# The Effects of Sound Stimulation on Allium cepa Root Growth

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#### **Abstract**

The effects of sound stimulation on mitosis, the extent of mitotic activity, and the characteristics of chromosomes and nuclei during mitosis in *Allium cepa* (onion) root tips were studied. Growing chambers were designed to allow one group of onion roots to grow with regular exposure to sound at a frequency of 5,000 Hz and an intensity of 75.9 dB. Another group of onions were grown without sound stimulation and served as the control group. It was shown that exposure to sound had adverse effects on *Allium cepa* root growth and mitotic activity. Sound-stimulated onions grew fewer and shorter roots and retained less mass. Analysis of mitotic abnormalities found in sound-stimulated root cells showed that sound exposure interfered with normal chromosome activity during mitosis, suggesting mitotic instability.

Keywords: Allium cepa, sound stimulation, mitotic activity, root growth

#### I. INTRODUCTION

In recent years, there has been an increased interest in the effects of sound on human health. Many studies address noise-induced health effects, particularly those that are the result of acoustic pollution. A number of researchers are studying the effects of noise and sound on biological systems as a result of a rise in acoustic pollution in rapidly urbanizing areas. Studies have shown acceleration of plant growth when exposed to frequencies at 10,000 Hz<sup>2</sup>, 5,000 Hz<sup>3</sup>, and *E. coli* grown exposed to 8,000 Hz sound.<sup>4</sup>

The idea that specific sounds, particularly that of music, can "heal" the human body is also prevalent. In Ekici et al., the effect of strong, complex, and rhythmic accent classical music on *Allium cepa* root growth was observed. The study yielded results suggesting exposure to this specific type of classical music increases growth and cell proliferation. While Ekici et al., found that sound stimulation increased cell proliferation, Tsen et al., observed that sound can also have destructive capabilities. In Tsen et al., sound frequencies were used to "shatter" simple viruses<sup>5</sup>.

The aim of this study was to investigate the effects of sound stimulation on *Allium cepa* root growth. Mitosis, the extent of mitotic activity, and the

characteristics of chromosomes and nuclei during mitosis were also studied. Results of this investigation may shed light on the potential impact that increasing noise levels in urbanizing areas might have on biological processes.

#### II. METHODS

In this study, *Allium cepa* plants were used specifically for their large chromosome size, making them practical subjects for viewing mitosis under a microscope. For 16 days, onion roots were grown in sound chambers designed to allow one group of 12 onion plants to grow roots under sound stimulation of 5,000 Hz for 30 minutes per day, and another group of 12 onions to grow roots without sound stimulation (Figure 1). Sound was generated by the Sonic V sound generator app. Sound intensity was measured



**Figure 1.** Sound chambers during root growth experiment: (left) sound stimulation chamber; (right) control chamber.

using Decibel 10<sup>th</sup>: Pro Noise Meter app to be an average of 75.9 dB with 5,000 Hz stimulation and an average of 54.9 dB without stimulation. Intensity of sound in the control chamber was measured to be an average of 54.1 dB (no sound stimulation). Root volumes were measured using water-displacement on the 7th, 14th, and 16th day of the experiment. The longest root of each onion was measured. Root masses were measured after roots were removed from their onion bulbs and patted dry. Length and mass measurements were made on the 17th day of experiment.

At the end of the root growth experiment, root tips were observed under the microscope using the squash method. 516x magnification was then used to examine root tip cells undergoing mitosis (Figure 2). In order to eliminate bias and maintain consistency



**Figure 2**. Stages of mitosis in *Allium cepa* meristematic root cells (516x): (a) prophase; (b) metaphase; (c) anaphase; (d) telophase.

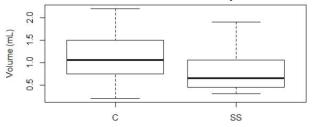




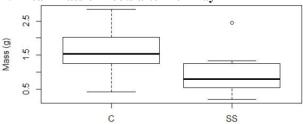
**Figure 3**. Root growth of *Allium cepa* after 16 days: (top) sound-stimulated (SS) group; (bottom) control (C) group.

during cell counts, the meristematic region was defined as the area of cells above the root cap and below the region of elongation using morphological differences in root cells. An average of 210 cells were counted from each onion root tip; a total of 2,595 cells were counted from the control group and a total of 2,518 cells were counted from the sound-stimulated group. Mitotic index was then determined for each onion root by dividing the sum of all cells in prophase, metaphase, anaphase and telophase in a given slide's field of view (FOV) by the total number of cells in the FOV.

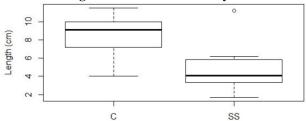
### A. Mean Volume of Roots after 16<sup>th</sup> Day



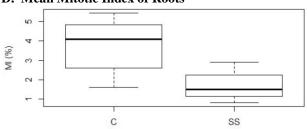
#### B. Mean Mass of Roots after 16th Day



## C. Mean Length of Roots after 16th Day



## D. Mean Mitotic Index of Roots

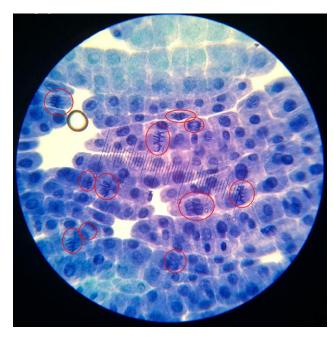


**Figure 4.** *Allium cepa* root growth (A) root volume; (B) root mass; (C) root length; (D) mitotic index.

Mean volume, length, mass, and mitotic indices for each group of onions were analyzed. Shapiro-Wilk tests were performed for normality and t-tests were performed to compare groups.

#### III. RESULTS AND DISCUSSION

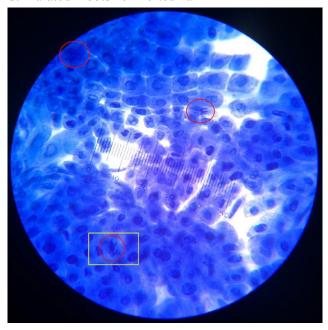
Results of this study suggest that sound exposure of 5,000 Hz and 75.9 dB has negative effects on *Allium cepa* root elongation. Sound-stimulated onions grew an average root length of 0.0472 m, while control onions grew an average length of 0.0846 m. More leaf growth was observed in the control group than in the sound-stimulated group (Figure 3). The mean root volume, length, and mass of the sound-stimulated



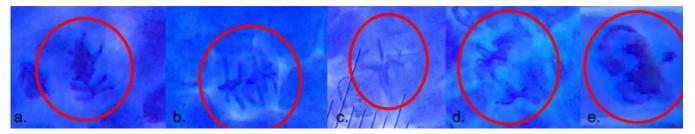
**Figure 5**. Micrograph of control *Allium cepa* root cells (516x): (red) mitotic figures; FOVs were created randomly within the meristematic region; mitotic figures were determined by chromosome distinguishability (condensing/fully condensed chromatin).

group were less than those of the control group after the 16th day of the growing period (Figure 4). There were statistically significant differences in root length (p < 0.005) and mass (p < 0.05). However, the difference in root volumes was not statistically significant.

The effects of sound exposure on mitotic frequency are shown in Figures 5 and 6. (Full-sized images of all <u>slides</u> can be found at www.isjos.org/pdfs/ISJOS\_v11\_p1-Data.pdf) Results suggest sound exposure may have negative effects on cell proliferation in *Allium cepa* roots, as a lower mean mitotic index for the sound-stimulated group was observed compared to that of the control group (Figure 4). Sound-stimulated roots exhibited an



**Figure 6.** Micrograph of sound-stimulated *Allium cepa* root cells in sound stimulated plant (516x): (red) mitotic figures; (yellow) mitotic abnormalities; determining mitotic abnormalities followed methods used by Pesnya and Romanovsky.<sup>6</sup>



**Figure 7.** Mitotic abnormalities in sound-stimulated root tip cells: (a) sticky metaphase; (b, d) lagging chromosomes; (c) unoriented chromosomes; (e) micronuclei.

average mitotic index of 1.69% while control roots had an average index of 3.76% (p < 0.0005). Furthermore, five root cells showed mitotic abnormalities in sound-stimulated onion roots while there was no sign of mitotic abnormalities in control roots, suggesting that sound exposure may also have influenced mitotic activity (Figure 7).

The results suggest that the sound exposure may have caused a form of mechanical stress in the *Allium cepa* roots, causing negative effects on biological processes associated with root growth and cell proliferation. Mitotic abnormalities are often associated with spindle disturbances.<sup>6</sup> As a mechanical wave, sound might have caused impairment of spindle microtubule formation through physical means. Some kinases that are key to the mitotic cycle are associated with mechanosensitive ion channels.<sup>2</sup> It is possible that the mechanical vibrations caused by the sound affected the function of the channels and, therefore, the kinases, resulting in decelerated root growth.

In further studies, limitations of the present study should be addressed. Image quality of FOV's in both sound-stimulated and control groups should be standardized, as more deeply tinted images make distinguishing chromosome and chromatin details difficult. As the cell cycle is a continuous process, it is also difficult to differentiate between certain mitotic stages—early prophase and interphase, for example—when using varying image qualities. Further research is suggested focusing on using a wider range of frequencies and the role that cyclindependent kinases (CDKs)—protein kinases that regulate the cell cycle—may have on mitotic activity in association with sound exposure. Multiple generations of onion plants should be experimented on to study the long-term effects of sound and a variety of species should also be investigated.

## IV. CONCLUSION

The results of this study suggest that sound stimulation of 5,000 Hz can have adverse effects on mitosis, the extent of mitotic activity, and the characteristic of chromosomes and nuclei during mitosis in *Allium cepa* root tips. Root growth and cell proliferation were significantly decreased after exposure to the sound. Mitotic abnormalities were also seen in sound-stimulated roots.

## V. REFERENCES

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