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The Effect of 900 Hz and 1200 Hz Audible Sound on the Germination and Development of Seeds of Oats, Wheat and Cucumber

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THE EFFECT OF 900 Hz AND 1200 Hz AUDIBLE SOUND ON THE GERMINATION AND DEVELOPMENT OF SEEDS OF OATS, WHEAT AND CUCUMBER

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INTRODUCTION

There are few experiments on plants in relation to audible sound in the literature. These are mostly on germination and growth. This investigation is to test the effect of 900 Hz and 1,200 Hz audible sound on the germination and development of seeds of oats, wheat and cucumber.

The sound frequencies which can be detected by man are termed the audible sounds. Chambers and Gaines (1932) suggested that the disruption of animal cells by audible sound may involve actual tearing apart of tissues as a result of rapid alterations of tension and compression produced in the surrounding medium by the vibration, or the explosion of the organism as a result of internal release of dissolved gases. The sound frequencies above the audible range are referred to as the ultrasonic sound. Ultrasonic waves are frequently used in the destruction of microbial cells and for the separation of intro-cellular particles (El'Piner, 1964). This destructive effect of ultrasonic sound is largely due to cavitation. Cavitation is the formation of partial vacuum within the cells due to the separation of intracellular structures (Clark and Hill, 1969).

Sound is measured in two ways. One is frequency, that is the pitch of the sound; and this is expressed in cycles per sound or

Hertz. The second way is by its intensity or loudness which is expressed in units called decibels (db). The higher the number of decibels the louder the sound.

LITERATURE REVIEW

The effects of audible sound on plants and plant cells has been investigated in a number of different ways. Northern and MacVicar (1939) showed that sound lowered the elasticity of the cytoplasm of *Spirogyra* by 20% to 88%. This could be seen by the displacement of chloroplasts in sound treated *Spirogyra* filaments. Gnanam (1959) reported an increase in the photosynthetic rate of *Spirogyra* when exposed to sound. Singh (1959) also observed an increased rate of photosynthesis in *Hydrilla verticillata* after exposure to sound waves from an electric bell. Both the rate and the total volume of oxygen evolved from sound -- excited *Hydrilla verticillata* were 60% to 100% higher than in control plants.

Ponniah (1958) played a single note to *Minosa pudica*, *Impatiens* balsamina, *Tagetes erecta* and *Hydrilla vercillata*. She found an increase in root development, total number of leaves and length of branches. Singh and Ponniah (1955) found that the musical sound of the *Veena* stimulated the growth of balsam plants in all parameters measured.

Lisenkov (1966) found that *Larix sibirica* seeds exposed to sound treated water had an increase in ground germination, increase frost resistance and better growth. Amylase activity increased in those seeds exposed to the sound for periods between 0.5 to 1.0 hours. Longer treatment decreased the enzyme activity.

Weinberger and Measures (1968) exposed wheat (Triticum aestivum), both spring and winter varieties, to a single audible frequency of sound at a given time. They found an increase in germination and better growth in winter wheat (Triticum aestivum var. Rideau) and in some cases this was temperature of frequency dependent. Spring wheat (Triticum aestivum var. Marquis) showed an increase in germination at 2°C and 10°C but not at 25°C. The growth response of the spring wheat was dependent upon treatment. The same workers in another experiment (Measures and Weinberger, 1970) using spring wheat noted a significant increase in some of the parameters measured when 300 Hz and 5,000 Hz were used. Growth was not significantly stimulated by treatment with either 1,250 Hz or 12,000 Hz sound frequencies.

Weinberger, Pearl and Graefe (1973) using four tunes and two random noise selections to determine whether variable sound frequencies may effect the growth and development of cucumber, corn and oats. The selections were subjected to a power spectral density analysis. They observed some growth changes, which gave the indication that a growth-audio action spectrum may be a wide spread general phenomenon.

There are some reports on negative growth effects of sound in the literature also. Weinberger and Das (1972) found that continuous exposure of synchronized cultures of Scenedesmus obtusiusculus to 4,000 Hz produced a decrease in the rate of cell division. The normal rate of cell division did not return until two life cycles had elapsed. Woodlief et al., (1969) found that tobacco plants subjected to random

noise showed a decline in the rate of growth by over 40 percent.

Ellis (1973) using, oats (Avena sativa var. AuSable) and wheat (Triticum aestivum var. Ionia) at 300 Hz, found that root elongation was inhibited. Walton (1974) also found root inhibition with Avena sativa var. AuSable and Cucumis sativis var. Markerter at 600 Hz. He however found no inhibition in Triticum aestivum var. Ionia at the same sound frequency.

A lot has been reported in the literature on the effect of audible sound on animals. Anichin (1972) using guinea pigs, found that after different periods of exposure to sound at 4,000 Hz and 100 db, the RNA content decreases in the cytoplasm of hair cells of the organ of Corti and neurons of the auditory cortex. Anichin (1968) also found that sound treatment caused the nuclei number to increase in the hair cells of the organ of Corti. Wustenfeld et al., (1970) found no effect in the nuclear volume or content of Nissl substances in the nerve cells of the cochlear nuclei of the guinea pigs, using four different frequencies.

Aleksandrovskaya and Chezhenkova (1970) found that the rabbit's cerebral cortex under 200 c.p.s. sound exposure had enhanced slow waves and spindles, particularly in the motor area, there was an increase in astrocycles while in the deep layers of the projection area (auditory cortex) there was a decrease. Bryshenskii and Meshaeleva (1969) found that animals subjected to the prolonged action of sound exhibited a lower coagulation activity of the blood. The devense reactions which develop after the injection of thrombin are weakened by sound.

Koitchev (1969) found that sodium distribution changed in the organ of Corti after exposure to sound. The increase of sodium was attributed to possible changes in the permeability of excited membranes. Sviderskaya (1968) in studying the effect of two frequencies on the motor activity of chicks found that different frequencies gave different responses and that the degree of response increased after the auditory systems of the chick had developed. Chambers and Harvey (1931) found that tadpoles *Bufo punctata* when held in shell vials filled with water, above the vibrator, were killed within one minute. Small fish were killed in three to four minutes and immature frogs were killed in about 10 minutes. Chambers and Gaines (1932) found that the water flea, *Daphnia pulex*, when exposed to sonic irradiation in a shell vial filled with water was killed with one second.

MATERIALS AND METHODS

Plant Material

Three types of seeds: oats (Avena sativa var. AuSable), wheat (Triticum aestivum var. Ionia), and cucumber (Cucumis sativis var. Markerter), were used in these experiments. These were obtained from Farm Bureau Services Inc., Kalamazoo, Michigan and stored in a refrigerator at 8°C when not in use.

Sound Source

Sound at a frequency of 900 Hz and 1,200 Hz were generated by an audio-oscillator[1] connected to an amplifier.[2] The audio-oscillator was adjusted to give sound of the intensity of 100 plus or minus 2 decibels. This sound intensity was measured by a sound level meter.[3] A nine inch speaker was suspended by strings in the experimental chamber. Using a system of hooks the speaker could be removed and returned to the same position in either chamber. These procedures were the same as those by Ellis (1973) and Walton (1974).

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[1]Audio-oscillator made by Hewlett Packard Co., Palo Alto, California (Model 200 CD).

[2]Amplifier made by The David Bogen Co., New York, New York (Model E 14).

[3]Sound level meter made by The General Radio Co., West Concord, Massachusetts.

Growth Conditions

For each type of seed five plastic dishes[1] (155mm x 63mm) were used for the experimental series and five for the control series. Each dish was lined with Armstrong No. 6 filter paper[2] and was moistened with 15mls of distilled water. Twenty seeds were then put in each dish and that dish was then placed in a clear polyethylene bag.[3] The bag was closed by means of a wire twist. The dishes were arranged in a circular pattern in Sherer Controlled Environment Chambers.[4] At the 900 Hz frequency dishes were run simultaneously. At the 1200 Hz each type of seed was run separately.

The experimental group were exposed to sound continuously and the control had no sound except for the normal background noise. All dishes were kept under constant darkness and at a temperature of 20 - 22 degrees C. The dishes were taken out and the seeds measured after five days. Growth chambers were interchanged for each succeeding run, so that an experimental chamber became a control chamber on the next run systematically. For the 900 Hz a total of four runs and two replications were made. At the 1200 Hz frequency eight separate runs and four replications were made for each of the three types of seeds.

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[1]Plastic dishes were obtained from Bradley Industries, Inc., Franklin Park, Illinois.

[2]Paper Manufactured by the Armstrong Cork Co., Lancaster, Pennsylvania.

[3]Bag made by Union Carbide Corp., Consumer Products Division, 270 Park Avenue, New York, New York.

[4]Sherer-Gillett Co., Marshall, Michigan.

Determination of Germination and Development

A seed with a root length 1mm or more was considered as germinated. Development was assessed, using the method of Thompson et. al., (1945) where the longest root of each germinated seed was measured in millimeters. A mean root length for both controls and experimentals was than calculated using the root lengths of all seeds that did germinate.

Statistical Analysis

Root elongation data were anlaysed by means of a two-way analysis of variance (unbalanced case) where factor 1 is the difference between control and experimental treatments, and factor 2 is the difference between runs. Analysis was carried out using Western Michigan University's PDP - 10 computer and library program #1.9.2. The per cent germination data were analysed by library program #1.9.1. (version 2). This is also a two-way analysis of variance. It shows the significance of control/experimental, replication, and growth chamber differences. The 'F' value generated in both programs were compared with the critical 'F' value at 5% level.

RESULTS

Germination

Tables 1 - 6 on pages 13 - 18, show the percent germination oats, wheat and cucumber at 900 Hz and 1200 Hz. Tables 7 - 12 on pages 19 - 24, show their statistical analysis.

In all cases, and at both 900 Hz and 1200 Hz sound frequencies, significant differences in percent germination were not found between the experimental and control group at the 5 percent level. Significant differences were not found between the two replications at 900 Hz and also between the four replications at 1200 Hz. Neither were significant differences found between growth chambers A and B in all cases at 5 percent level of significance.

Root Elongation

Tables 13 - 18 on pages 25 - 30, show the average length of the longest root of germinated oats, wheat and cucumber at 900 Hz and 1200 Hz. Tables 19 - 24 on pages 31 - 36, show the results of their statistical analysis.

<u>Oat Seeds</u>

At 900 Hz the grand mean of oats control group is 99.64mm and that of the experimental series is 99.63mm. (see Table 13, page 25). The difference observed is not significant (see Table 19, page 31) at the 5 percent level. A significant difference was not found be-

tween the four runs of the experiment. Neither was interaction significant at the 5 percent level.

At the 1200 Hz the grand mean of oats control group is 111.53mm and that of the experimental group, 110.41mm (see Table 16, page 28). The difference between these two is not significant at the 5 percent level (Table 22, page 34). Unlike the 900 Hz experiment a significant difference is found between the eight runs of the experiment at the 5 percent level. However, a significant interaction at the 5 percent level was not observed.

Wheat Seeds

The grand mean of wheat control group at 900 Hz is 96.25mm and that for the experimental group is 96.23mm (Table 14, page 26). This difference is not significant at the 5 percent level (see Table 20, page 32). A significant difference between the runs and a significant interaction at the 5 percent level were not found.

At 1200 Hz, the grand mean of wheat control group is 105.64mm and that of the experimental series is 105.32mm. (Table 17, page 29). The differences observed are also not significant at the 5 percent level. A significant interaction is found at the 10 percent level but not at the 5 percent level.

Cucumber Seeds

At 900 Hz, the grand mean of cucumber control series is 57.45mm and that of the experimental series is 57.42mm. (see Table 15, page 27). The difference observed is not significant at the 5 percent

level (Table 21, page 34). Also significant differences were not found between all the four runs of the experiment. Neither was a significant interaction found at the 5 percent level.

At 1200 Hz, the grand mean of cucumber control group is 58.05mm and that of the experimental group is 60.19mm (Table 18, page 30). The grand mean of the experimental group, unlike all the others, is slightly higher than the control series. This difference is significant at the 10 percent level but not at the 5 percent level. (Table 24, page 36). The difference between the runs is also significant at the 10 percent level but not at the 5 percent level. Interaction was found not to be significant at the 5 percent level or at the 10 percent level.

		Runs			
	GM	1	2	3	4
Control	90.00	90	93	83	94
Sound	90.00	92	93	88	87
		1		2	
		Replications			

Table 1. Per cent germination of oats for control and sound treated seeds at 900 Hz.

		Runs			
	GM	1	2	3	4
Control	99.00	98	99	100	99
Sound	97.25	95	97	98	99
		Replications 1		Replications 2	

Table 2. Per cent germination of wheat for control and sound treated seeds at 900 Hz.

		Runs			
	GM	1	2	3	4
Control	99.25	99	100	100	98
Sound	96.75	97	96	99	95
		1		2	
		Replications			

Table 3. Per cent germination of cucumber for control and sound treated seeds at 900 Hz.

Runs

	GM	1	2	3	4	5	6	7	8
Control	94.00	96	96	94	92	97	91	96	90
Sound	94.13	91	95	95	96	97	91	95	93
		1		2		3		4	

Replications

Table 4. Per cent germination of oats for control and sound treated seeds at 1200 Hz.

	Runs								
	GM	1	2	3	4	5	6	7	8
Control	98.00	100	97	96	96	98	98	99	100
Sound	95.50	95	98	95	96	95	95	95	95
		1		2		3		4	
		Replications							

Table 5. Per cent germination of wheat for control and sound treated seeds at 1200 Hz.

Runs

	GM	1	2	3	4	5	6	7	8
Control	97.50	95	95	97	97	98	100	99	99
Sound	98.38	97	97	98	99	99	99	98	100
		1		2		3		4	

Replications

Table 6. Per cent germination of cucumber for control and sound treated seeds at 1200 Hz.

Factor	F Value
Difference between control/experimental	2.513
Difference between replications	3.283
Difference between chamber A and B	0.462

Table 7. Two-way analysis of variance of oats germination data at 900 Hz using mean values only (library program #1.9.1., version 2).

Factor	F Value
Difference between control/experimental	0.529
Difference between replications	2.882
Difference between chamber A and B	0.529

Table 8. Two-way analysis of variance of wheat germination data at 900 Hz using mean values only (library program #1.9.1., version 2).

Factor	F Value
Difference between control/experimental	1.200
Difference between replications	0.000
Difference between chamber A and B	1.200

Table 9. Two-way analysis of variance of cucumber germination data at 900 Hz using mean values only (library program #1.9.1., version 2).

Factor	F Value
Difference between control/experimental	0.012
Difference between replications	1.814
Difference between chamber A and B	0.008

Table 10. Two-way analysis of variance of oats germination data at 1200 Hz using mean values only (library program #1.9.1., version 2).

Factor	F Value
Difference between control/experimental	0.000
Difference between replications	1.855
Difference between chamber A and B	0.001

Table 11. Two-way analysis of variance of wheat germination data at 1200 Hz using mean values only (library program #1.9.1., version 2).

Factor	F Value
Difference between control/experimental	0.000
Difference between replications	2.278
Difference between chamber A and B	0.001

Table 12. Two-way analysis of variance of cucumber germination data at 1200 Hz using mean values only (library program #1.9.1., version 2).

		Runs			
	GM	1	2	3	4
Control	99.64	96.47	97.89	102.82	101.37
Sound	99.63	96.66	97.71	101.71	102.45
		1		2	
		Replications			

Table 13. Mean length in mm of longest root of germinated oats for control and sound treated seeds at 900 Hz. GM = Grand mean.

Runs

	GM	1	2	3	4
Control	96.25	93.85	93.50	97.84	99.79
Sound	96.23	97.01	90.25	97.51	100.14
		1		2	

Replications

Table 14. Mean length in mm of longest root of germinated wheat for control and sound treated seeds at 900 Hz. GM = Grand mean.

	GM	Runs 1	Runs 2	Runs 3	Runs 4
Control	57.45	59.27	53.49	56.63	60.39
Sound	57.42	58.33	54.37	57.57	59.42
Replications		1		2	

Table 15. Mean length in mm of longest root of germinated cucumber for control and sound treated seeds at 900 Hz. GM = Grand mean.

Runs

	GM	1	2	3	4	5	6	7	8
Control	111.53	109.13	110.92	120.24	110.14	115.10	117.42	107.58	101.43
Sound	110.41	122.65	104.73	109.99	116.86	110.93	99.38	109.72	108.94
		1		2		3		4	

Replications

Table 16. Mean length in mm of longest root of germinated oats for control and sound treated seeds at 1200 Hz. GM - Grand mean.

Runs

	GM	1	2	3	4	5	6	7	8
Control	105.64	100.58	112.52	97.44	100.45	102.04	112.87	113.00	106.08
Sound	105.32	105.51	100.53	101.74	111.64	112.01	104.69	103.20	103.29
		1		2		3		4	

Replications

Table 17. Mean length in mm of longest root of germinated wheat for control and sound treated seeds at 1200 Hz. GM - Grand mean.

Runs

	GM	1	2	3	4	5	6	7	8
Control	58.05	63.82	62.81	69.70	53.04	49.33	59.02	55.89	51.24
Sound	60.19	66.22	56.67	49.00	58.05	67.15	56.50	61.34	66.40
		1		2		3		4	

Replications

Table 18. Mean length in mm of longest root of germinated cucumber for control and sound treated seeds at 1200 Hz. GM = Grand mean.

Factor	F Value	Probability
Weighted means analysis of variance		
Difference between control/ experimental	0.00	0.975
Difference between runs	0.15	0.701
Interaction	0.70	0.402

Table 19. Two-way analysis of variance (unbalanced case) for root elongation data of oats at 900 Hz (library program #1.9.2.).

Factor	F Value	Probability
Weighted means analysis of variance		
Difference between control/experimental	0.19	0.660
Difference between runs	1.20	0.273
Interaction	0.75	0.386

Table 20. Two-way analysis of variance (unbalanced case) for root elongation data of wheat at 900 Hz (library program #1.9.2).

Factor	F Value	Probability
Weighted means analysis of variance		
Difference between control/experimental	0.38	0.538
Difference between runs	0.41	0.523
Interaction	1.85	0.174

Table 21. Two-way analysis of variance (unbalanced case) for root elongation data of cucumber at 900 Hz (library program #1.9.2).

Factor	F Value	Probability
Weighted means analysis of variance		
Difference between control/experimental	0.48	0.486
Difference between runs	7.38	0.007
Interaction	0.71	0.401

Talbe 22. Two-way analysis of variance (unbalanced case) for roon elongation data of oats at 1200 Hz (library program #1.9.2).

Factor	F Value	Probability
Weighted means analysis of variance		
Difference between control/experimental	0.06	0.810
Difference between runs	2.18	0.140
Interaction	3.62	0.057

Table 23. Two-way analysis of variance (unbalanced case) for root elongation data of wheat at 1200 Hz (library program #1.9.2).

Factor	F Value	Probability
Weighted means of analysis of variance		
Difference between control/experimental	3.17	0.075
Difference between runs	3.71	0.054
Interaction	0.47	0.493

Table 24. Two-way analysis of variance (unbalanced case) for root elongation data of cucumber at 1200 Hz (library program #1.9.2).

DISCUSSION

From the results of these experiments there was no observed effect of audible sound at 900 Hz on the germination of oats, wheat and cucumber. The results also failed to show that either 900 Hz or 1200 Hz had a significant effect on the development of oats, wheat and cucumber.

The germination results agreed with those of Ellis (1973) and Walton (1974), who used 300 Hz and 600 Hz respectively. The effect on root elongation however showed some differences. Ellis found a significant root inhibition in both oats and wheat treated with sound. Walton also found significant root inhibition in oats but not in wheat. He also found a significant root inhibition in cucumber. From these results and the differences, one can say that sound follows fairly the normal distribution of curve. The low frequencies cause inhibition, the middle frequencies have no effect and the higher frequencies stimulate. It also appears that every species follows its own curve. Perhaps more investigation along the biochemical line will throw more light on these. The work by Weinberger, Pearl and Graefe (1973) also gave the indication that a growth-audio action spectrum may be a widespread general phenomenon. There are other examples in the literature where sound either inhibits, stimulates or has no effect on the growth of plants.

In all four runs of the experiment at 900 Hz, there were no considerable variations in either control or experimental groups. In oats the control group showed a low mean of 96.47mm and a high

mean of 102.82mm. The experimental group showed a low mean of 96.66 and a high mean of 102.45mm and a high mean of 100.14mm. In cucumber the control group showed a low mean of 53.49mm and a high mean of 60.39mm. The experimental group showed a low mean of 54.37mm and a high mean of 59.42mm.

At the 1200 Hz level, there was quite a bit of variation in all the runs of the experiment. The control group in oats had a low mean of 101.43mm and a high mean of 120.24mm. The experimental group showed a low mean of 99.38mm and a high mean of 122.65mm. In wheat, the control group showed a low mean of 97.44mm and a high mean of 113.00mm and a high mean of 112.01. Cucumber control group showed a low mean of 49.33mm and a high mean of 69.70mm. The experimental group had a low mean of 49.00 and a high mean of 67.15mm.

Further analysis by a consultant using Western Michigan University Advanced Analysis of Variance Program failed to show that the sound at 1,200 Hz had an effect at the 5 percent level on oats, wheat and cucumber. The pair-wise differences in this analysis showed that there were some differences in the two growth chambers. These differences might be the cause of the wide variation found in all the runs of the experiment at 1,200 Hz. It was surprising to find chamber differences, for the experiment was designed to remove any chamber differences.

No interaction was observed at the 5 percent level in all cases.

It is interesting to note that root stimulation was observed in cucumber treated with 1,200 Hz sound frequency at 10 percent

level. Further work may thus use higher frequencies for future experiments in cucumber.

On the basis of the statistical analysis of these results, one may reasonably conclude that sound at a frequency of 900 Hz and 1,200 Hz has no observed effect on the germination and development of oats, wheat and cucumber at the 5 percent level.

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