tubing wrapped in black cloth. The cloth would protect the roots from the influence of light during growth and be removed at the end of the experiment. We could then examine the original configuration of the root system with greater ease. This would also give more certainty in the various mass measurements.

While our conclusion satisfies the initial research question, it does not provide full insight into how or why plants are able to target flowing water. Our preliminary experiment seemingly suggests the movement of water specifically influences growth. However, our final experiment only tested flowing water against no stimulus, making it difficult to conclusively support this claim. In the future, it would be interesting to bring the ideas of our preliminary experiment to our final experiment's setup and methods, explicitly testing stagnant water against flowing water. And further extensions could include the use recordings of running water as opposed to real water or soundproofed choice chambers to truly determine if sound is the primary influence.

But from the study done by M. Gagliano, the main theory appears to be that plants use vibrations to help detect the movement of water. Sound waves originate from vibrating objects and are transmitted by the oscillation of particles in a medium. Plants have been shown to change their behavior when exposed to multiple different types of vibrations (5). Certain frequencies influence germination, elongation, and cell cycling (7-9). This shows that vibrations can influence growth and directionality. In wheat and rice, exposure to vibrations increases overall yield, nutrient content, and resistance against pests (10). Plants have also been shown to produce glucosinolate and anthocyanin, defensive components, when exposed to frequencies mimicking feeding (11). These effects heavily imply that vibrations cause chemical changes which regulate growth. More specifically, streamside trees have been found to grow their roots into deeper layers of soil rather than shallower streams (12). Deeper layers of soil provide a steadier source of water than shallow streams, and differences in vibration could explain how roots are able to direct themselves past the closest source of water and instead choose the most effective.

The current concept of the influence of sound vibrations on a plant cell is a combination of research and hypothesis. It appears that sound vibrations increase the membrane tension of the plasma membrane of cells through microfilament rearrangement (3). The changes in the plasma membrane caused by these vibrations allow for the movement of a "messenger" through stretch-activated channels, most likely the calcium ion $Ca^{2+}$. Through either $Ca^{2+}$ sensors or calcium dependent protein kinases, the generated $Ca^{2+}$ "message" is passed through proteins or transcription factors, eventually resulting in gene expression (3). Cells exposed to vibrations also synthesize more proline and reactive oxygen species (ROS), auxin and ethylene, and ATP. Proline and ROS increase activation of $Ca^{2+}$ and $K^+$ channels, further increasing gene expression, while auxin and ethylene may target specific response genes (3). This aspect is of particular interest as ethylene and auxin are both known to regulate plant growth. Auxin is found in shoot and root tips and promotes stem and root growth as well as controls orientation. ATP production increases in order to support these processes (3). This general pathway of sound-induced gene regulation is most likely how the plant roots in our experiments differentiated the source of flowing water.

We can therefore see that the behavior of tree roots is most likely intentional. With the most supported hypothesis being that plants can detect vibrations, this could solve the issue of root invasion of sewer pipes. Rather than the typical treatment of harsh chemicals, actions like soundproofing could present an eco-friendly solution.

As plants have evolved to be so tuned in to their environment, it is inevitable that human development will affect them. Besides the obvious concerns of deforestation and degradation of soil quality, growing knowledge of the sensitivity of plants raises other concerns as well. Plants make up 80% of Earth's biomass, yet they are often overlooked because they operate at a different pace than other organisms (13). However, time lapse videos of plants have shown them competing for territory and nutrients and even communicating through various signals (14). The effects of noise pollution is well documented in animals, but its effects on plants remain unknown. If plants can detect vibrations as small as chewing caterpillars, noise pollution could prove to have larger consequences than expected, blocking off sonic information channels (11). It is therefore important to further understand how plants respond to external stimuli. If plants are truly able to distinguish between vibrations with as much subtlety as current data suggests, this leaves unknown concerns for the impact of sound pollution on plant behavior. Neglecting such a large part of our ecosystem could therefore prove detrimental in the future.

**Figure 5: Root branching example.** This image shows the secondary branching of roots growing towards the left side of the image, which was the side exposed to flowing water. This branching is not present on the side exposed to no stimulus.

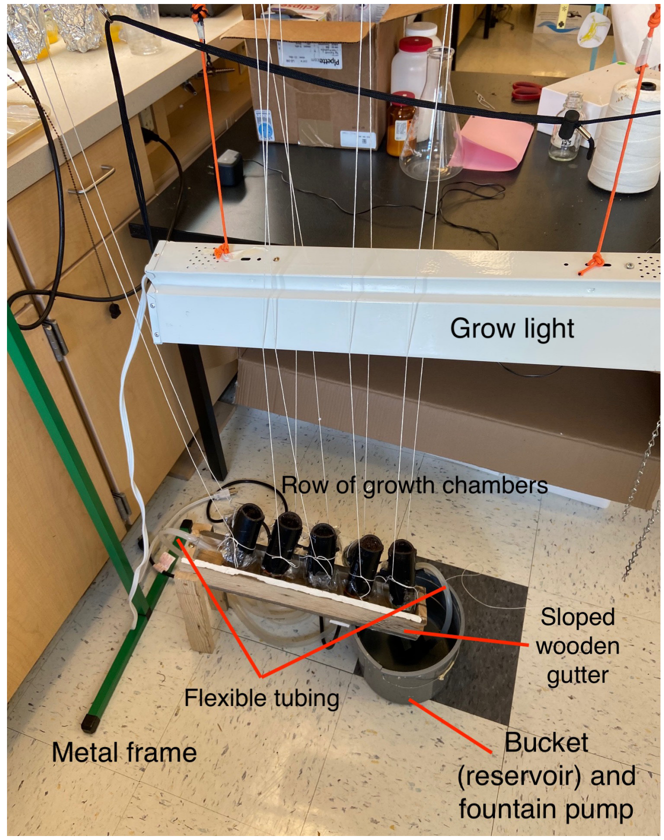


**Figure 6: Experimental setup.** This labeled image shows the entire setup of the experiment. It shows how each component was hung from the metal frame, as well as the setup of the gutter system to ensure that a consistent water flow was delivered to one end of each choice chamber.

## MATERIALS AND METHODS

To test the question of whether plants could detect the external presence of running water, a double ended choice chamber was created to grow each plant in. Each chamber had one end suspended above a gutter with water flowing through it, while the other end was exposed to no sound. To analyze root growth, plants were removed from their chambers and the roots were grouped into those that had grown down the end exposed to flowing water and the end exposed to no sound. Root growth was quantified through mass, with groups being weighed individually. The results were standardized by converting the values into percentages of the total root mass of the plant. A greater percentage of total mass was taken as a sign of increased growth.

The manipulated variable was the presence of running water. The responding variable was the amount of root growth, measured by mass. As controls, two chambers were grown: one where both ends were exposed to running water and one where neither end was exposed to running water. This allowed for a better determination of whether changing the plants' exposure to flowing water was the specific factor that influenced growth. The controlled variables were the type of plant grown, orientation and build of each chamber, and general growing environment (such as light and temperature). Different species of plants have different affinities for water and could therefore react differently in this experiment. These variables were managed by growing the same species of bean throughout each trial under an overhead grow light, which kept the light source consistent. The plants were also grown indoors at a stable temperature of around 18˚C. Each chamber was leveled to ensure that stronger influences on growth, such as gravitropism, had a minimal effect on the root directionality. When building the chambers, the separation angle between the two prongs was kept as consistent as possible. This standardized the degree of separation between the paths the roots could take, improving clarity of results. Keeping these variables consistent increased certainty that the presence of running water was the main condition changing the directionality of root growth.

To create the chambers, PVC pipes were cut into 10 five-inch segments. Angled portions were then cut off each segment. Pairs of segments were joined with PVC cement, creating five chambers with an inverted "Y" shape, before the cement was left to harden. Potting soil was then placed in a bucket and mixed with water until it was moist to the touch. The two open ends at the bottom of each choice chamber were wrapped with plastic wrap, and each chamber was filled with soil. Five seeds were germinated between damp paper towels within a sealed bag. Once each seed had germinated, they were removed from the bag and each was placed within its own choice chamber.

In the preliminary experiment, one end of each of the five choice chambers was placed in a tray filled with water. Flexible tubing was connected to a fountain pump and wrapped around the other end of each chamber. Supports were added under the ends of each chamber to ensure they were all level. The pump was then switched on and each plant was allowed to grow until its stem had reached 10 inches. At that point, each plant was removed, and the longest root on either side of the chamber was measured. The number of roots that had grown longer than 3.33 inches (two thirds the length of each arm of the chamber) was measured as well.

In this experiment, a cord was used to hang a grow light from a metal frame. The gutter system was set up underneath. One end of the gutter was elevated, using blocks as support. The lower end of the gutter was placed on top of a bucket filled with water containing the fountain pump, which served as the reservoir. Flexible tubing was connected to the fountain pump, and the other end of the tubing was secured to the elevated end of the gutter. Four plastic dividers were placed within the gutter to differentiate spaces for each chamber to occupy, and small pebbles were added throughout to create more disturbances and vibrations in the water (Figure 6). Using string, each chamber was hung from the metal frame with one end placed in the gutter above the running water (Figure 7). After the plants had grown to around 10 inches, a hose was used to flush the soil out. Roots that had grown in the same direction were grouped together and massed. After recording this data, the chambers were refilled, five new seeds were germinated, and the process was repeated.

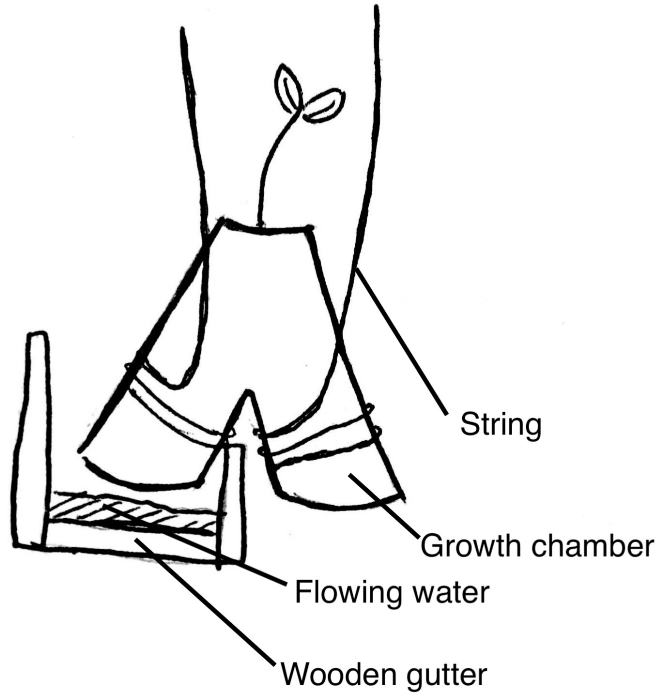


**Figure 7: Cross sectional gutter diagram.** This labeled drawing shows a cross sectional view of the wooden gutter, giving a closer view of the placement of each choice chamber.

For the control tube with no stimulus, the chamber was hung underneath the grow light in a similar fashion as mentioned above, but the gutter system was not placed beneath the tube. For the control tube with running water, the chamber was hung underneath the grow light and the gutter was aligned to expose both ends of the chamber to running water. Both ends of the gutter level were kept level to ensure that both ends of the chamber were the same distance away from the running water. The fountain pump maintained the movement of water through the system. The same standards of growth and methods of data collection were used for both control tubes as well.

## REFERENCES

1. M.A., Pohls, O. "Study of Root Invasion of Sewer Pipes and Potential Ameliorative Techniques." Acta Horticulturae, vol. 643, 2004, pp.113-121, doi: 10.17660/

ActaHortic.2004.643.13.
2. Biggs, Alton. "Plant Structure and Function." Glencoe Biology, 1st, McGraw-Hill, 2009, pp. 648, 651.
3. Mishra, Ratnesh C., et al. “Plant acoustics: in the search of a sound mechanism for sound signaling in plants.” Journal of Experimental Botany, vol. 67, no. 15, 2016, pp. 4483-4494, doi: 10.1093/jxb/erw235.
4. Gagliano, Monica, et al. “Tuned in: plant roots use sound to locate water.” Oecologia, vol. 184, no. 1, 2017, pp: 151-160, doi: 10.1007/s00442-017-3862-z.
5. Dietrich, Daniela. “Hydrotropism: how roots search for water.” Journal of Experimental Botany, vol. 69, no.11, 2018, pp. 2759-2771, doi: 10.1093/jxb/ery034.
6. Atkinson, Jonathan A., et al. “Branching Out in Roots: Uncovering Form, Function, and Regulation.” Plant Physiology, vol. 166, no. 2, 2014, pp. 538-550, doi: 10.1104/pp.114.24542.
7. Gagliano, Monica. “Green symphonies: a call for studies on acoustic communication in plants.” Behavioral Ecology, vol. 24, no. 4, 2013, pp. 789-796, doi: 10.1093/beheco/ars2006.
8. Chowdhury, Emran K., et al. “Update on the effects of sound wave on plants.” Research in Plant Disease, vol. 20, no. 1, 2014, pp. 1-7, doi: 10.5423/RPD.2014.20.1.001.
9. da Silva, Jaime A.T., et al. “Sonication and ultrasound: impact on plant growth and development.” Plant cell Tissue and Organ Culture, vol. 117, no. 2, 2014, pp. 131-143, doi: 10.1007/s11240-014-0429-0.
10. Hassanien, Reda H.E., et al. “Advances in effects of sound waves on plants.” Journal of Integrative Agriculture, vol. 13, no. 2, 2014, pp. 335-348, doi: 10.1016/S2095-3119(13)60492-X.
11. Appel, Heidi M., et al. “Plants respond to leaf vibrations caused by insect herbivore chewing.” Oecologia, vol. 175, no. 4, 2014, pp. 1257-1266, doi: 10.1007/s00442-014-2995-6.
12. Dawson, Todd E., et al. “Streamside trees that do not use stream water.” Nature, vol. 350, 1991, pp. 335-337, doi:10.1038/350335a0.
13. Thompson, Andrea. "Plants Are the World's Dominant Life-Form." Scientific American, Springer Nature, 1 Aug. 2018, www.scientificamerican.com/article/plants-are-the-worlds-dominant-life-form/.
14. Gowan, Jessi. "How Much Can Plants Hear?" Permaculture Research Institute, 28 Aug. 2017, www.permaculturenews.org/2017/08/28/much-can-plants-hear/.

**Article submitted:** May 6, 2020
**Article accepted:** June 14, 2020
**Article published:** November 10, 2020