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THE EFFECT OF AUDIBLE SOUND FREQUENCY ON THE GROWTH RATE OF YOUNG WHEAT PLANTS

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THE EFFECT OF AUDIBLE SOUND FREQUENCY ON THE GROWTH RATE OF YOUNG WHEAT PLANTS

by

Cathleen Barczys

A Thesis Submitted to the Faculty of the

DEPARTMENT OF PLANT SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE
WITH A MAJOR IN AGRONOMY AND PLANT GENETICS

In the Graduate College

THE UNIVERSITY OF ARIZONA

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This thesis has been approved on the date shown below:

Albert K. Dobrenz
A. K. Dobrenz
Professor of Plant Sciences

August 15, 1985
Date

DEDICATION

To my parents,

Daniel and JoAnn Barczys

PREFACE

Plants, like all living things, live in an environment rich in energy and information, yet science has progressed little in its understanding of how living things interact with their environment and other living things, beyond the chemical energy level. As the sun's light overpowers the moon's during the day, science's emphasis on the obvious--light and chemical energy--has clouded our perception of the much larger picture of unceasing interactions which comprise life in any of its forms. Largely by default rather than by active investigation, it has been assumed that energy systems of lesser intensity play little, if any, role in the life of living systems--despite the omnipresence of such energy systems as non-visible electromagnetic radiation, magnetic fields, and sound.

But all forms of knowing begin with what can most easily be perceived, and Western science, at present, places almost total reliance on conscious sensory perception. The preoccupation with the "sledgehammer" energy forms, therefore, is natural, has been immediately useful in improving the human standard of living, and has given us the tools to move on now to the more elusive energy interactions that occur among living things and their environments. The emergence of the field of ecology is a direct recognition of Life's intertwining with itself; the struggle towards a unified field theory in physics strengthens the belief that forces and energies beyond our present understanding do exist. And the growing body of investigation into such minute or low-level phenomena as hormones, microwave radiation and low-level radioactive emissions is proof that very small doses of certain energy forms can profoundly affect living things.

It is in this context that the present work was undertaken. It is hoped that this work will stimulate us as scientists to give more credence to the inner wonderings and intuitions which so often are mistakenly dismissed as non-rational or non-scientific. For it is from these inner whisperings that science will come to understand the true depth of the connections underlying all life forms and their environments.

ACKNOWLEDGMENTS

Perhaps more than most theses, this research project is truly a result of many people's efforts. The sound equipment and technical expertise were the outward signs of Dr. William Bickel's support; just as valuable was the enthusiasm with which he supported this work. Dr. Brooks Taylor and Mrs. Charlotte Brooke were the magicians who found laboratory space in the crammed Agriculture Building for a graduate student with a 90 db liability. Mr. Robert Sackett of the Arizona Crop Improvement Association donated the seed. Dr. Robert McDaniel found time in his busy schedule, at late notice, for one more graduate student. Dr. Albert Dobrenz was the best advisor a graduate student could have--always supportive yet critical, always inspiring in his enthusiasm for life and for learning. Dr. Kaoru Matsuda provided the elegantly simple photographic technique which is the core of this work; his immense plant physiology background, his patience and perseverance, and his belief in students are a constant source of inspiration to me.

Two people -- Loretta Jacobs-Schwartz and Greg Ohlsen -- kept me going through thick and thin, which in the end was just as important as the technical support. Kate McMillan gave me the good company and working atmosphere in her home this past year without which I would not have been able to work. Marya Lowe and company gave me belief in my ideas. A deep, very special appreciation goes to Dennis Kensil for contributions too far-reaching to describe here. Finally, though the research was nearing the end by the time he arrived, it never would have been finished on time, nor the ideas and analysis nearly as developed if it were not for Robert Simons. For this and more, I owe him special appreciation.

To all of these people and to the many other helpful friends and colleagues I encountered on my path here in Tucson, many thanks.

CB
August 1985

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ABSTRACT

Research into the effect of sound on plants has been hindered by the lack of a systematic way of selecting the sound treatment. This research tested one possible screening method: an action spectrum of growth rate as a function of frequency. Four-day-old wheat seedlings were exposed to 15-minute treatments of pure tone. The audible sound spectrum was scanned systematically from 100-20,000 Hz. Several variable-frequency treatments -- including music -- were tested. Growth rate was measured directly using time-lapse photography. Growth of the controls was not constant over short time intervals (minutes); up to 20% variation (relative to the mean) was observed. No effect larger than the largest variation in controls was observed consistently for any one sound treatment, but there were statistically significant differences between the control and experimental growth rate curves. Also, the overall action spectrum showed a pattern of regular oscillation every 3000 Hz between 7000 and 13,000 Hz.

INTRODUCTION

Sound and Light

Sound is two things: 1) an auditory sensation in the ear, and more generally, 2) a disturbance in a medium which can cause this sensation (Rossing, 1982). A sound wave is a pressure wave. A disturbance such as the plucking of a string or a breeze hitting a leafy tree causes the string or leaves to oscillate, inducing the molecules around the oscillating object to be alternately compressed and relaxed, which in turn alternately relaxes and compresses adjacent molecules until frictional losses reduce the signal to background levels.

Since $$\text{Pressure} = \frac{\text{Force}}{\text{Unit area}}$$

$$\text{Force} = \text{Mass} \times \text{Acceleration}$$

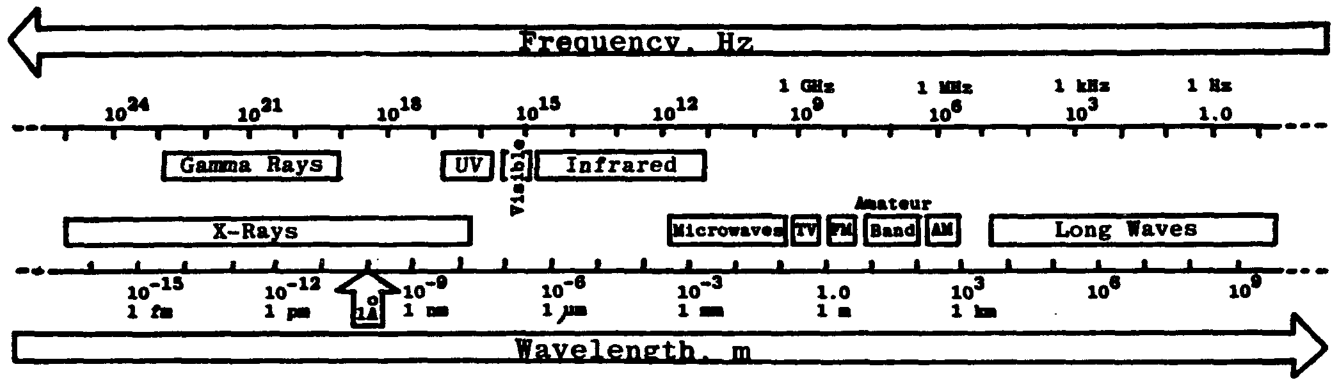
$$\text{Mass} = \text{Density} \times \text{Volume},$$

pressure is related to molecular density and acceleration:

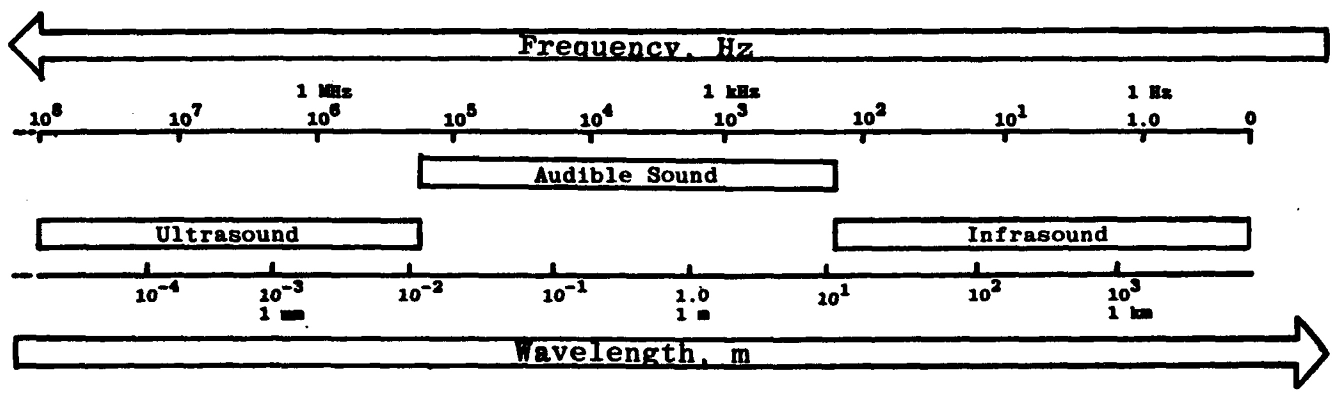
$$\text{Pressure} = \frac{(\text{Density} \times \text{Volume}) \times \text{Acceleration}}{\text{Unit area}}$$

Therefore, changing either the density of air or the molecular acceleration causes a pressure change; sound waves do both.

Figure 1 and Tables 1 and 2 describe the relevant parameters of sound energy and compare it to the dominant energy source in a plant's environment--light. Note that, like visible light in the electromagnetic spectrum, what is popularly called "sound" is



A. The electromagnetic spectrum.
(Redrawn from Halliday & Resnick, 1981, p.661.)



B. The sound spectrum.

Figure 1. The electromagnetic and sound spectra.

Table 1. Sound Treatment Parameters in Terms Relevant to Plant Physiologists

Frequency	Comparable sound	Wavelength
20 Hz	Between D# and E, 1 octave lower than lowest piano note	17.15 m
300	Between D# and E, just above middle C	1.14 m
5,000	Between D# and E, just above the upper limit of the piano	6.86 cm
20,000	Not heard by many people past childhood.	1.72 cm

Sound Level db	Sound Pressure Pa[a]	Bars	Sound Intensity W/m²	Comparable Sound[b]
0	2.0×10^{-5}	2.0×10^{-10}	10^{-12}	Threshold of hearing for humans
20	2.0×10^{-4}	2.0×10^{-9}	10^{-10}	Average whisper (at 1 m)
50	6.3×10^{-3}	6.3×10^{-8}	10^{-7}	Office, classroom
90	6.3×10^{-1}	6.3×10^{-6}	10^{-3}	Pneumatic drill (at 3 m)
120	20.0	$2. \times 10^{-4}$	1	Threshold of pain for humans

a. These pressure changes are very small. The human ear can respond to pressure fluctuations as small as 1 billionth of atmospheric pressure. Even at the pain threshold the pressure fluctuation is less than 1 thousandth of atmospheric pressure.

b. From Halliday and Resnick, 1981, p.323.

Table 2. Key Quantities in Sound and Electromagnetic Energy.

	Sound	Electromagnetic
Speed: in air[a]	343 m/sec	3×10^8 m/sec
in water[b]	1486 m/sec	2.25×10^8 m/sec
in brass	3354 m/sec	Opaque--no transmission
Energy	$E = \frac{1}{2} m \omega^2 A^2$	$E = h\nu$
	where: m = mass of vibrating particle ω = angular velocity $= 2\pi f$ f = frequency A = amplitude	where: h = Planck's constant $= 6.63 \times 10^{-34}$ J sec ν = frequency = f

a. In dry air at 20°C.

b. In distilled water.

actually only a small portion of the entire sound spectrum, chosen to reflect the human limits of perception (Figure 1). Like electromagnetic radiation (of which visible light is only a part), sound is a wave and therefore can be described by frequency and wavelength. Further, like all waves sound transmits energy and information through a medium, yet the medium itself is not transported. Unlike electromagnetic radiation, sound is a longitudinal mechanical wave. Longitudinal refers to a wave whose oscillations are parallel to the direction of propagation of the sound wave; transverse waves such as electromagnetic radiation oscillate perpendicular to the direction of propagation. Mechanical refers to a wave that requires matter--specifically, an elastic medium--for its transport; sound cannot travel in a vacuum, for example, as light can (Halliday and Resnick, 1981, Chapter 18; Rossing, 1982, pp. 31-39).

As with light, where it is frequency which produces the different colors, it is frequency which produces the different tones or notes in sound. Loudness as perceived by humans is related to sound intensity which is the rate of energy transfer per unit area. Sound intensity, however, is usually not measured directly; rather a simpler measurement--sound pressure level--is taken. A third quantity -- sound power level--is used to describe the strength (or power output) of a sound source. Thus sound level in decibels (db) can be expressed in terms of intensity, pressure or power:

$$\text{Sound level (db)} = 10 \log \frac{I}{I_o}$$

where I = intensity
I_o = standard reference intensity
= limit of human hearing
= 10^{-12} W m^{-2}

$$\text{Sound level (db)} = 20 \log \frac{P}{P_o}$$

where P = pressure amplitude
P_o = standard reference pressure amplitude
= 20×10^{-6} Pa

$$\text{Sound level (db)} = 10 \log \frac{W}{W_o}$$

where W = power
W_o = standard reference power
= 10^{-12} watts

A 6 db change in sound pressure level[1] is roughly equivalent to a halving (or doubling) of the loudness perceived by humans.

The energy difference between light and sound waves is substantial. The intensity level at the human threshold of pain is only 1 W m^{-2}, compared with the 480 W m^{-2} of sunlight most places in the U.S. typically experience.

---

1. Loudness is a psychophysical quality which depends on such factors as frequency, quality of sound (pure tone vs. musical), and individual variation in ear sensitivity. Hence there is some disagreement over the precise sound level (db) change required to double or halve loudness as perceived by humans. Some studies have suggested that a 10 db change rather than 6 is required.

Interest in sound and plants has come from three diverse fields[1]:

1) noise pollution control

2) ecology

3) agriculture.

Noise Pollution Control Research

Noise pollution control with plants has emphasized outdoor urban applications (Aylor, 1972; Martens, 1980; Bullen, 1982; Palmieri, 1982; Baumgardt, 1971), with occasional attempts at indoor sound reduction (Rusak et al., 1980). Figure 2 shows the noise frequencies and pressure levels typically encountered in traffic environments. From roughly 250 to 4000 Hz, sound pressure levels in traffic can be above 90 db--well into the annoying and unsafe range. Sound pressure level (and loudness) drops off rapidly outside these frequencies.

The bulk of the research has concentrated on the attenuating, or sound-weakening, characteristics of foliage. Attenuation is operationally defined as the reduction in loudness, as measured by sound pressure level (SPL), obtained when a sound treatment delivered by a source at point A is measured by a microphone at point B:

$$\text{Attenuation} = \frac{SPL_A - SPL_B}{\text{distance from A to B}}$$

1. A computer literature search was conducted using the following databases: Agricola, CAB Abstracts, Biosis, and Food Science and Technological Abstracts.

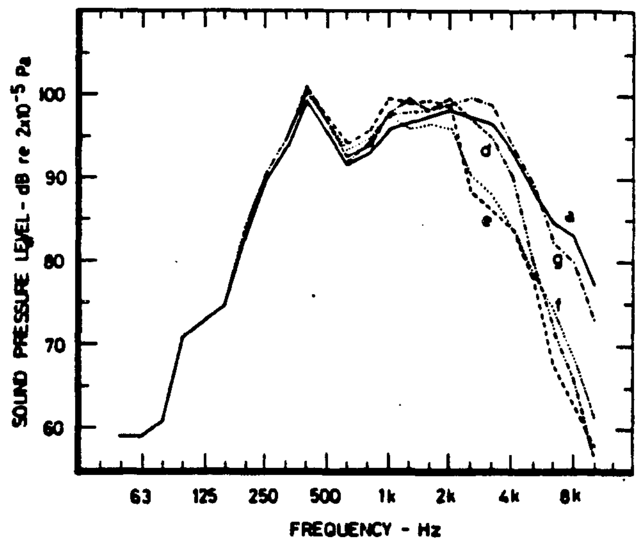


Figure 2. Standard traffic noise spectrum used in The Netherlands (curve a), and how this spectrum can be changed by the foliage of different plant species: d) spectrum with fully foliated birch trees, e) hazeltrees, f) tropical plants, and g) privets. From Martens, 1980, p.70, Journal of the Acoustical Society of America.

Most studies have examined the attenuation of forests and trees (Arabadzhi,1969), but one crop species (corn) has been studied. Table 3 provides an overview of the magnitude of the sound-attenuating effects and of some of the variables involved. Note that the effect varies with season, with summer being the more attenuating at 4000 Hz, for example. Since a 6 db drop in sound pressure level is equivalent to a halving of the loudness perceived by humans, significant noise reduction can be achieved with fairly narrow belts of vegetation.

Measurement methodologies vary but in general, forests appear to attenuate sound more strongly at the lower and higher frequencies. Various deciduous and coniferous forest communities in The Netherlands, when compared to an open sandfield control, were shown to more strongly attenuate sound at frequencies around 200 Hz and also for some forests, at high frequencies (8000 or 16,000 Hz) (Figure 3) (Linskens, et al., 1976). Enhanced sound transmission, however, occurred in the middle frequency range (500-2000 Hz) in some forests (Curves B and D in Figure 3). There were differences among the various communities in frequencies of maximum attenuation and in seasonal effects.

The ground and its condition (hard-packed vs. soft, for example) also appears to play a significant role in attenuation at the lower frequencies (roughly 200-1000 Hz) (Figure 4). The mechanism is thought to be two-fold:

1) acoustic interference between the sound traveling directly from the source and that which arrives at the receiver

Table 3. Various Excess Attenuation (Ae) Values Reported in the Literature, as Described by Linskens, et al., 1976.

Plant	Frequency of Measurement	Ae	Season	Report cited
Corn	200 Hz	15 db/100 m	Summer	Aylor (1972)
	4,000	90	"	"
Brush	4,000	45	"	"
	4,000	15	Winter	"
Dense grassland	10,000	63	NA	Eyring (1946)
Dense jungle	10,000	73	NA	"
Deciduous forest	NA	17	NA	Meister & Ruhrberg (1959)
Dense hedge	NA	30	NA	"
Forests/oak, beech, pine,	250-500	15-25 (max)	NA	Haupt (1973)
	4,000-8,000	2-20	NA	
Dense woods	350	3.5[a]	NA	Weiner & Keast (1959)
	5,000	25		

a. Absorption coefficients, rather than Ae values, were reported in this study.

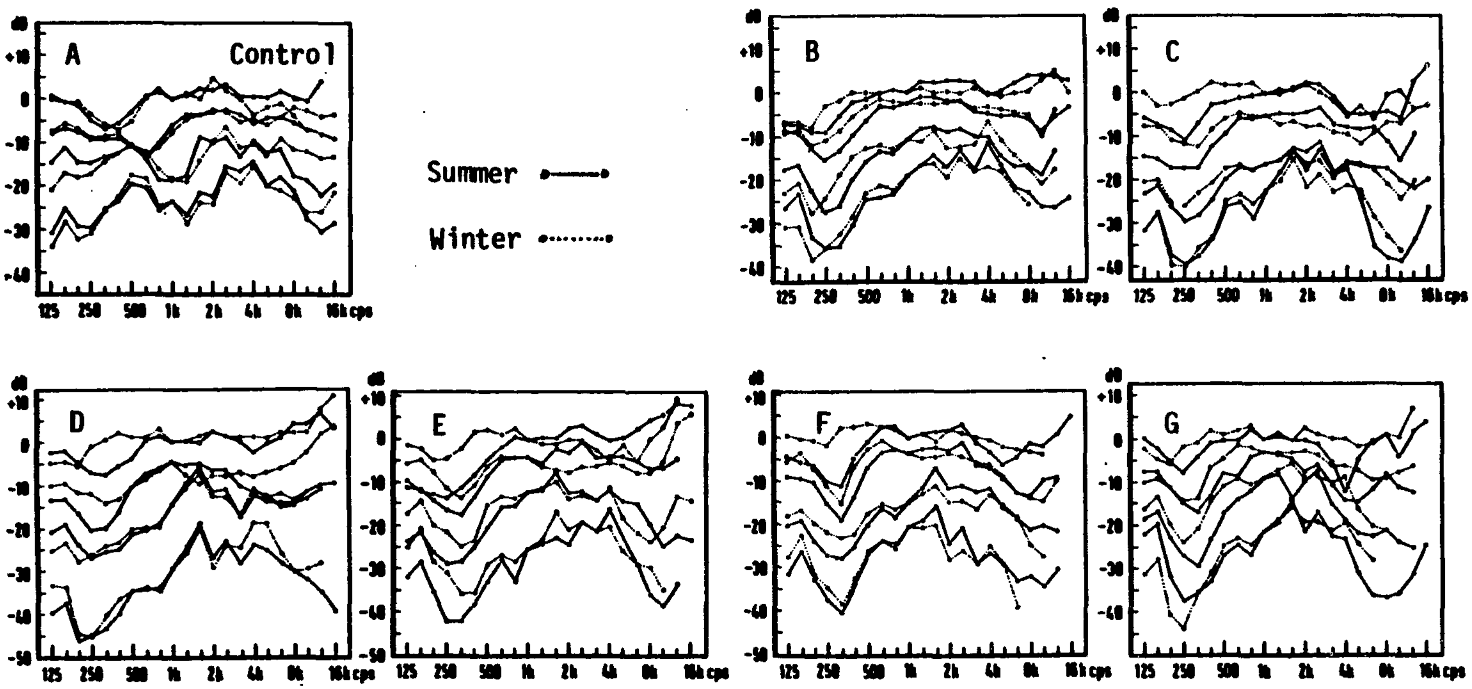


Figure 3. Attenuation of sound as a function of frequency, for 2 seasons (summer and winter) and at 4 distances from the sound source (4, 8, 12 and 16 m). Measurements taken at 1 m above ground. A) Control, an open sand field. B-G) Various deciduous and coniferous forests. From Linskens et al., 1976, pp. 168-169. Oecologia.

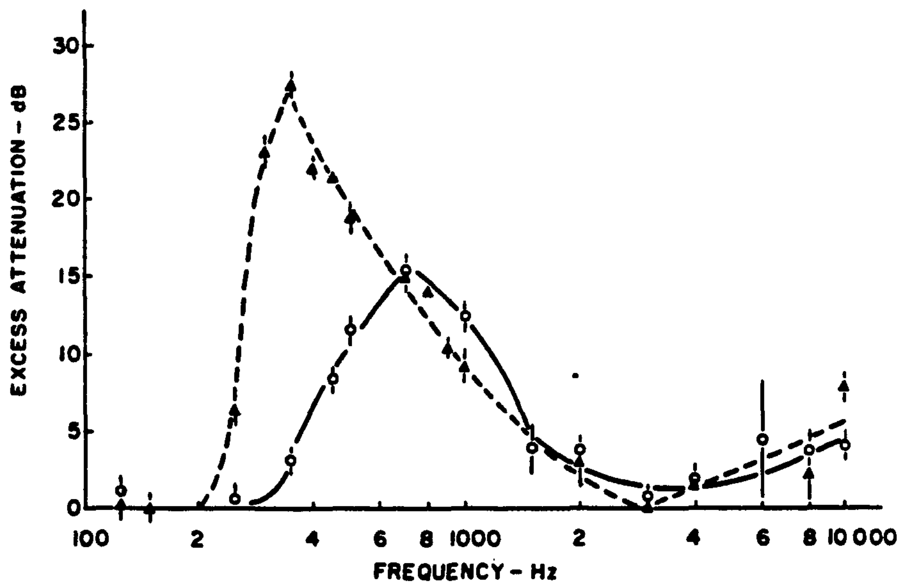


Figure 4. Excess attenuation in decibels for a weather-slaked fine sandy loam (o) and for the same soil after disking (Δ) at frequencies of 100 to 10,000 Hz. The source and receiver heights were 1 m and sound was measured at a distance of 52 m from the source. From Aylor, 1972, p. 201, Journal of the Acoustical Society of America.

after reflection from the ground;

2) a phase lag due to interaction of the sound with the soil surface; the more porous the soil, the longer the delay and therefore, the greater the attenuation and the greater the shift of the attenuation peak to a lower frequency (Figure 4).

Ecological Research

For ecologists the interest lies in the effect of plant communities on animal communication. Such studies, like the noise pollution control studies, have been complicated by varying soil, air, temperature and humidity conditions--all of which influence sound propagation. Yet the general conclusion is clear: plants do attenuate sound and the effect varies with frequency, season, and type of vegetation.

Each plant community has its own acoustic climate, characterized by a particular sound attenuation spectrum as Figure 3 above illustrated. Animals appear to have adapted their communication systems to take advantage of the acoustic climate of the environment (Richards and Wiley, 1980; Theiss and Prager, 1984; Wells and Schwartz, 1982). To be effective, communication requires that the receiver not only detect the presence of a signal but also discriminate significant information-carrying variations in the sound transmission. Plants can weaken (attenuate) a signal by absorption as well as degrade the pattern in a signal by causing reverberations[1]. Further, the acoustic climate of a calling site such as a broad leaf can impart directionality to a signal, as is the case for at least one frog species (Wells and Schwartz, 1982). Hence communication patterns would be expected to differ for animals in forests versus open grassland and for those who communicate near the ground versus farther above the ground. It is also expected that the emphasis on frequency modulation versus amplitude modulation would vary with the animal's environment. These kinds of relationships have been variously investigated and largely confirmed for North Carolina forest passerine birds (Richards and Wiley, 1980), bush crickets (*Tettigonia cantans*) (Keuper and Kuehne, 1983) and *Corixa dentipes* Thms. (Theiss and Prager, 1984), among others.

An interesting sidenote in this last study with pond insects was that sound attenuation varied tremendously (from "slight" to 80 db at 10 kHz) due, according to the authors, to variation in photosynthesis rates which caused large variation in the quantity of gas bubbles in the pond. Their measurements of "low" and "high" photosynthesis, however, were taken in winter and summer, respectively, and thus did not control for seasonal variation; yet gas bubbles would be expected to distort sound transmission in liquid.

Apart from sound attenuation, plants can affect animal communication by actually serving as the signal transmitter.

---

1. Reverberations are reflected sounds, and thus increase with increased availability of scattering surfaces such as leaves.

Animals such as cicadas (Dictyophara Europaea Homoptera Cicadina Fulgoroidea) (Struebing, 1977), bush crickets (Keuper and Kuehne, 1983), leafhoppers (Amrasca devastans Dist.) and plant hoppers (Nilaoarvata lugens Stal) (Saxena and Kumar, 1980; Saxena and Kumar, 1984), induce vibrations in the plant via the legs and sometimes the abdomen. These vibrations can be directly perceived by other species members up to 1.5 - 2 m away; transmission depends greatly on the mechanical properties of the plant. In one study (Saxena and Kumar, 1980), sound applied externally via an harmonium or audio-oscillator was shown to be picked up by the plant, causing it to oscillate. Mating success of males and females communicating on the same plant was drastically reduced. The effect was frequency dependent, with mating percentages dropping from 100% to 10% at 200 and 300 Hz. The intensity level was 72-76 db measured at the plant.

Both the noise pollution control and ecology fields have studied plant-sound interaction largely on the macroscopic and descriptive level. The search is now underway for the mechanism by which vegetation alters sound transmission. Possible mechanisms include soil absorption, scattering from trunk, branch, and leaves, absorption by the boundary layer of air at leaf surfaces, and/or vibration of the branches and leaves themselves. Such induced vibration actually represents a transfer of kinetic energy of the vibrating air molecules in a sound field to the plant, and thus is sound absorption. This kinetic energy is later lost as heat due to friction.

Martens et al. (1981, 1982) measured the sound absorption of leaves from numerous species using a laser-Doppler-vibrometer system (later combined with an interferometer), to examine vibration patterns. Sound absorption was found to vary with leaf frequency, species, and leaf orientation relative to the sound source (Figure 5). Although the amount of sound energy absorbed in this way by a single leaf is very small, the authors felt the attenuation could be significant in nature given the large number of leaves present.

Agricultural Research

The noise pollution control and ecology studies on sound attenuation by plants provide support for the idea that plants are affected by sound. Direct effects of sound on plant growth have been measured by agricultural researchers, but the interest has been primarily in ultrasound. The distinction between audible and ultrasound is man-made, chosen to coincide with the human range of hearing, yet the energy levels of the sound treatments typically used for ultrasound vs. audible sound studies are so different (less than 1 W cm^{-2} for audible vs. up to 20 W cm^{-2} for ultrasound) that it is probably correct to assume that their overall effects are likewise quite different. Since work reported herein is primarily concerned with the audible sound range, the ultrasound research will be treated only briefly below, followed by a more detailed discussion of the audible sound literature.

Ultrasound

Ultrasound (20 kHz - 600 MHz) was initially investigated by

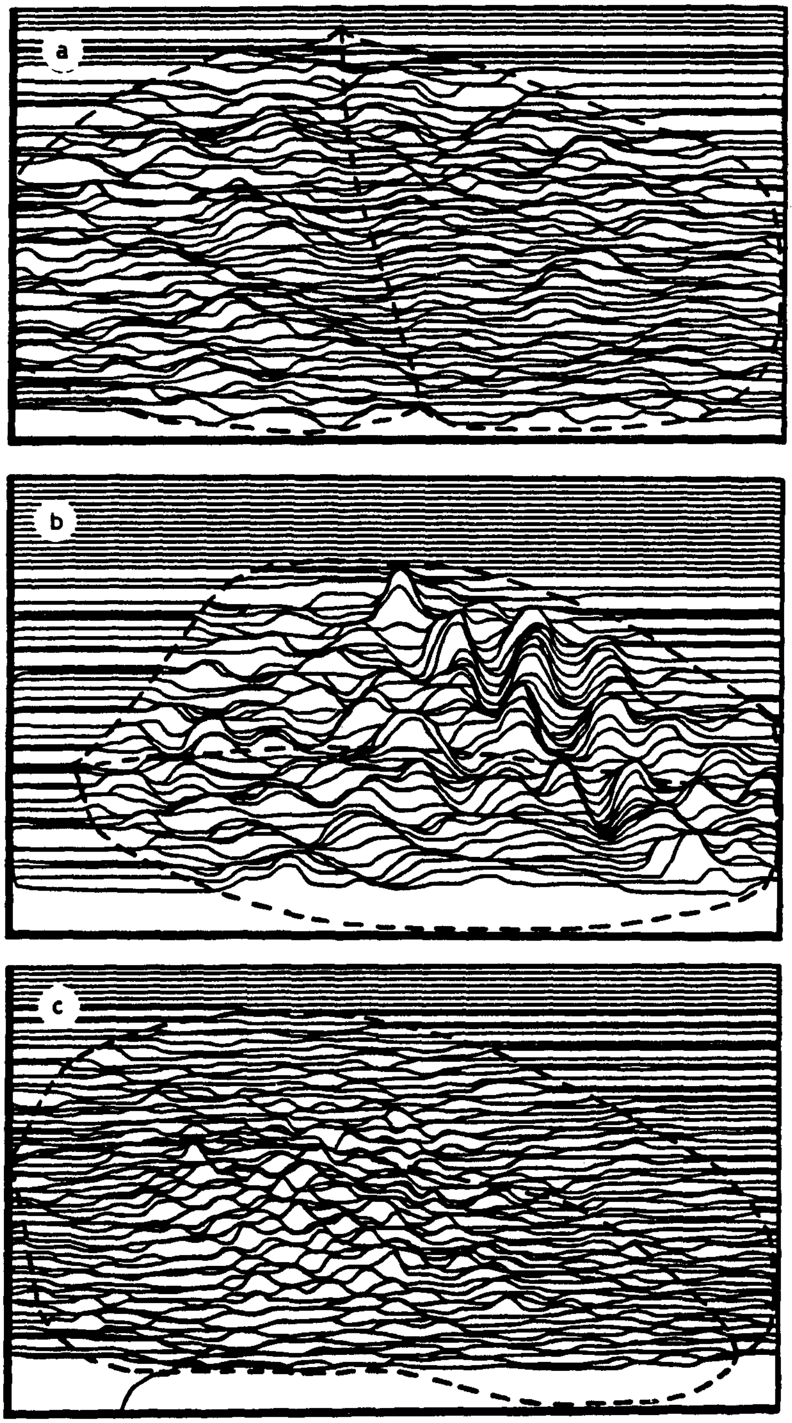


Figure 5. Direct evidence of frequency-dependent sound absorption by plant leaves. Vibration velocity pattern of Tilia platyphyllos Scop. leaves as a function of frequency: a) 440 Hz, 108 db; b) 1200 Hz, 122 db; c) 6750 Hz, 104 db. From Martens et al., 1982, p.291.

the USSR and Eastern European countries to stimulate germination and crop productivity, especially for wheat. Western interest in ultrasound awakened in the 1960's; however, its interest lay more in the killing potential of ultrasound--for pest control in grain storage bins, for example, or for weed control. Ultrasound has been reported to have a wide variety of beneficial effects on plants (Gordon, 1971; Bebee, 1983), including:

- improved germination
- breaking of dormancy
- increased growth rate
- increased yield.

Improved germination has been reported for a wide variety of species--for example, sugar beets (*Beta vulgaris* L.), corn (*Zea mays* L.), potatoes (*Solanum tuberosum* L.), sunflowers (*Helianthus annuus* L.), wild rice (*Zizania aquatica* L.), conifers, and rye (*Secale cereale* L.). At least in barley (*Hordeum vulgare* L.), ultrasound appears to increase the rate of diffusion of water into seeds. Pre-soaking of seed reduces the sound dosage time required for beneficial effects, but also for a lethal reaction.

Breaking of dormancy has been shown to occur with ultrasound in wild rice, for example, although the same treatment was found to reduce barley germination.

Increased growth rates have been reported with ultrasound in maize, barley, millet (*Pennisetum americanum* L., K. Schum), rice (*Onyza sativa* L.), wheat, peas, beans, lucerne (*Medicago sativa* L.), sunflowers, and conifers, yet decreased growth rates were also reported for some of the same species in other studies. One unusual effect occurred in a study with peas. Seeds soaked for 6 hours, left to stand for 12 hours, and then treated with 10-30 seconds of high intensity ultrasound (20 W cm^{-2}) showed a decrease in total germination but a doubling of the height and primary root length of 20% of the survivors. The increased growth was not accompanied by an increase in yield of peas, however.

Increased yield was reported in studies with field-grown sugar beets (38% and 14% increases), peas and pods (32%), potatoes raised from seed (45%), and potatoes raised from tubers (15%), as well as lesser increases in cucumbers, lettuce, lucerne, sunflower seed, and numerous cereals.

The mode of action of ultrasound on plants is not clear. There are a number of physical effects associated with ultrasound:

- shearing, caused by the fluctuating, uneven pressure distribution which is created within cells and molecules by wavelengths close to or smaller than the dimensions of the cells and molecules;
- heat production, from frictional losses;
- cavitation, which is the formation of regions of partial vacuum caused by high frequency ultrasound[1].

---

1. Cavitation is also used in plant-water relations to indicate the formation of an air bubble in a xylem element whose water contents were previously under tension. In either case the term indicates a switching between positive and negative pressure.

These physical effects probably play a significant role in the numerous physiological and molecular effects of ultrasound on plants which have been noted:

- an increase in the rate of certain biochemical reactions, as measured by the activities of the following enzymes: amylase, cellulase, peroxidase, catalase, RNase, some respiratory and photosynthetic enzymes;
- chromosomal abnormalities and degradation of DNA;
- formation of nitrous acid (a known mutagen) when proteins and nucleic acids have been irradiated;
- oxidation of macromolecules, perhaps due to cavitation-induced formation of free radicals and ions;
- changes in membrane permeability;
- disruption of chloroplast ultrastructure (Bartels, 1969);
- changes in the fatty acid composition of oils.

In summary, the effects of ultrasound are often inconsistent. Different investigators have not been able to reproduce others' results, and different species have not reacted similarly to the same treatments. Treatments have varied widely in frequency, intensity and duration of exposure and thus total energy input[1], as well as in plant conditions (age, variety, dry versus pre-soaked seed, growth environment temperature and light). Thus consistent patterns among successful beneficial ultrasound

---

1. Ultrasound treatments typically employ sound frequencies of 1-2 MHz applied at energy levels ranging from 0.5 to 20 W cm^{-2}, for a duration of 1-5 minutes.

treatments have not emerged.

Audible Sound

The audible sound spectrum is detailed in Figure 6. Table 4 summarizes the audible sound research and results. The largest effects occurred with two kinds of treatment:

1) 5000 Hz applied at 92 db to Rideau winter wheat during 4 weeks of chilling of the grains at 2^{0} C in water, followed by sound treatment for the next 8 weeks; the effects included a 200% or greater increase in all growth parameters measured: plant height, dry and wet weight of root and shoot, and number of roots;

2) a flute agrarian tune, applied to cucumbers for 8 hours a day until the plants were 8 weeks old; plant height, fresh weight and dry weight of tops, and number of buds increased by 200% or more.

As illustrated in Table 4, the following variables are relevant:

<u>Sound variables</u>	<u>Plant variables</u>
frequency	species and variety
intensity	age
duration of treatment	growth conditions
total energy input	
pure tone vs. variable frequency	

Each variable is discussed separately below.

<u>Frequency.</u> Sound effects are clearly frequency-dependent. In the Measures and Weinberger studies (1968), 5000 Hz practically doubled the growth of Rideau winter wheat and Marquis spring wheat. Three hundred hertz stimulated the Marquis wheat to a lesser degree,

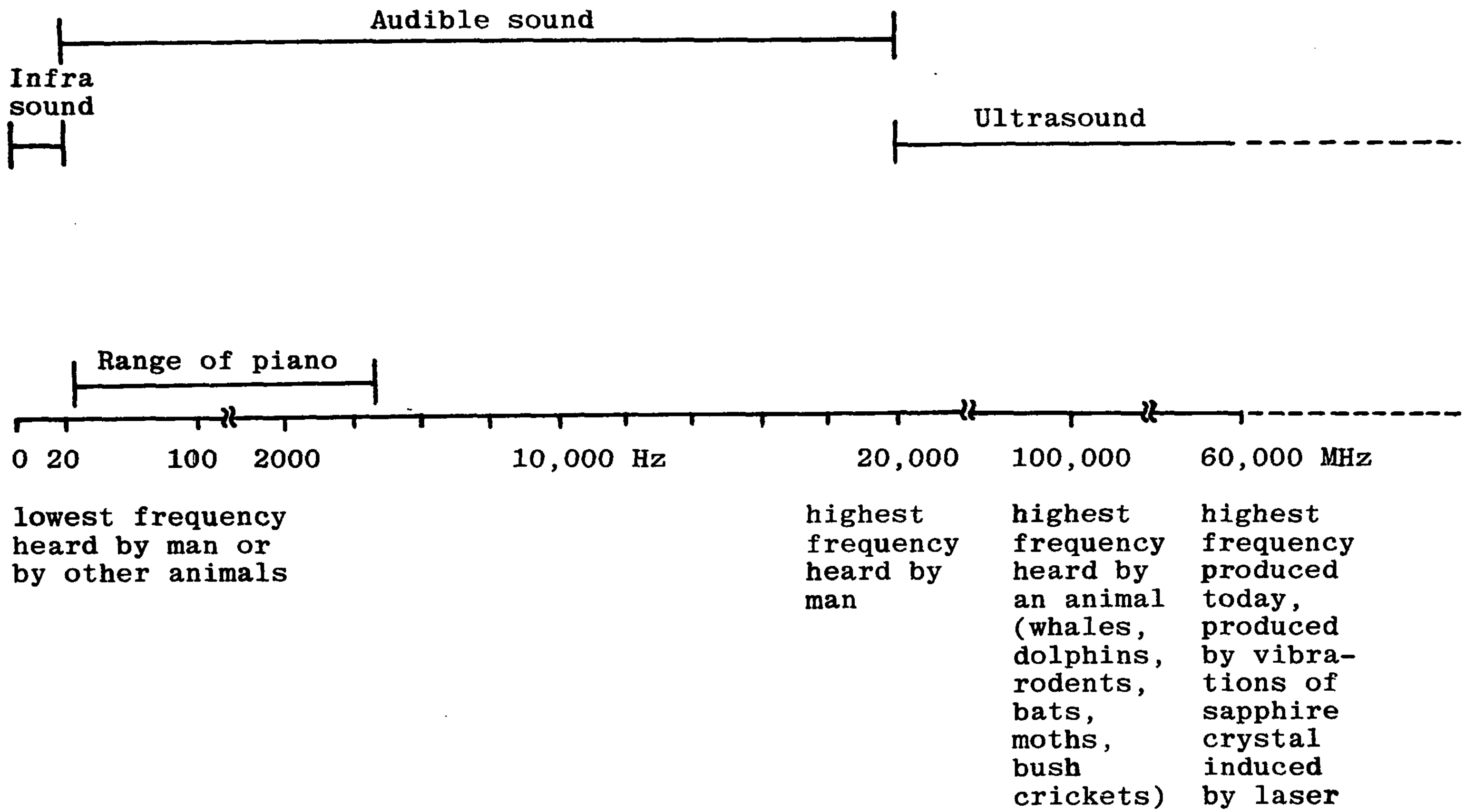


Figure 6. Details of the sound spectrum.
(From Shepard, 1983, Ch. 16; & Bickel, 1984, pp. 8,13.)

Table 4. Summary of Audible Research and Results Reported in Literature.

Plant Name	Age	Sound Treatment Type	Frequency	Intensity	Duration	Other Test Parameters Temp.	Variables Measured	Results
Algae (Scenedesmus obtusiusculus)	Synchro- nized cultures exposed at different phases of 24-hour life cycle	Pure tone	4,000 19,000	160 db	216 hrs	26±1 °C	Cell division rate (initial and final number of cells) Cell dry weight Number of nuclei per cell	4,000 H: whi 19,000 H dec Normal c (48 Generall div Most ser cel sep
Wheat (Triticum aestivum L.) Opolska (Spring) Dankowska Biala (winter)	From seed to 7, 9, and 14 days	Pure Tone	100 Hz 150 250 300 1000	90 db	Continuous; #hrs/day not avail.	10 °C 15 °C	Shoot height Root total length	Effects tem At 10°C, mos At 15°C, spr win At 15°C, max and
Wheat: Marquis (Spring) Rideau (Winter)	1-4 weeks seed pre- treatment to 1, 2, 4, 6, 8 weeks	Pure tone	5,000 Hz 12,000	92±1 db 95±1 db	Continuous apparently day and night; begun during 4-week chilling pre-treatment of seed	2 °C 10 25	Germination rate Plant height Dry and wet weights of root and shoot Number of roots	Effects tem Germinat for no Growth, yie for
Wheat: Marquis (spring)	4 weeks seed pre- treatment to 8 weeks	Pure tone	300 1250 5000 12,000	93 db 89 db 92 db 95 db	Continuous for 4 weeks pre-treatment only, not during growth	2 °C pre- treatment then day=25 °C night=18C	Height Number of tillers Number of roots FW and DW of roots FW and DW of shoots	5000 Hz 200 300 Hz - 1250 Hz 12000 Hz
Wheat: Rideau (winter)	4 weeks seed pre- treatment and 8 weeks growth	Pure tone Random white noise	300 1250 5000 12000 60- 32000	89-95, 105, 120 92-56	Continuous for 4 weeks pre-treatment only	25±2 °C	Height Number of roots Number of tillers Number of emergent leaves DW roots and shoots Floral initiation	Effect o 105 and Mor 92 db - whe all Floral i
Wheat Rideau (winter)	Seed - 4 week cold pre-treatment	Pure tone	5000 Hz	92 db	Continuous for 4 weeks	2 °C	Amino acid composition: free and total alcohol-soluble amino acids	Free amir Tota Rela Less Total ami Tota Rela
Alfalfa Bean Corn Cucumber Flax Lettuce Oats Pea	From seed to 8 weeks	Variable frequency: - music, agrarian tunes, vocal and instrumental - random noise	 20- 20,000 Hz 60- 32,000 Hz	 78-92 db 92-56 db	Continuous 8 hr/day	25±1 °C	% germination (hourly) FW and DW of tops Number of leaves Height Number of buds and flowers	Germinati but at l drop Growth: c 200% 300% slig Some less and

ted in Literature.

ntensity	Duration	Other Test Parameters Temp.	Variables Measured	Results	References
60 db	216 hrs	26±1 °C	Cell division rate (initial and final number of cells) Cell dry weight Number of nuclei per cell	4,000 Hz - 15% decrease in cell devision rate after 48 hrs which persisted until sonication stopped 19,000 Hz - initial 10% increase in cell division rate, but decreased after 48 hours to 10% below initial rate. Normal cell division rates restored after 2 generations (48 hours) after sonication stopped. Generally fewer multinucleate cells (preparing for division) in sonicated cultures. Most sensitive time in life cycle for depressing cell division rate: 0-4 hours after autospore separation.	Weinberger and Das (1972)
) db	Continuous; #hrs/day not avail.	10°C 15°C	Shoot height Root total length	Effects vary with plant variety, age, plant part, temperature and frequency. At 10°C, 14 days: sound generally inhibited growth (40-70%) most inhibitory frequency = 100 Hz (spring wheat) 300 Hz (winter wheat) At 15°C, 14 days: sound stimulated growth spring wheat roots: max = 20% at 300 Hz winter wheat shoots: max = 14% at 100 and 250 Hz At 15°C, 9 days: all sound stimulated growth; max= 35% at 250 Hz for winter wheat shoots and 30% at 300 Hz for spring wheat roots.	Tomaszewski and Weryszko (1976)
±1 db ±1 db	Continuous apparently day and night; begun during 4-week chilling pre-treatment of seed	2°C 10 25	Germination rate Plant height Dry and wet weights of root and shoot Number of roots	Effects vary with plant variety, age, plant part, temperature and frequency. Germination: 2-3 fold increase at 2°C, 5000 Hz, for both spring and winter wheat; no effect at 25°C. Growth, effective frequency: for winter wheat = 5000 Hz, yielded 200% increase in all growth parameters; for spring wheat - no consistent effect.	Weinberger and Measures (1968)
db db db db	Continuous for 4 weeks pre-treatment only, not during growth	2°C pre-treatment then day=25°C night=18°C	Height Number of tillers Number of roots FW and DW of roots FW and DW of shoots	5000 Hz - most effective; all but 1 parameter increased by 200% 300 Hz - effective, but to lesser degree 1250 Hz - no effect 12000 Hz - no effect.	Measures and Weinberger (1970)
·95, ;,) ·56	Continuous for 4 weeks pre-treatment only	25±2°C	Height Number of roots Number of tillers Number of emergent leaves DW roots and shoots Floral initiation	Effect of intensity: 105 and 120 db: Reduced root and shoot weights at 5000 Hz Morphological anomalies in 3% of 2nd and 3rd leaves 92 db - Normal growth for all frequneces except 5000 Hz where growth stimulated by at least 50% in almost all parameters. Floral initiation not affected in any treatments.	Weinberger and Measures (1979)
db	Continuous for 4 weeks	2°C	Amino acid composition: free and total alcohol-soluble amino acids	Free amino acids: Total amount - no effect Relative proportions altered, however: 60% more alanine in sonicated embryo, 50% less in endosperm. Lesser differences for arginine, serine, and proline Total amino acids: Total amount - less in sonicated embryos and endosperm for all amino acids except methionine Relative proportions similar, however.	Measures and Weinberger (1973)
92 db 56 db	Continuous 8 hr/day	25±1 °C	% germination (hourly) FW and DW of tops Number of leaves Height Number of buds and flowers	Germination: No clearly significant effect, but beans and corn showed 90% and 38% more germination at 18 hrs with a female solo melody; dropped to 20% and 27% at 24 hours Growth: cucumbers and flute melody - 200% increase in height and weight (wet and dry) 300% increase in number of buds slight but significant increase in number of leaves Some lesser but significant effects with flute on corn and oats.	Weinberger and Graefe (1973)

and 1250 and 12,000 Hz were shown to have no effect. Tomaszewski and Weryszko (1976) found that 150 Hz stimulated wheat seedling growth at 10° C and 100 Hz inhibited growth. Their results illustrate that at low frequencies even a 50 Hz frequency difference can be significant[1]. Also illustrated by these two studies was the variable effect a particular frequency can have depending on the plant variety used, the plant organ studied, and the temperature at which the experiments are conducted. The effect of a particular frequency is not necessarily constant over time; Tomaszewski and Weryszko (1976) found that effects at 9 days of age were not necessarily the same as those at 7 or 14 days, and Measures and Weinberger (1968) found no significant growth differences until the plants were 4 weeks old. Harmonics do not necessarily produce the same effect; for example, Measures and Weinberger (1970) found that 1250 Hz had no effect on Marquis wheat whereas 5000 Hz, the fourth harmonic, nearly doubled growth. (To fully test the effect of harmonics, however, 5000 Hz would have to be treated as the fundamental and its overtones --i.e., 10,000 Hz, 15,000 Hz, etc.-- tested).

<u>Intensity.</u> It is important to remember that the sound

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1. At high frequencies a 50 Hz difference may not be significant, however. More likely it is the number of octaves or half-tones separating two tones which determines whether the frequency difference is significant regarding its effect on plants. The 50 Hz difference between 100 and 150 Hz, for example, represents 7 half steps whereas between 2000 and 2050 Hz it is equivalent to less than 1 half-step.

intensity measurement scale is logarithmic; hence, an increase of 6 decibels in sound pressure level corresponds to a doubling of loudness as perceived by the human ear. In the only reported study of intensity effects, Weinberger and Measures (1979) found that wheat seedlings treated with 4 audible frequencies or random white noise at an intensity level of 105-120 db exhibited reduced root and shoot weight and abnormalities in the morphology of some of the leaves. Those plants treated with 92 db intensity showed either normal or stimulated growth. Thus it appears that a critical intensity threshold exists above which sound can be harmful.

Duration. No studies have been reported which examined the effect of sound duration. Both the Tomaszewski (1976) and Weinberger (1968,1970,1973,1979) groups applied sound continuously for at least 8 hours. Because duration affects total energy input, it is reasonable to assume that duration will influence sound's effect on plants. That sound can have a lingering effect was demonstrated in the 1970 Measures and Weinberger study, where wheat grains pre-treated with sound only for 4 weeks at 2^{o} C prior to planting produced plants with significant growth differences.

Total Energy Input. As given earlier in Table 2, the energy of a sound wave is:

$$E = \frac{1}{2} m \omega^2 A^2$$

Thus, the energy imparted by a sound treatment upon a plant can be changed by changing the amplitude (A) (i.e., loudness) or the frequency ($f = \omega/2\pi$), or the duration. Thus it is important to control these 3 variables. The intensity study by Weinberger and Measures (1979) was actually a comparison of different energy dosages; it showed that too high of a dose of even a low-energy form such as audible sound can be detrimental.

Pure Tone vs. Variable Frequency. Pure tones are much easier to quantify and control than variable frequency sound treatments such as random white noise or music. Further, the precedent exists in plant physiology--in light energy research--for energy analysis in terms of single frequencies. Lastly, there is the underlying belief that even if music, for example, was found to benefit plants, pure tone studies would eventually be required in the search for the mechanism. Hence all the audible sound studies in the scientific literature except one have used pure tone treatments. There is a fairly strong popular belief, however, that music does affect plants, with rock being detrimental and classical being beneficial. Numerous experiments have been reported in the popular literature which support these contentions (Tompkins and Bird, 1973).

The one scientific investigation into variable frequency sound examined 6 agrarian tunes and 2 random noise selections for their effect on germination and growth of alfalfa, beans, corn, cucumber, flax, lettuce, oats, and peas (Weinberger and Graefe, 1973). No clear statistically significant effects (at the 5% level) were obtained for germination rate, although germination of bean and corn seed treated with one tune--a flute piece--increased 90% and 38%, respectively, at 18 hours compared to the untreated; by

24 hours, the advantage over the controls dropped to 20% and 38%, respectively. In the growth studies only 4 of the tunes, including the flute melody, were tested along with the 2 random noise selections. Cucumber tops doubled in wet and dry weight and in height, and the number of flower buds tripled; the number of leaves increased by 73% (Table 5). Lesser increases in wet weight of corn and oat tops were also obtained with the flute melody. Neither the standard randomized white noise nor a second random noise selection created by randomizing one of the agrarian tunes produced any discernible effect.

Plant Species and Variety. Sound effects appear to be species- and variety-specific. Of the eight species tested in the Weinberger and Graefe (1973) variable-frequency study, two- to three-fold, statistically significant increases in measured growth parameters were obtained for cucumbers, whereas none of the other species appeared affected. Different varieties of wheat were shown to respond differently to sound treatments in both the Weinberger and Measures (1968) and Tomaszewski and Weryszko (1976) studies. Five thousand hertz stimulated growth of both Rideau winter and Marquis spring wheat (Weinberger and Measures, 1968), whereas only Marquis was stimulated by 300 Hz. Dankowska Biala winter wheat at 10^{0} C was found to be strongly inhibited by 100 Hz whereas Opolska spring wheat was inhibited only by the 300 Hz treatment (Tomaszewski and Weryszko, 1976). Species-specificity has been more widely documented in the ultrasound literature.

Table 5. The effect of a flute agrarian tune on cucumbers (var. Straight Eight) after 8 weeks growth. From Weinberger and Graefe, 1973, p.1853.

Treatment	Height cm	Wet wt. tops, g	Dry wt. tops, g	Number buds	Number leaves
Control	25.4	6.9	0.6	20.2	10.8
Music-treated	51.0	12.7	1.0	61.3	18.7

Plant Age. Weinberger and Measures (1968) found no significant differences in growth of sound-treated vs. control wheat seedlings until after 4 weeks of age. Tomaszewski and Weryszko (1976) observed differences in effects between 9-day-old and 7- and 14-day-old wheat seedlings. In short, what may be beneficial at one developmental stage, may inhibit or have no effect at another stage.

Plant Growth Conditions. Both temperature and light can drastically affect the growth and development of plants. While all the studies described here carefully controlled light and temperature throughout the experiments, no systematic investigation of light effects on plant response to sound has been undertaken. Because plant growth rate can only be expected to change if there is room for change--that is, if the plant is not already growing at the maximum possible rate--it may be that response to sound of plants under "stressed" conditions such as low light (as in growth chambers) differs from that under unstressed or normal field conditions.

Temperature effects have been studied, however. Weinberger and Measures (1968) found that 5 kHz sound stimulated germination of their spring and winter wheat at 2^{o} and 10^{o} C, but not at 25^{o} C. The sound-treated plants in Tomaszewski and Weryszko's (1976) study showed a general sound stimulation of growth for shoots and roots of both the spring and winter wheat at 15^{o} C, but a strong sound inhibition at 10^{o} C for the spring wheat and partial inhibition for the winter variety.

Possible Mechanisms for Sound Effects

The most comprehensive, systematic treatment of the problem is given by Measures (1971). A reproducible, statistically significant, frequency and intensity dependent effect of audible sound on wheat was demonstrated in both laboratory and field experiments; up to two-fold increases were obtained in numerous growth parameters (shoot and root length, dry weight, and fresh weight). Measures then conducted a series of investigations into the possible mechanism(s) for the effect. She examined:

- leaf morphology, including stomata and trichome number
- respiration rate
- photosynthetic rate
- amino acid composition
- nucleic acid content
- mitosis in root apical meristems
- peroxidase activity
- P-32 uptake
- effect of sound on the growth of two Rideau wheat seed contaminants.

Leaf morphology, respiration, photosynthesis, peroxidase activity, and DNA content and mitotic activity of the root tips were unaffected by the 5 kHz sound. P-32 uptake by both grain and roots was reduced. Free and total amino acid composition was lower, but RNA concentrations were higher in the sound-exposed series. The proportions of the free amino acids were altered as follows:

Free amino acids	% change compared to control
Alanine, in the embryo	60% more
Alanine, in the endosperm	50% less
Leucine, in the embryo	50% less
Amides	13% less

The relationship between sound and plants is thus not clear.

Strong evidence for plants affecting sound transmission comes from the noise abatement and ecology literature; if plants attenuate sound, the reasoning continues, they must absorb it or at least be affected by the sound in some way. Direct studies of audible and ultrasound effects on plant germination, growth and development have shown that:

1) Sound can help or hurt plants, or not affect them at all. Besides frequency, a critical intensity level may be involved, above which a negative effect is observed, but below which a positive effect occurs. Too long of an exposure may also be detrimental.

2) Results may not be consistently observable -- for example, some experiments have given no results on germination but significant, easily observable difference 4-8 weeks later in wheat. At other times, the reverse has occurred. The effect may depend on what time of the plant cycle the sound is applied. In some cases, it appeared that the rate of plant development was affected, but not the end product.

Up to now the approach has been to almost randomly select a sound frequency and intensity and a plant and see what happens, with very little systematic rationale behind those choices. What is needed is a systematic screening technique by which the sound frequency and intensity ranges can be quickly scanned with a few test plants, as a preliminary to later, longer-term tests. Any significant peaks or valleys in this preliminary sound scanning curve could then be used as starting points for longer-term field or laboratory studies.

PURPOSE

The purpose of this research was to examine the effect of audible sound frequency on the growth rate of young wheat plants, in order to provide an action spectrum which can serve as one possible screening method for longer-term field or laboratory studies.

METHODS

Overview of Procedure

Four-day-old "Yecora Roja" (*Triticum aestivum* L.) wheat seedlings in Hoaglands solution under fluorescent lighting were exposed to pure-tone sound treatments at 90 ± 2 db (10^{-3} W/m^2). The audible sound spectrum was scanned systematically from 100-20,000 Hz. Growth rate was measured directly using time-lapse photography; plant height was measured from the enlarged film projections, converted to real height (by multiplication with a conversion factor), and then used to calculate growth rate. The controls were tested separately on another day in the same experimental set-up minus the sound treatment.

Planting and Growth Procedures

Yecora Roja, a hard red spring wheat, was chosen for the testing because it is presently the most common wheat grown in Arizona. Certified seed from the 1984 crop grown in Yuma, Arizona was obtained from the Arizona Crop Improvement Association. Preliminary experiments were conducted to determine optimum lighting and planting conditions (embryo up vs. down, for example) to maximize growth and uniformity and to minimize root disturbance upon transfer to the experimental set-up. The procedure which resulted was as follows: for each test, 32 morphologically similar seeds were planted in 2 square plastic trays (19.5 x 19.5 x 5.5 cm), in medium-weight vermiculite, with 16 seeds per tray spaced 3.8 cm apart. The seeds were planted vertically 0.8 cm deep with the embryo ends down. All seeds were oriented so that they faced the same direction to improve the morphological uniformity of the seedlings.

The seeds were grown in a laboratory under fluorescent light banks whose intensity was 200-240 μE (PAR) at the location of the trays. For most experiments the lighting was an alternating combination of "Gro-Lux" (Sylvania Lifeline Gro-Lux F40-GRO) and cool white (Westinghouse F40 CW) lights. A 13-hour-day, 11-hour-night lighting schedule was maintained. In the few instances when this light bank was not available, the all-cool-white bank or all-warm-white (Sylvania Lifeline F48 T12-WW) bank was used. No significant differences in morphology or seedling height of seedlings at this age (4 days) were observed among plants grown under the three different light banks, as determined in a separate comparison study. Plants were watered to field capacity every day or every other day.

Temperature and relative humidity in the laboratory were typically 22-25^{o}C and 30-40%, respectively, for both day and night.

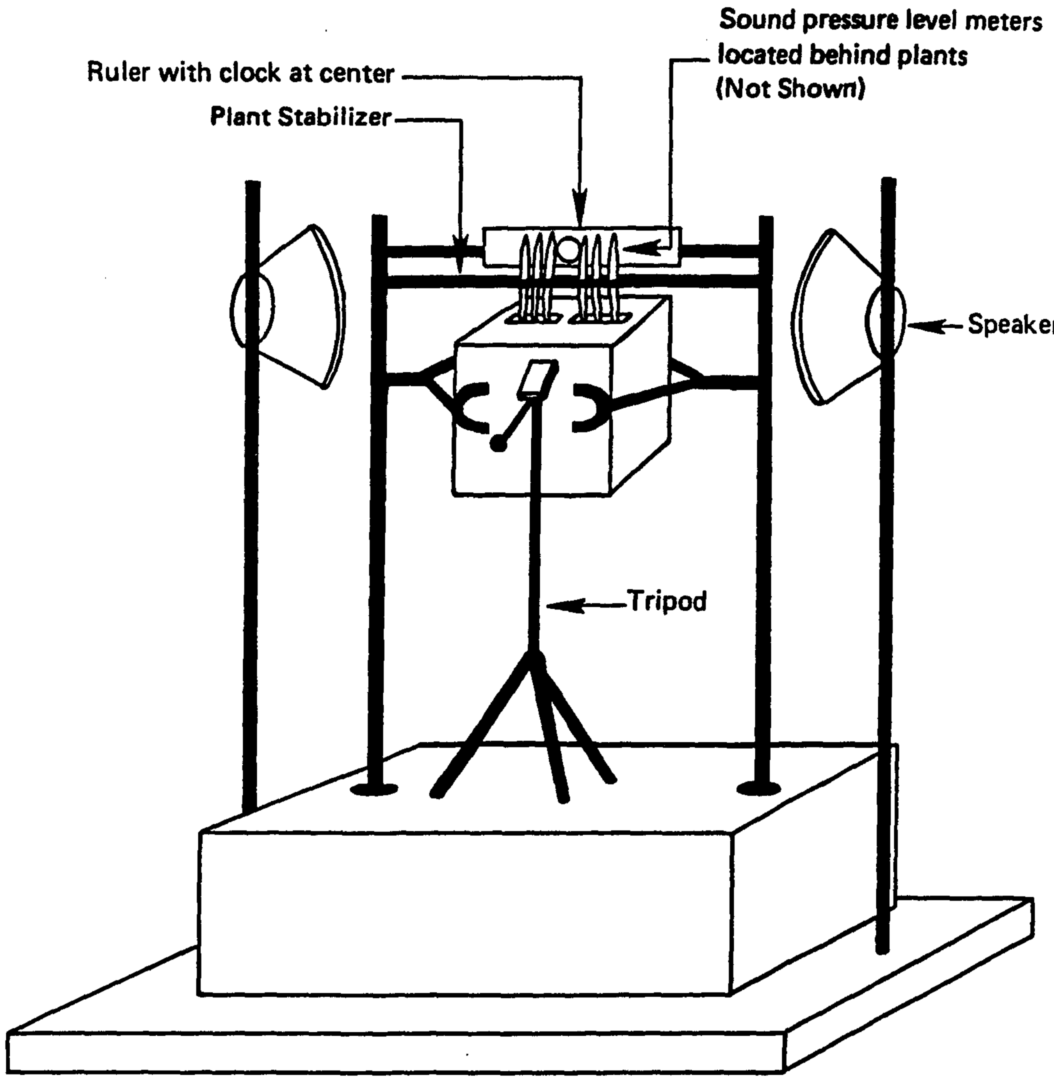
Experimental Set-Up

Experiments were conducted in a separate room (2.5 x 2.5 x 3.0 m). A sound-isolated anechoic chamber of sufficient size to minimize temperature fluctuations during the day was not available. The room used had hard surfaces (cement) on all sides except one, which had acoustical tile; hence, the surfaces were highly sound reflective. The approach, therefore, was not to try to eliminate reflection but rather to maintain the sound characteristics of the room as constant as possible.

The experimental set-up is outlined in Figure 7. The mounting system (Figure 8) was designed to ensure that: 1) the exposure of the shoot, including the growing region (basal end), to the sound was maximized; 2) disturbance to the seedling during set-up was minimal; 3) friction between the growing leaf and the mounting system surfaces was minimal; 4) stability of the mounting system and plant position were maintained over the course of a test.

The plants were placed in the set-up in modified Hoaglands solution so that the growing region faced front (towards the speakers) and the seedling tips were 15 cm below the cool-white fluorescent lights at the start of the experiments; light intensity was 320 μE (PAR). The speakers were angled to maximize the sound intensity delivered at the plants.

Temperature and relative humidity were monitored using wet and dry bulb thermometers. Over the course of the entire investigation temperature and relative humidity ranged from 21.7 - 26.1 ^{o}C and from 28 - 49%, respectively. For any one test day, however, temperature remained constant within $\pm$ 1.1^{o}C (comparable to a growth chamber) and relative humidity usually remained within $\pm$ 3.5 percentage points of the average; maximum variation in relative humidity for any test day was ten percentage points.



Dimensions:

From lights to seedling tips: 15cm

From center of speaker to plants: 26cm
(between plants 3 and 4)

From plants to back wall: 1.07cm

From sound pressure level meters to plants: 4.0cm

Size of plant solution container: 9.5 x 12.5 x 14.5cm

Figure 7. The experimental set-up.

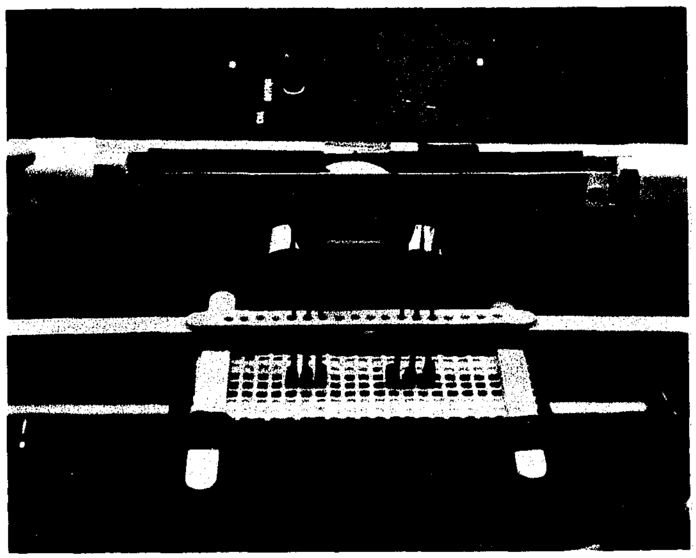


Figure 8. Close-up of the mounting system.

Sound Equipment

An audio sweep generator (Production Devices Model 140) connected to a Yamaha amplifier (Model A-700)[1] provided the pure-tone sound. The two speakers were Realistic models 40-1271C and 40-1272C. Frequency was determined with a Hewlett-Packard 5210 A frequency meter. Two sound pressure level meters (Realistic) were placed 4.0 cm behind the plants.

Photography

Photographs of the growing plants against a metal ruler of fixed width were taken every five minutes using a Pentax K1000 camera with a close-up lens. The Kodak Plus-X-Pan film (black and white) was developed with Microdol-X. Plant heights were read from the projections of the negatives on a projection screen which had a superimposed grid. The projector was a Leitz Model Wetzlar Prado 500.

Test Procedure

For each test day, six 4-day-old seedlings were transplanted from the vermiculite to the Hoaglands solution in the experimental set-up. After one half hour the photography began and shots were taken every five minutes thereafter; another 1-1.5 hours of equilibration was allowed before the sound treatments began in the experimental trials. A quiet period of about 1 hour was inserted

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1. For the earliest experiments a Realistic SA-10 amplifier was used.

after approximately 2 hours of sound in most tests. At the end of the day, another quiet period of 1 hour was inserted. Total test length was typically 8 hours. A photograph was taken the next morning before dismantling the test to obtain the night growth rate.

Table 6 summarizes the experiments. Five control and nine experimental tests were conducted. The following types of sound treatment schedules were tested:

1) 15 minutes or longer of sound (one frequency) + 15 minutes quiet,

2) 15 minutes of sound with no quiet periods between tones,

3) 5 minutes of sound with no quiet between tones.

For all tests the sound pressure level measured at the plants was 90 ± 2 db except at the highest frequencies (beyond 17,000 Hz) where the maximum attainable was 75 ± 3 db.

In addition to the pure tone experiments, tests with variable frequency sound were run for 1 full day plus several short periods in other tests. The variable-frequency sound treatments included the human voice, pure tone sweeps (produced by the audio oscillator), and music (on cassette tapes). The music included:

1) a series of Michael Jackson selections, to represent rock music,

2) a series of bassoon duets and solos, and a Mozart Piano Concerto (#23 in A) to represent classical music.

Table 6. Summary of Experiments.

Experiment	Sound Treatment: Frequency (Hz)	Duration
Series 0		
Control		
Experimental 1	100-1000 in 100 Hz intervals, 1000-20,000 in 500 Hz intervals	5 min, no quiet between tones
Series 1		
Control		
Experimental 1	60, 100, 1250, 5000, 8000, 11,000, 14,000	15 min sound + 15 min quiet
Experimental 2	14,000, 17,000, 20,000, rock & classical	15 min sound + 15 min quiet
Series 2		
Control		
Experimental 1	100, 1000-10,000 in 1000 Hz intervals	15 min sound + 15 min quiet
Experimental 2	10,000-20,000 in 1000 Hz intervals	15 min sound + 15 min quiet
Experimental 3	100, 1000-20,000 in 1000 Hz intervals	15 min sound, no quiet between tones
Experimental 4	Slow sweeps @ 60-1000 & 60-20,000, 5000, 8000, 20,000	15 or 30 min sound + 15-60 min quiet
Series 3		
Control		
Experimental 1	Music -- rock & classical	50 min music + 1 hr quiet
Series 4		
Control		
Experimental 1	Slow sweep @ 60-1000, 60, 1250, 5000-20,000 in 3000 Hz intervals	15 min sound, no quiet between tones

Data Analysis

Data was analyzed using the SuperCalc 3 spreadsheet program for graphs and CoStat (Version 1.00) for the statistical analysis.

RESULTS AND DISCUSSION

Growth

The growth of 4-day-old wheat seedlings over the course of a day is illustrated in Figure 9. After the plants are transferred from the planting trays (vermiculite) to the experimental set-up (Hoaglands solution) growth first slows and then rapidly recovers, as the variation in slope during this time suggests. Growth rates can slow to as much as 50 % of the typical daily average at this time. Thus, although photographic measurements were begun 1/2 hour after immersion into the Hoaglands solution, sound treatments generally were not initiated until two hours after immersion, when the transplant effects diminished.

Typical daily growth rates for these wheat seedlings ranged from 20-25 μm/minute, or 1.4 mm/hour. Thus during the test day, plants grew a total of 14 mm.[1] Night-time growth rates were 15-20% lower. Comparing the two curves for the theoretical and actual plant growth, plants under these experimental conditions initially had slower than average growth (probably due to transplant shock)

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1. These experiments examined only one aspect of plant growth -- that of the first leaf; to obtain a picture of the plant's total growth activity during this day, growth of the roots and of the second leaf within the culm of the first leaf would also have to be considered.

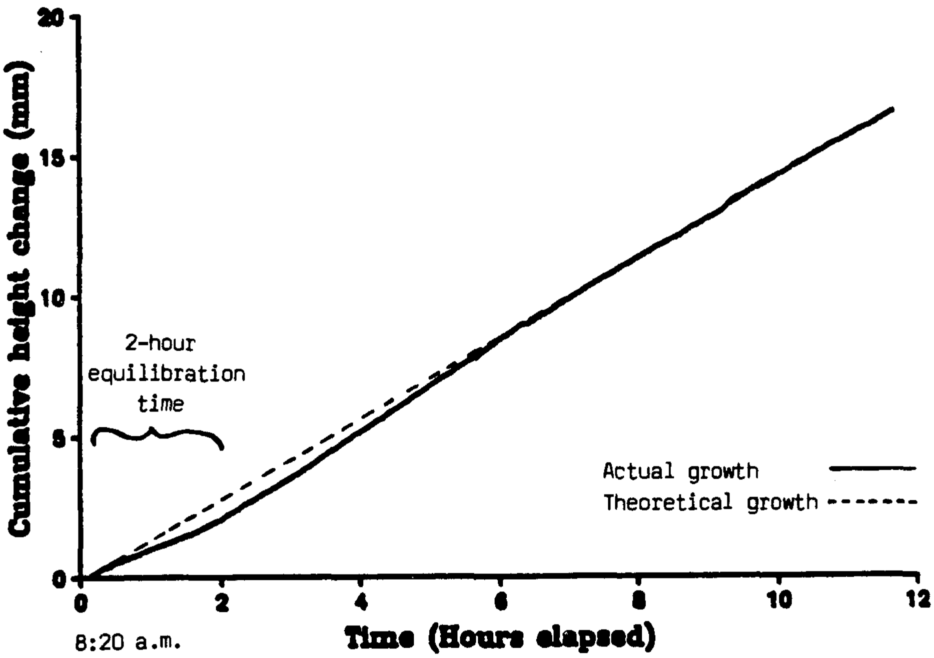

Figure 9. Theoretical and actual growth of 4-day-old wheat seedlings over the course of a day under laboratory growing conditions. The curves represent averages for 6 plants. Dotted straight line is the theoretical plant growth which would be obtained if plants grew all day at a constant rate equal to the actual average growth rate observed in the test plants.

but then increased their growth rate to as much as 15%[1] faster for up to six hours into the test. At this time the growth rate then levelled off.

The three types of graphs used for the bulk of the data analysis are shown in Figure 10.[2] The graphs all plot growth rate as a function of time but the method of determining growth rate varies. In Figure 10a, the growth rate was calculated for each five-minute interval between photographs; the point plotted at 10 minutes, for example, is the average growth rate for the five minute interval ending at 10 minutes. In Figure 10b, growth rate is plotted as a moving average of three 5-minute intervals; the point plotted for 10 minutes, for example, represents the average of the three growth rates for the intervals 0 - 5, 5 - 10, and 10 - 15 minutes. Thus it is a way of smoothing out the curve of Figure 10a to emphasize the growth rate trends. In Figure 10c, growth rate is a simple average over a 15-minute interval. The difference in height between the beginning and end of the interval is divided by 15 minutes. Thus, the point plotted at 30 minutes is the average growth rate for the interval from 15 - 30 minutes. It produces the smoothest curve of the three types of graphing methods and is the clearest way of delineating the fifteen minute sound treatments, but

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1. Percentages are calculated relative to the average daily growth rate.

2. Note that t=0 minutes on these and all subsequent graphs refers to the official start of the test, which was 30 minutes after the plants had been transferred to the experimental set-up. Thus, 90 minutes on these graphs actually represents the end of the 2-hour equilibration time denoted in Figure 9.

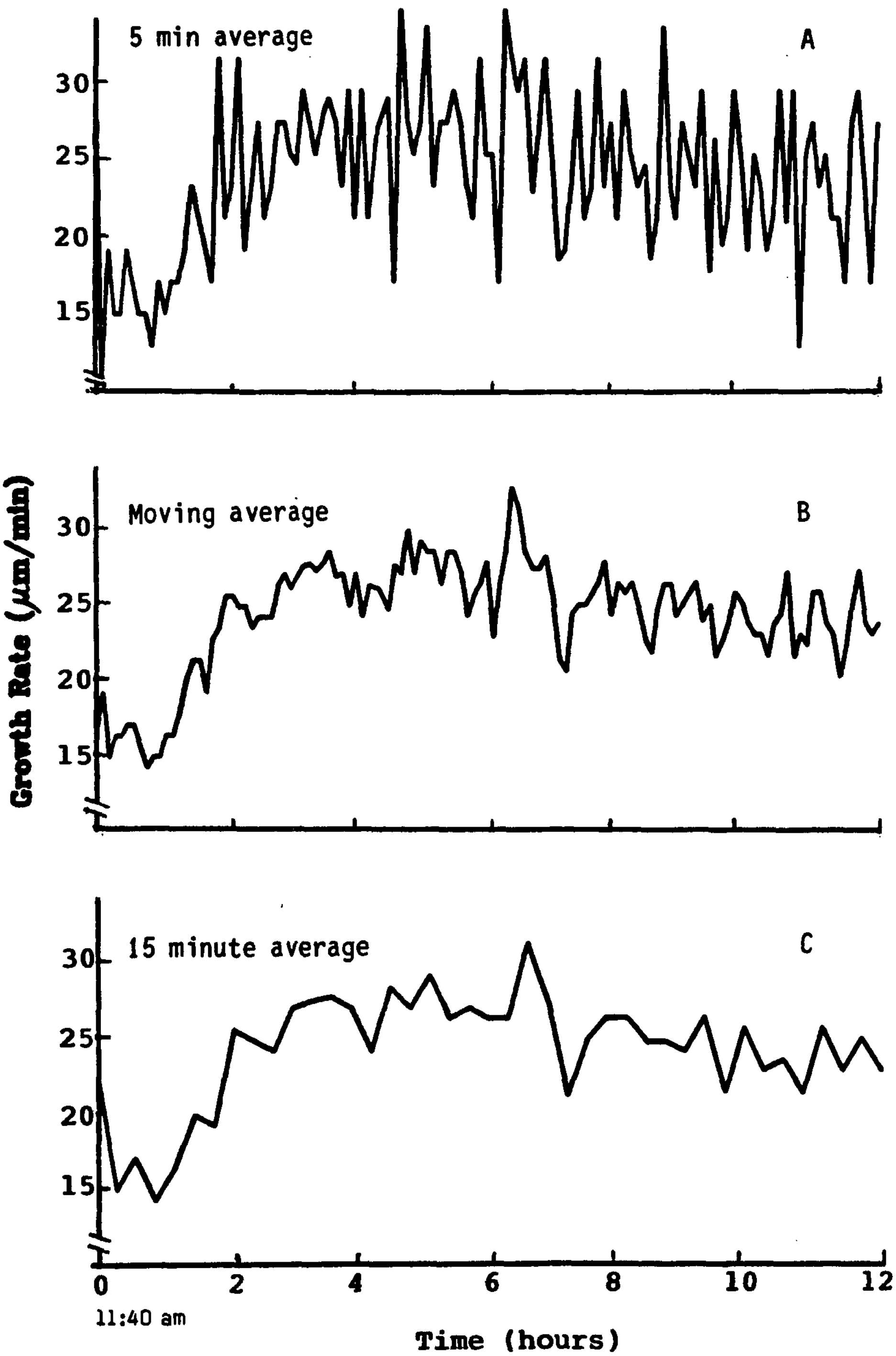

Figure 10. The three types of graphs used for data analysis. All describe the growth rate of wheat seedlings as a function of time, but vary in the method of determining growth rate.

it sacrifices sensitivity; two-thirds of the data in the moving average graph, for example, is eliminated in this third graph type.

Figure 11 gives the growth rate curves for all four control tests using moving averages, since this method was considered to be the most appropriate balance between smoothing out experimental noise and retaining sensitivity to short-term changes in growth rates.[1] All controls show the typical initial pattern of low growth rates followed by recovery in the first two hours. In Controls 3 and 4, a general decline in growth rates over the course of the day is evident. For Controls 1 and 2 this trend is not evident although it is probably in part due to the shorter test length; the growth rate decline in Controls 3 and 4 does not become clearly evident until after 7-8 hours. Each control test shows evidence of a major short-term fluctuation in growth rate between 7 and 8 hours, which is further evidence that daily plant growth is not constant. It also suggests that a minimum growth rate change of at least 25% (relative to the preceding interval) must be surpassed for an experimental treatment to be considered effective in altering plant growth. Typical growth rate fluctuation during the day was much less -- the coefficient of variation (standard deviation/mean) was approximately 10%.

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1. The degree to which one wishes to smooth out a curve depends on how much of the variation one believes is real and how much is experimental error. This issue is discussed more fully below under "Noise vs. Growth."

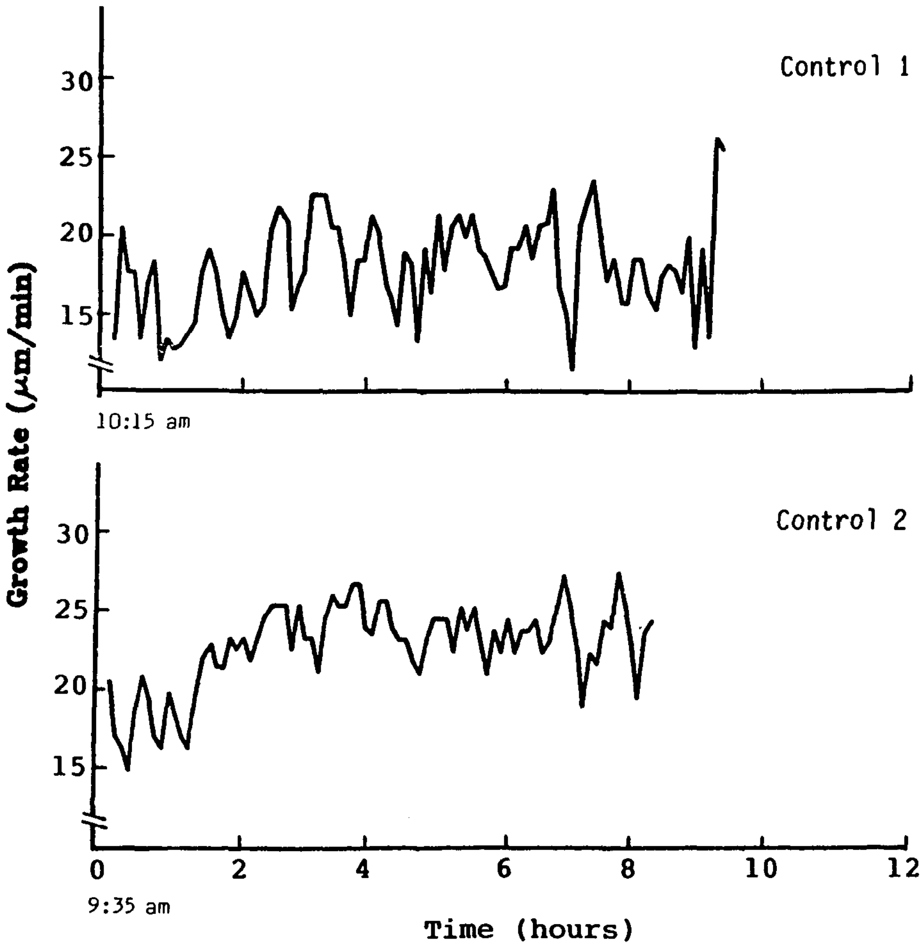
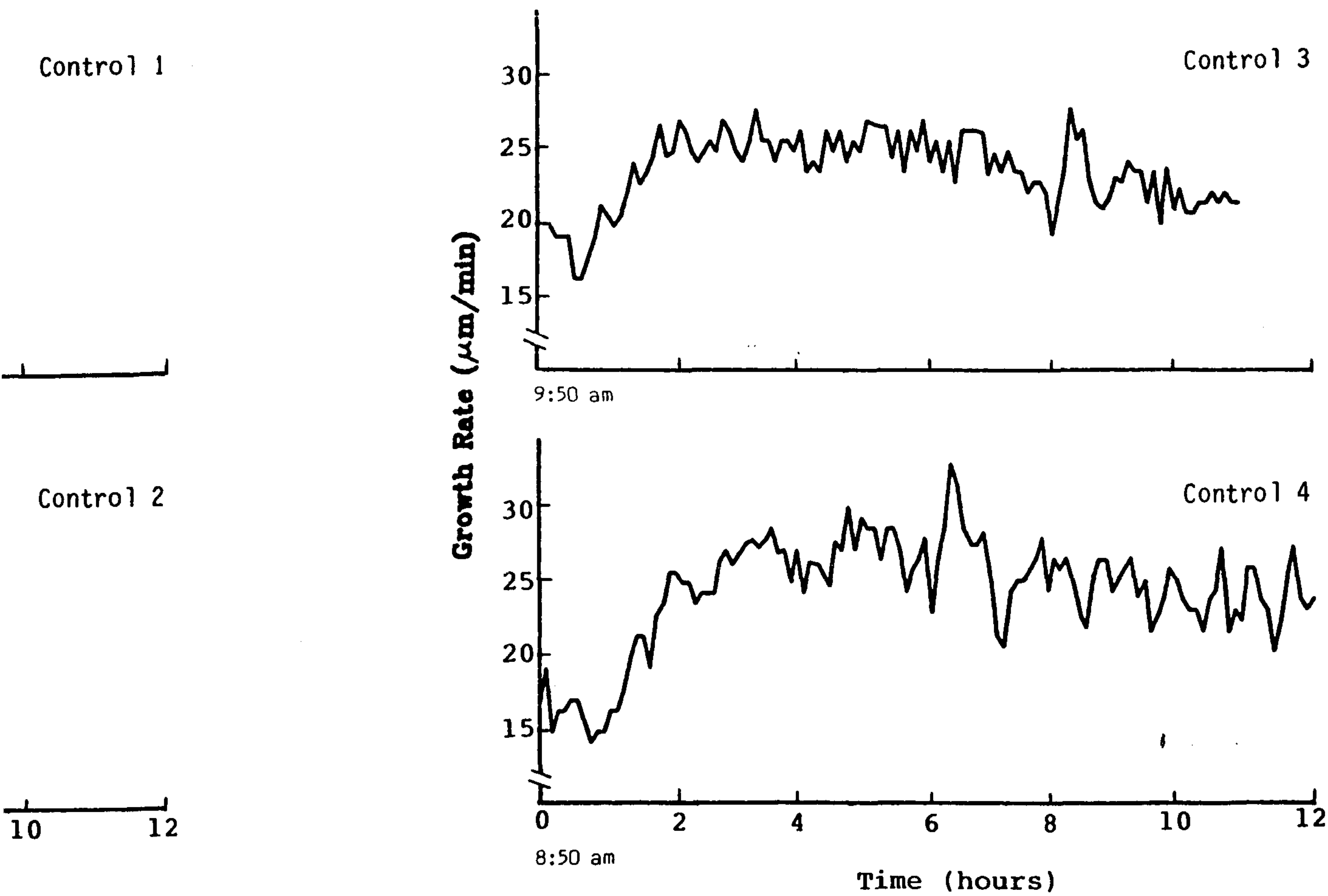


Figure 11. Growth rate in terms of movin
time for all four control tests.



. Growth rate in terms of moving averages as a function of
ll four control tests.

A statistical comparison of the four controls is summarized in Table 7, and confirms the general patterns described above. The mean for Control 1 -- the test under lower light conditions, as explained below --is significantly lower than the others. The mean for Control 4, taken from the second seed lot, as explained below, is higher than that for Controls 2 and 3. Both linear and quadratic curve fits are highly significant for Controls 3 and 4, but Controls 1 and 2 were not well characterized by any polynomial up to the fourth order. Combining all the controls into one group, however, a linear regression is significant at the 5% level.

Because of the variation among the controls, it was considered most appropriate to compare the experimental tests in each series with the one control tested during that series, rather than with the combined controls.

The growth results can be summarized as follows:

- The average growth rate of the first leaf of Yecora Roja 4-day-old wheat seedlings under these laboratory conditions was 23 μm/min, or 1.4 mm/hr.

- Growth rates vary over the course of a day.

- The general trend is a decline after 7 hours of testing, which is roughly after 5-6:00 pm.

- Superimposed on the general trend are shorter-term growth rate fluctuations of typically within $\pm$ 5% of the mean. There is also at least one maximum fluctuation --lasting less than an hour and representing at least a 20-30% change in growth rate--occurring per day.

Table 7. Summary of Statistics for the Four Control Tests.

Statistics for Growth Rate[a]	Control #1	Control #2	Control #3	Control #4	All Controls
Constant growth model:					
Mean	18.6	23.9	24	24.8	23.2
Standard deviation	2.92	1.72	2.01	2.46	3.3
n	68	64	91	117	340
Student-Newman-Keuls test for non-significant ranges[c]	a	b	b	b	b
Maximum value	26.1	27.8	27.5	32.5	32.5
Minimum value	11.3	19.2	19.1	19.1	11.3
Linear model:					
y-intercept	19.4	24.1	27.3	27.9	24
slope	-.00337	-.000533	-.0102	-.0083	-.0025
F	.8839	.05193	76.45	50.49	4.23
P	.3506	.8205	5.45 e-9	3.06 e-8	.04
Polynomial model:					
Order of best fit[b]	4	4	2[d]	3[d]	1
P of best fit	.1563	.1883	2.63 e-9	8.68 e-9	.04

a. Growth rates are reported as 15-minute moving averages.

b. Fits up to the fourth order were tested.

c. Tests with the same letter entry are not considered significantly different at the 5% level, according to this test based on means, standard deviations, and n.

d. All fits from the first to the fourth order were highly significant ($\leq .01$) for this test. The order reported here was chosen because probabilities for the terms added after this order dropped off considerably.

• The controls differ among themselves. Graphically, the characteristic magnitude of the fluctuations varies for each day as well as the mean for the first 8 hours, around which the fluctuations occur. These graphical differences are supported statistically by the SNK test and polynomial fit results.

The Individual Test Series

The statistics for all tests are summarized in Table 8. One test series -- Series 0 on Table 6 in the Methods section -- and Experimental Test 1 in Series 1 were dropped from the analysis because problems in the mounting system rendered the results unusable.

Series 1

This test series was conducted in a different location from the rest of the tests, since the latter location was not yet available. The series basically scanned the frequency spectrum in 3000 Hz intervals over the course of 2 tests, but the first test had to be dropped from the analysis as noted above; thus data are available only for 14, 17 and 20 kHz. In addition three music selections -- one each of jazz, rock and classical -- were tested. Pure-tone sound treatments were applied for 15 minutes followed by 15 minutes of quiet; the music treatments were applied for 30 minutes, followed by 30 minutes of quiet. The results are graphed in Figure 12. Both the experimental and control show considerable variation, but the overall pattern in the control is fluctuation about a mean of 19 μm/min. The experimental plants, in contrast, showed a clear decline over the day interrupted by a large spike around 5 hours, 15 minutes into the rock music treatment. The sloping aspect of the experimental curve is largely due to higher growth rates at the beginning of the day during the time from 1.5 to 3 hours; the average growth rates for the experimental and control

Table 8. Summary of Statistics for the Four Test Series, Including Experimental and Control Tests.

Statistics for Growth Rate[a]	Series 1 Cont	Series 1 Exp	Series 2 Cont	Series 2 Exp 1	Series 2 Exp 2	Series 2 Exp 3	Series 2 Exp 4	Series 3 Cont	Series 3 Exp	Series 4 Cont	Series 4 Exp
Constant growth model:											
Mean	18.6	19.6	23.9	23.2	24.8	22.9	23.5	24	25	24.8	23.9
Standard deviation	2.92	3.8	1.72	2.43	2.43	2.03	2.13	2	1.52	2.44	1.56
n	68	55	64	83	81	84	63	91	57	116	44
Student-Newman-Keuls test for non-significant ranges[c]	a	a	a	ab	ab	bc	c	a	b	a	b
Maximum value	26.1	31.3	27.8	28.2	29.7	28	27.5	27.5	28.2	32.5	28.2
Minimum value	11.3	10.3	19.2	17.4	17.7	17.7	18.4	19.1	21.8	19.1	19.1
Linear model:											
y-intercept	19.4	23.9	24.1	25.7	28.7	25.7	25	27.3	25.2	27.8	23.4
slope	-.0034	-.0175	-.0005	-.0084	-.0131	-.0093	-.0058	-.0103	-.0008	-.0078	-.0002
F	.8732	10.466	.0519	16.93	55.62	37.09	5.084	17.07	.1213	46.52	.3524
P	.3535	.0021	.8205	.00009	3.7e-8	4.2e-8	.02775	6.4e-8	.729	6.0e-9	.5559
Polynomial model:											
Order of best fit[b]	4	1	4	1	1	1	1	2[d]	4	3[d]	4
P of best fit	.1563	.0021	.1883	.00009	0	5.9e-8	.0274	2.6e-8	.1153	8.6e-9	.4185

a. Growth rates are reported as 15-minute moving averages.

b. Fits up to the fourth order were tested.

c. Tests with the same letter entry are not considered significantly different at the 5% level, according to this test based on means and standard deviations. The SNK test was applied to each series individually (not to the entire set of tests as a group.

d. All fits from the first to the fourth order were highly significant (≤ .01) for this test. The order reported here was chosen because probabilities for the terms added after this order dropped off considerably.

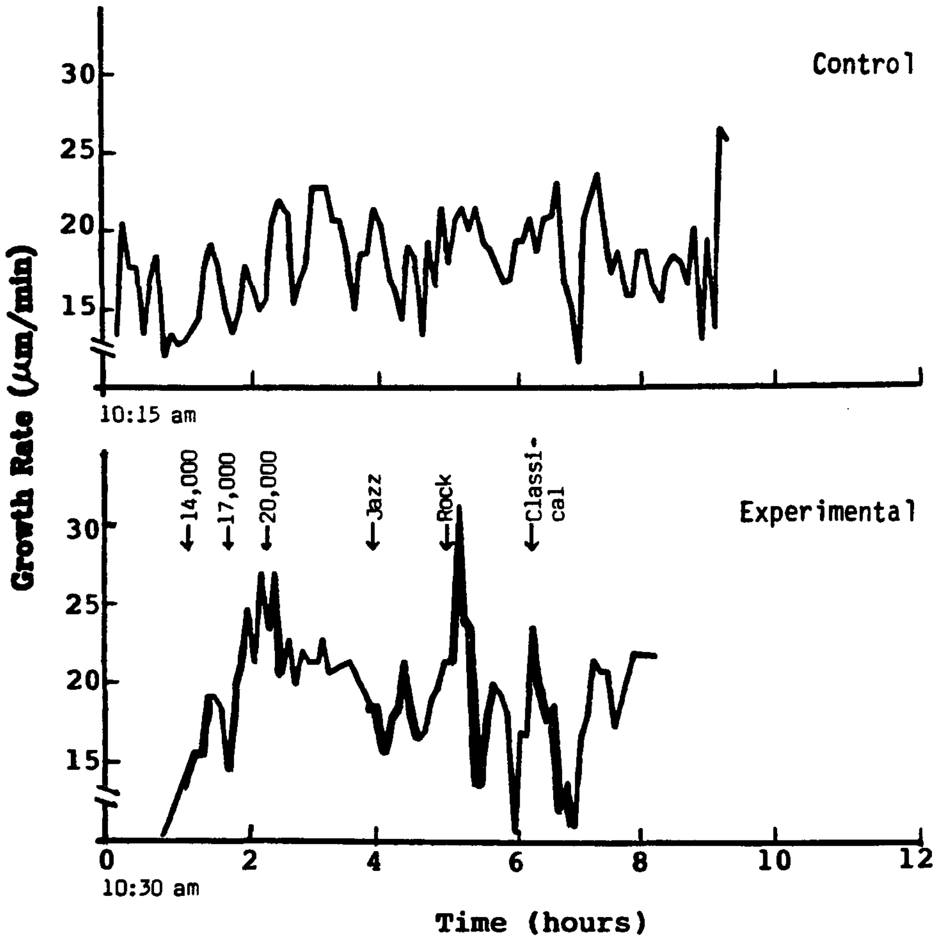


Figure 12. Results of Series 1.

were 20.7 and 17.9 μm/min, respectively. Sound treatments at 14, 17, and 20 kHz were being applied during the interval from 1.5 to 3 hours.

Growth rates for the times when the sound treatments were applied can be characterized as follows:

Net increase in growth rate:

14,000 Hz *
17,000 Hz *

Net decrease in growth rate:

classical music *

No net change:

20,000 Hz
jazz music
rock music

The net increase in growth rate at 14,000 and 17,000 Hz could be due to recovery from transplant shock. The "no net change" for the rock music treatment obscures the most dominant feature of the day's test, which was the spike mentioned earlier. A spike of this magnitude occurred only once during the entire testing. The growth rate was 22 μm/min at the beginning of the treatment, rose to 32 μm/min (a 50% increase), fell to 13 μm/min and then returned to 20 μm/min at the end of the period. These changes were larger than the largest change in the control, which occurred at 7 hours. A similar large dip in growth rate also occurred in the experimental test at 7

* Asterisk denotes a very large growth rate change ($\geqslant$ 5 μm/min). A change of 5 μm/min represents a 20% shift in growth rate when compared to the mean for these test series.

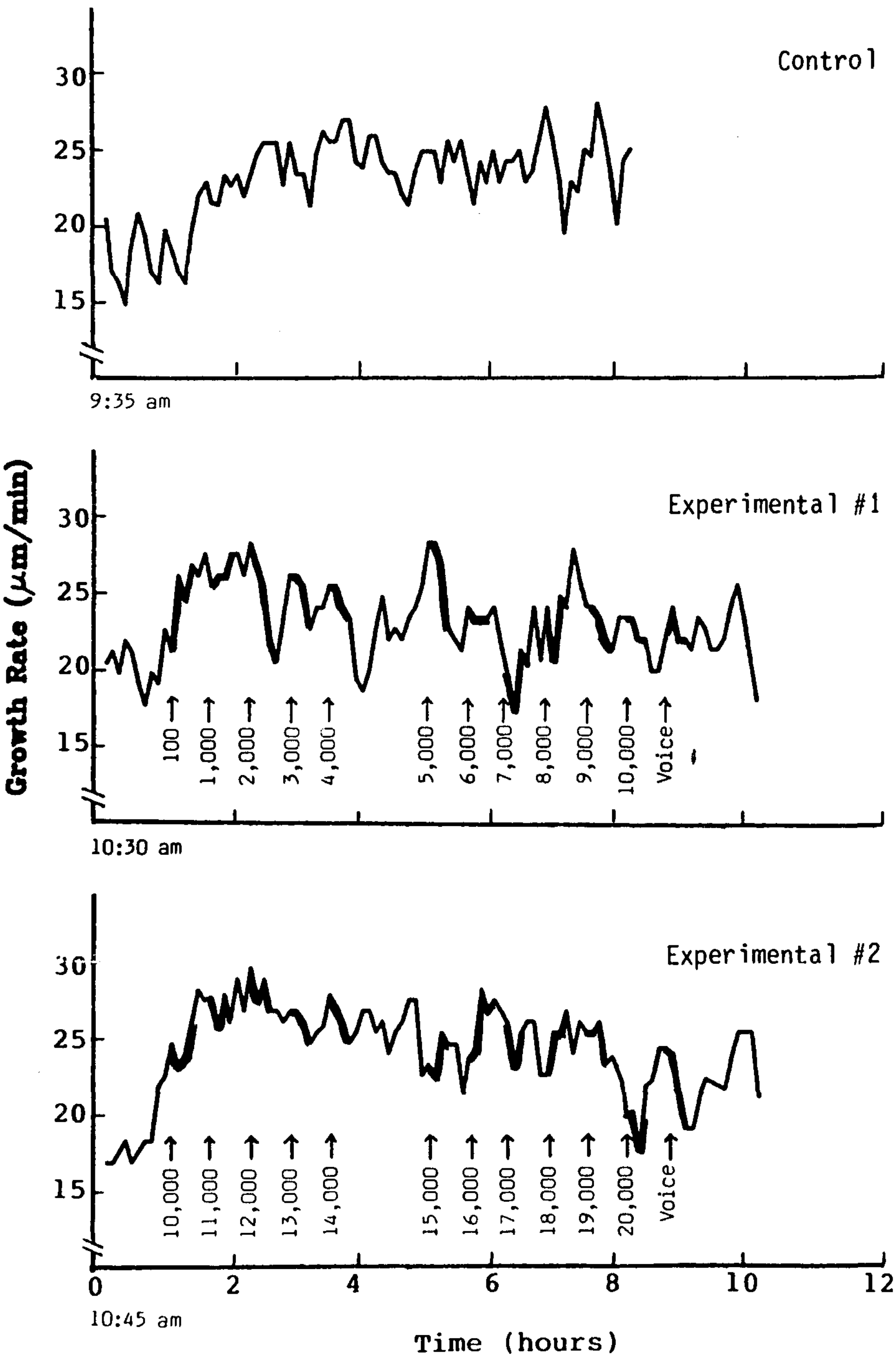
hours, during the classical music treatment. In both cases the growth rate dropped 50% (23 to 12 μm/min) within 20 minutes.

These observations are partly supported by the statistics. While the means for the two tests are not that different (19.6 and 18.6 μm/min for the experimental and control, respectively), the standard deviation is 30% higher for the experimental test (3.80 vs. 2.92 for the control). The Student-Newman-Keuls (SNK) test, however, found the differences in the means non-significant at the 5% level. The experimental data regressed well to a linear fit (p=.00210) but the control did not (p=.3535, F=.8732). The best fit for the control was a fourth order polynomial (p=.1563).

Series 2

This series is the principal one for testing the entire frequency spectrum. The curves for the moving averages for all five tests in this series are given in Figure 13. The control for this series was less variable and with a substantially higher average growth rate than the Series 1 control; the difference in means (23.9 μm/min for Series 2, 18.6 μm/min for Series 1) is probably a direct result of the higher light intensity for Series 2, as described earlier. A large dip in growth rate at 7 hours occurred in Series 2 as in Series 1.

Experimental tests 1 and 2 represent an entire frequency scan spread out over two tests. Test 1 covers the range from 100 - 10,000 Hz in 1000 Hz intervals (except for the 100 Hz treatment). Test 2 scans the 10,000 - 20,000 Hz range. Two voice treatments,



Control
30
25
20
15
9:35 am
Experimental #1
Growth Rate (μm/min)
100
1,000
2,000
3,000
4,000
5,000
6,000
7,000
8,000
9,000
10,000
Voice
10:30 am
Experimental #2
10,000
11,000
12,000
13,000
14,000
15,000
16,000
17,000
18,000
19,000
20,000
Voice
0
2
4
6
8
10
12
10:45 am
Time (hours)

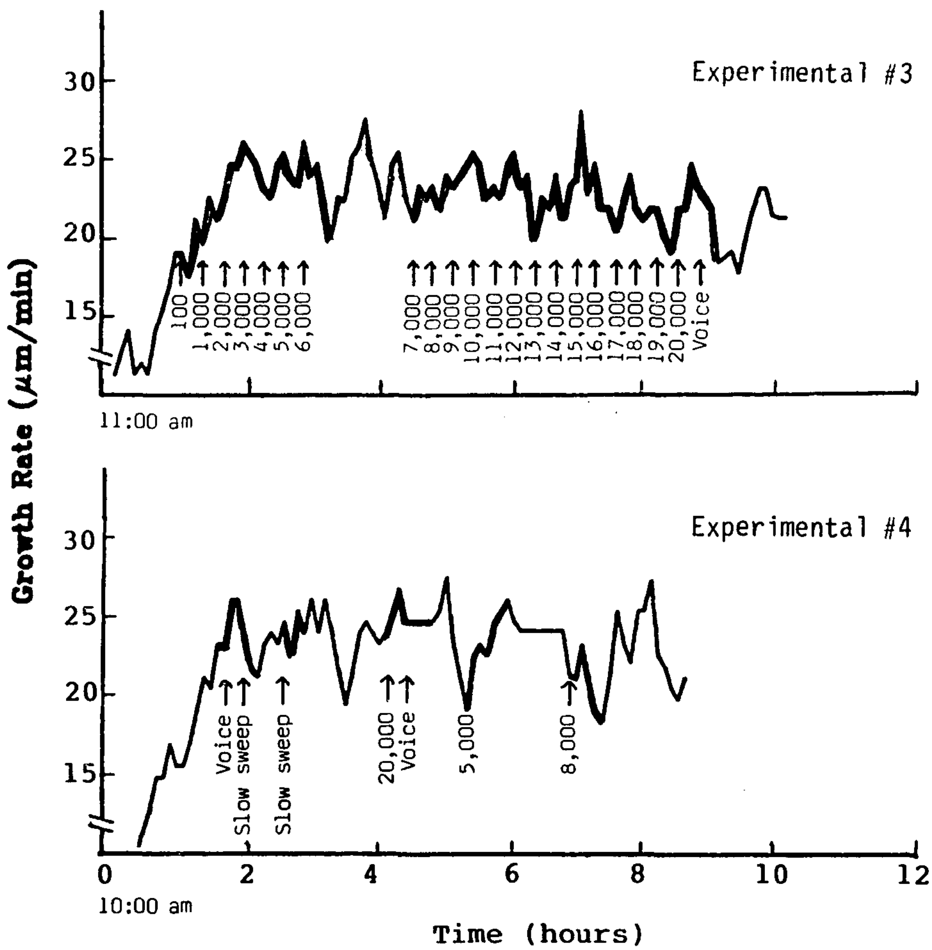


Figure 13. Results for Series 2.

principally singing, were also tested. Sound was applied for 15 minutes followed by 15 minutes of quiet. Overall the experimental curves are similar to the control curve. The SNK test shows the differences in mean and standard deviation to be non-significant. There are some differences in the curves, however. After one hour the control basically fluctuates around the mean, whereas the two experimental curves show a general sloped trend. Again, this slope was due principally to higher growth rates in the early part of the day (1.5-3 hours). The slope was greater in Experimental Test 2 than in Test 1. The magnitude of the individual fluctuations, however, was greater in Test 1 than in Test 2, and it also surpassed that of the control. The standard deviations reflect these trends: for both Experimental Tests 1 and 2 the standard deviation is 2.43, which is 40% higher than the 1.72 value for the control. The larger standard deviation for Test 1 derives from the greater magnitude of the individual fluctuations; for Test 2 it is the greater slope which accounts for the larger deviation from the mean.

The sound treatment periods were characterized by the following growth rate patterns:

Net increase in growth rates:

100 Hz *	15,000 Hz
1000	16,000
10,000 (Test 2)	18,000

---

* Asterisk denotes a very large growth rate change ($\geqslant 5$ μm/min).

Net decrease in growth rates:

2000 *	11,000
3000	12,000
4000	13,000
5000 *	14,000
9000	19,000
10,000 (Test 1)	voice (Test 2)

No net change:

6000	17,000
7000	20,000
8000	voice (Test 1)

Frequency and time are united in Experimental Test 3, where the entire audible frequency was scanned in 1000 Hz intervals in one day, by eliminating the quiet periods between treatments. Test 3 looks intermediate between the control and Tests 1 and 2, and the values for the standard deviation and linear regression slope follow this pattern. The growth rate trend in the early part of Test 3 is like that of the other 2 experimental tests -- higher between 1.5-3 hours -- but to a lesser degree than for Tests 1 and 2. The sound treatment periods showed the following growth rate trends (treatments with a check mark showed the same trends in Tests 1 and 2):

Net increase in growth rate:

✓100	9,000
✓1000	11,000
2000*	13,000
4000	20,000
7000	

Net decrease in growth rate:

✓3000	✓12,000*
6000*	16,000
✓10,000	✓voice*

No net change:

5000	✓17,000
✓8000	18,000
14,000	19,000
15,000	

As the check marks indicate, a few frequencies produced the same net effect in both the frequency scans, but there were no consistent strong effects. The voice treatment did produce a large (20%) decrease in growth rate in both Tests 2 and 3, but this decrease was no greater than the largest fluctuation found in the control. (This voice treatment decrease is counter to what would be expected if higher transient CO_2 levels caused by the experimenter's breath were significant.)

One phenomenon which occurred during these tests was guttation in the late afternoon during the voice treatment. Half of the six plants had easily visible guttation drops at their tips which disappeared within 15 minutes after the sound treatment ceased. Guttation had also occurred in the variable frequency treatments of the first series, but it was less consistent. One plant began at 1:50 during the jazz treatment; a second began at 3:10 during the rock treatment and on the third plant guttation appeared within the first few minutes of quiet after the rock treatment. The guttation in all three cases persisted until about 4:45 that day, after the sound treatments had ceased. In all of the controls guttation was observed only once, on one plant during Control 1.

The growth rates during the sound treatments for the various frequencies will be discussed further below in the "Action Spectrum" portion of the results.

At this point in Series 2 a change in direction of the testing was made. It appeared after these 3 experimental tests that there were few, if any, sound effects, contrary to what had been observed in Series 1 and in the preliminary tests. Further, the overall growth rate variation in general seemed greatly reduced, particularly after Control 3 (described below under "Series 3") was run. The seed had been inadvertently exposed to toluene vapors during storage for 6 weeks between Series 1 and 2, and thus there was a strong possibility that seed vigor had been reduced. Thus replication of the frequency scans of Series 2 was postponed in order to:

1) attempt to find some kind of reproducible sound effect, using not only pure tones but variable sound sweeps[1] and voice (Series 2, Test 4) and music (Series 3, Test 1);

2) compare the test seed with a new batch of seed (Series 4 control), to see whether the exposure to toluene vapors could have altered the seeds.

---

1. The variable sound sweep used in this study scanned the designated frequency range continuously from low to high frequency every 10 seconds.

Thus the final test in Series 2 examined 3 variable frequency treatments applied for 15 minutes:

- voice
- slow sweep, 20-20,000 Hz
- slow sweep, 20-1,000 Hz,

one pure tone applied for 15 minutes:

- 20,000 Hz

and two pure tones applied for longer durations than previously (30 minutes):

- 5,000 Hz
- 8,000 Hz.

Quiet periods of the same length as the treatment period were applied before and after each treatment. The purpose of this test was to see if any kind of sound effect could be demonstrated. The voice treatment had shown a sharp decrease in growth rates in tests 2 and 3 of this series and was accompanied by the guttation phenomenon discussed above. The slow sweeps were a way of systematically scanning the entire audible frequency range within minutes in a more ordered way than applying random noise.[1] Five thousand hertz was included because it was the frequency which Weinberger and Measures (1968, 1970, 1973, 1979) found effective in stimulating plant growth in their work with wheat. Eight thousand hertz showed a large decrease in Test 1 of Series 1 (which was later rejected for data analysis as explained earlier). Twenty-thousand

---

1. Random noise had been suggested earlier as a sound treatment for these experiments but was not included principally because both the Weinberger and Graefe (1973) and Weinberger and Measures (1979) experiments showed no effects.

hertz, while showing no big net change in growth rate, had the lowest actual growth rate of all the frequencies in Tests 1 and 2.

The most immediate feature one observes comparing Test 4 with the others (Figure 13) is the two periods of flatness in the middle portion of the curve and the equidistant division of the curve into three parts, each 90-95 minutes wide. The second flat period, unfortunately, is due to an experimental artifact since no photographs were taken for 40 minutes when the experimenter was not present. The middle flatness is real, however, and the growth rate pattern on the 5-minute interval level which produced this flatness on the moving average graph is shown in Figure 14. The series of regular truncated oscillations over the period in which the 20,000 Hz and voice treatments were applied yielded the flatness in the moving average curve.

Looking at the statistics for the entire Series 2 only Tests 3 and 4 were considered significantly different from the control on the basis of mean and standard deviation. A striking feature was the decided nonlinearity of the control in contrast to the experimental tests for which linear regression was highly significant ($p < .01$) for all but the last test which was significant at the 5% level.

Series 3

Series 3 tested three music selections -- 1 rock and 2 classical -- at longer durations than previously used (50 and 40 minutes), again as part of the effort to find a large, reproducible

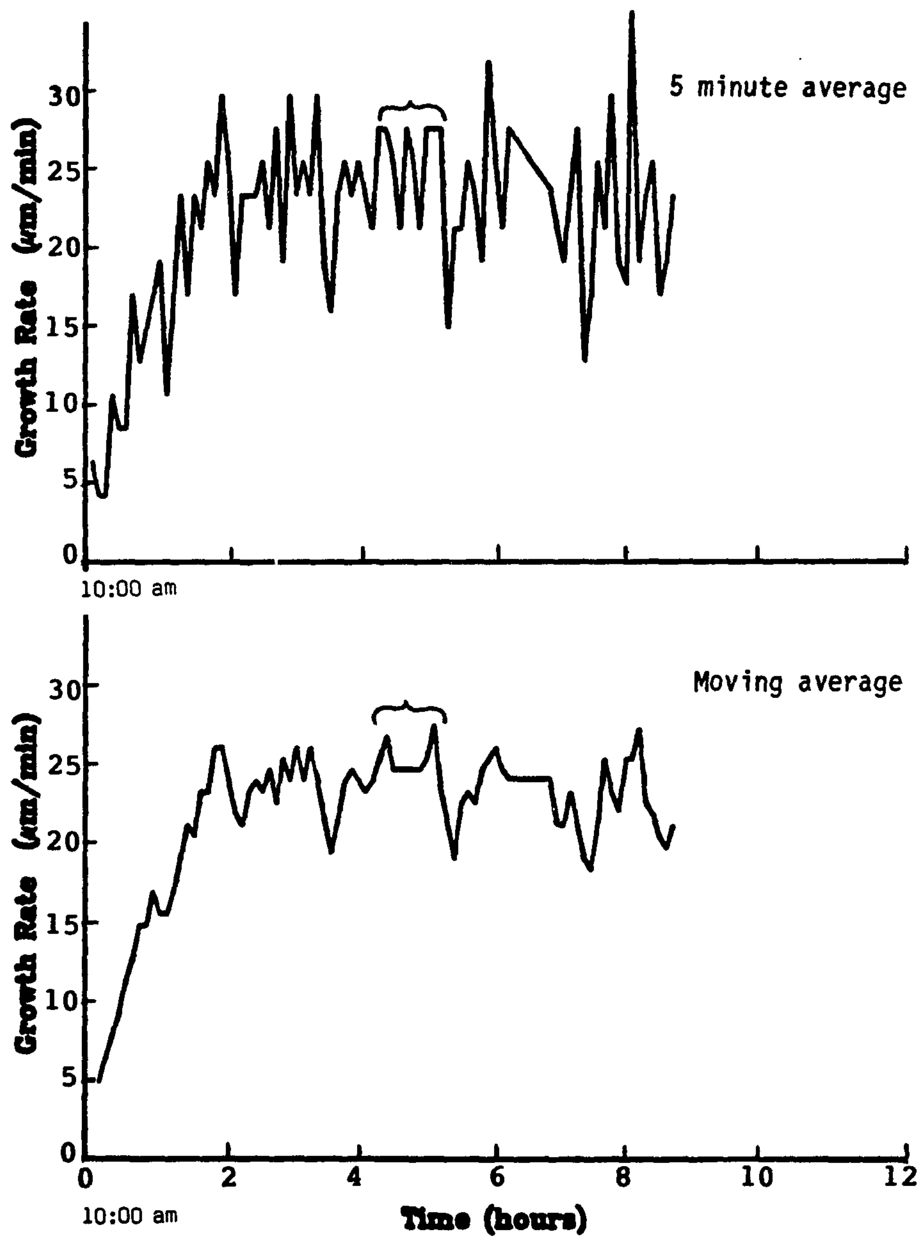


Figure 14. The pattern on the five-minute-average graph corresponding to the unusual flat pattern in the moving average graph of Test 4 in Series 2.

sound effect. The outstanding feature of the Control 3 graph (Figure 15) is the low range of variability throughout most of the test except for the dip at 8 hours, similar to that noted in the other two controls. The curve was an excellent quadratic and linear fit ($p=2.6x10^{-8}$ and $p=6.4x10^{-8}$, respectively). In both of these aspects the experimental test differed quite substantially. The fluctuations were more variable in magnitude and period in the experimental test, and no polynomial fit the data particularly well. The best fit was a fourth order polynomial, with p=.1153. The SNK test results reflect the obvious visual difference -- the two tests were found to be significantly different at the 1% level.

The rock selection and the classical bassoon selection both resulted in a net increase in growth rate by the end of the 50-minute period, whereas the final classical music selection -- a Mozart piano concerto -- resulted in no net change. The growth rate increase for the first two selections was about 20% (relative to the mean daily growth rate).

Omitting the small non-fluctuating linear portion of the curve just before 4 hours which was an experimental artifact (2 photographs were missing), the graph for this experimental test is quite similar to those of Series 2. It is the control in Series 3 that is different, and this difference motivated the investigation into possible seed deterioration, tested in Series 4.

Series 4

The plants from the new seed batch had the highest average

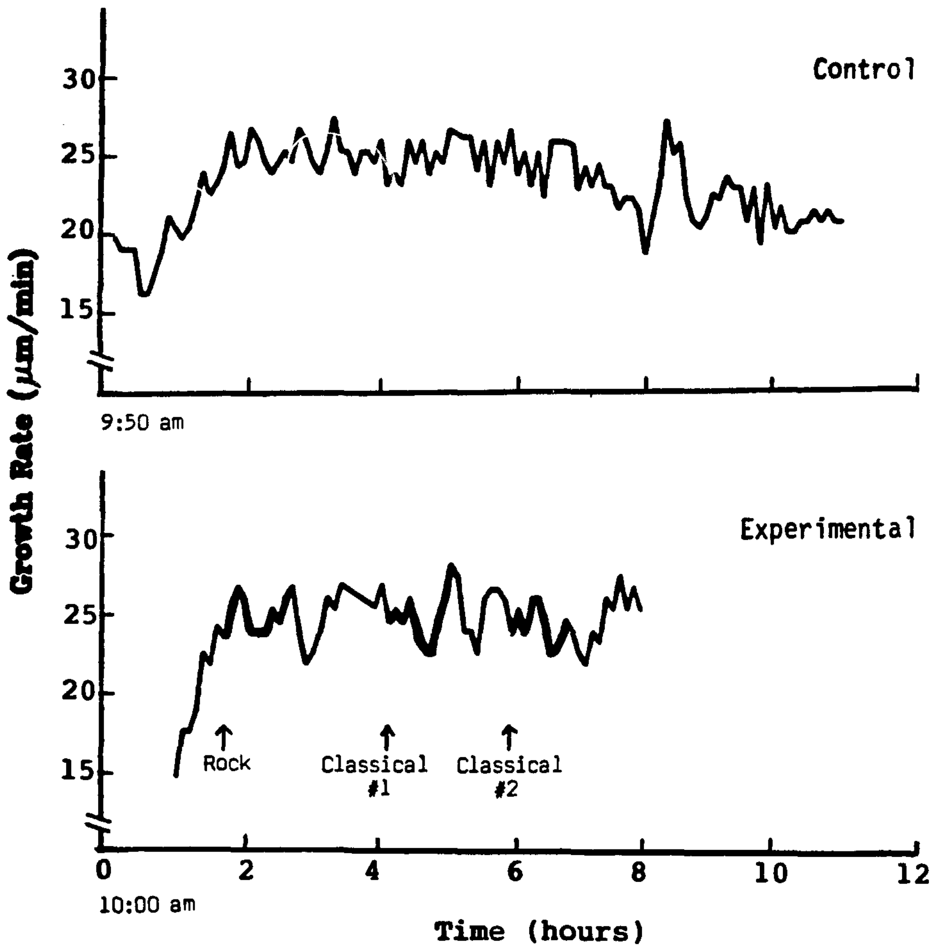


Figure 15. Results for Series 3.

growth rate and standard deviation of the 3 controls tested in the permanent laboratory (a mean of 24.8 μm/min vs. 23.9 and 24.0 for Controls 2 and 3, and a standard deviation of 2.46 compared to 1.72 and 2.01 μm/min for Controls 2 and 3). The SNK test, however, found these differences non-significant. They were nevertheless considered support for the idea that the seed had been altered by the toluene vapors in light of the graphical differences. Examining the four control curves in Figure 11, the overall appearance from Controls 1 to 3 is one of declining magnitude of the fluctuations. Control 4, with the new seed, returns to a more widely variable pattern more similar to that of Control 1. In a separate test which compared heights of 32 seedlings of each seed batch at 3 and 4 days of age, the new seed heights were 17% greater on the average than the old.

Contamination and reduced seed vigor were considered important issues for these tests because an increased growth rate is only possible if the plants have the capacity to increase their growth rate. Since the first leaf which was used for these height measurements is still largely dependent upon seed reserves (only when the second leaf emerges does photosynthesis become the primary energy supplier), the condition of the seed is critical to the growth capacity of the plant. The inadvertent six-week exposure of the seed to toluene vapors may have affected seed respiration rates causing an increase, as is common in disease and wound response. Increased respiration rates depletes seed reserves and hence, reduces seedling vigor. Thus the higher average growth rate and the greater variability of the new seed compared to the old were considered evidence that the old seed had indeed been damaged by the toluene vapors.

Given the higher growth rate and greater variability of the fluctuations in the control test with the new seed, the results of the frequency scan with the new seed were therefore unexpected: it was the least variable in its fluctuation of all tests, control and experimental (Figure 16). The only large dip occurred at 5.5 hours (5:15 pm), which corresponds fairly well in real time to the large dip in late afternoon characteristic of the controls. The unusual 5-minute average growth rate pattern which produced this moving average curve is depicted in Figure 17. The interval of truncated periodic oscillation on the 5-minute graph coincides well with the sound treatment; it extends over almost the entire frequency scan, from 1250 through 17,000 Hz. During this time the oscillations were regular, with a period[1] of 15-20 minutes. The individual sound frequencies were characterized by the following growth rate patterns during the treatment period:

Net increase in growth rate:

5,000 Hz
✓11,000
voice *

---

1. Period is the time required for one complete oscillation to occur.

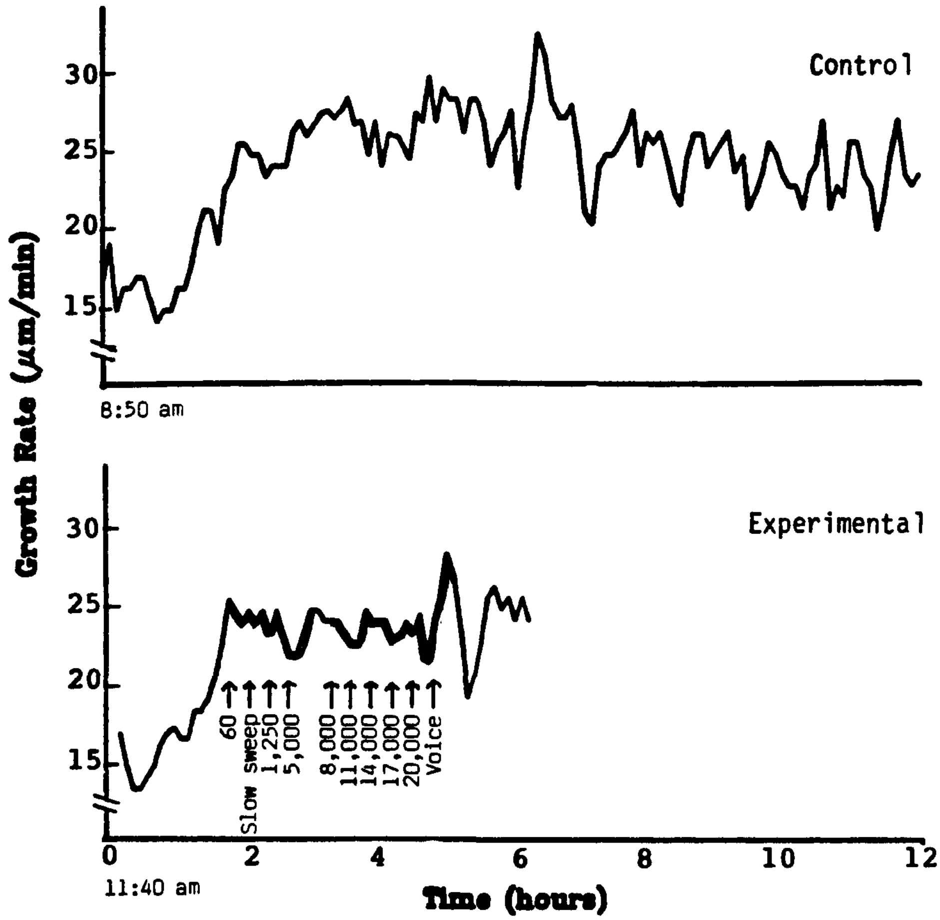


Figure 16. Results for Series 4.

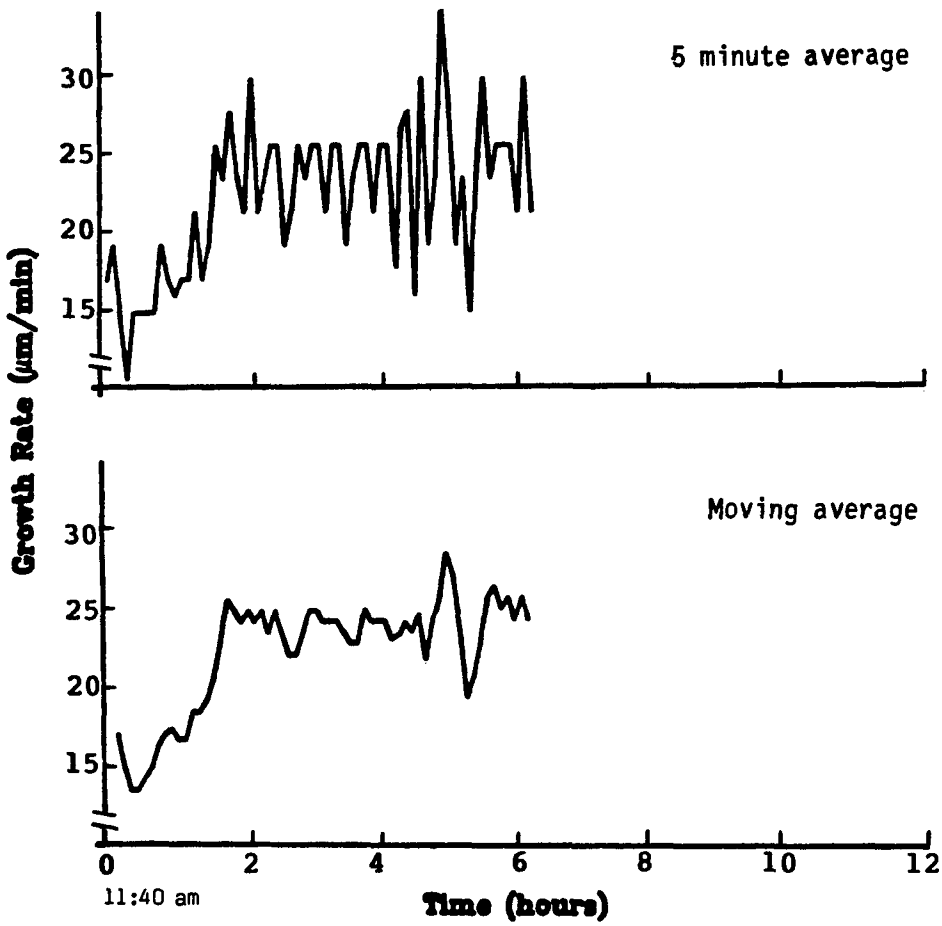


Figure 17. The pattern on the 5-minute-average graph corresponding to the unusual truncated pattern in the moving average graph for the experimental test of Series 4.

Net decrease in growth rate:
1250
8000
14,000

No net change:

60
slow sweep, 20-20,000 Hz
✓17,000
✓20.000

This frequency scan was in 3000 Hz intervals, unlike the 1000 Hz scans of Series 2. Three sound treatments produced the same overall pattern as they did in at least one of the other frequency scans, as the check marks indicate. Only one of these -- 17,000 Hz -- showed the same pattern (no net change in growth rate) in all three frequency scans.

Surprisingly this experimental test curve does not regress well to a linear fit ($p=.5559$). The closest fit was a fourth order polynomial ($p=.4188$). The SNK test found the experimental test significantly different from the control which confirms the graphical dissimilarity.

To summarize the results presented thus far, differences between the sound-treated and control tests can be seen statistically and graphically, although further replications are needed to confirm the differences. At the level of individual frequencies, however, no consistent trends are observed. The fluctuation in growth rate as measured by this technique is considerable (up to 20% variation), and complicates analysis of short time intervals. Consistent with the original purpose of the research, however, an action spectrum was prepared, to examine further the frequency variable.

Action Spectrum

Figure 18 depicts growth rate as a function of frequency for the three frequency scans conducted. Figure 18A describes the scan with the new seed in Series 4 (15 minutes sound, no quiet periods). Figure 18B presents the results from Series 2, Tests 1 and 2 (15 minutes sound + quiet). Figure 18C shows the Series 2, Test 3 results (15 minutes, no quiet). A comparison of A with B and C illustrates the importance of testing in smaller intervals. It appears that there are no frequency effects in Figure 18A, which comes from the day with the least amount of fluctuation -- the Series 4 test described just previously. When Figure 18A is superimposed on the highly periodic[1] Figure 18C, however, there is excellent agreement between the two curves (Figure 19), and it is apparent that the appearance of no effect in Figure 18A derives mainly from the selection of frequencies tested.

From 7000-19,000 Hz the curve in Figure 18C oscillates regularly with a period of 60 minutes, and a spread of 3.5 μm/min. The biggest difference in this test occurred between 19 and 20 kHz, from 19 μm/min at 19,000 to 24 μm/min at 20,000 Hz.

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1. Periodicity, which requires time on the x-axis, can be reported for this test even though frequency is along the x-axis because on this day the frequency scan was continuous over the entire day with no quiet periods. Hence, frequency and time were concurrent along the x-axis.

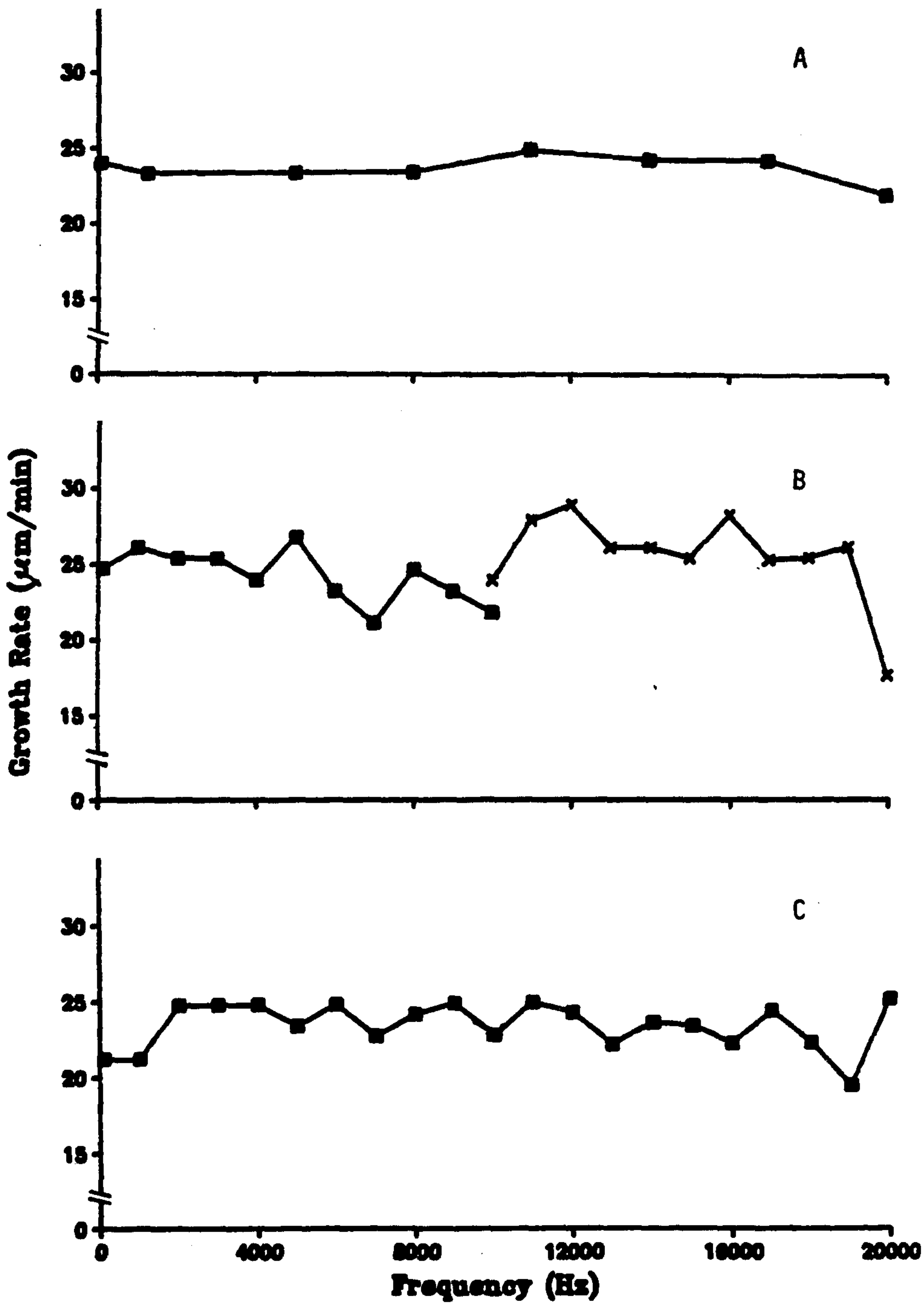

Figure 18. Growth rate as a function of frequency for three individual scanning tests.

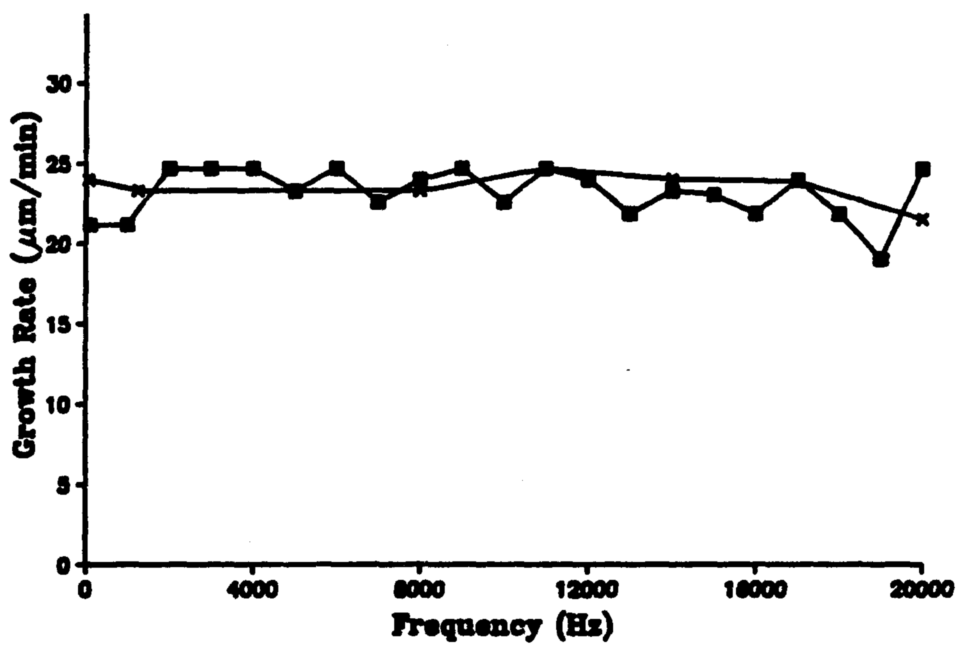

Figure 19. The two curves from Figures 18A and 18C -- growth rate as a function of frequency -- superimposed to illustrate the importance of frequency scanning interval in the overall growth rate trend observed.

In Figure 18B the central portion of the spectrum -- from 4000-13,000 Hz -- repeats the regular pattern seen in the central portion of Figure 18C. The general decline in growth rate over the course of the day can be easily seen in the 100-10,000 (Series 2, Test 1) and 10,000-20,000 (Series 2, Test 2) portions of the spectrum (Figure 18B).

Combining all 3 frequency scans the following trends are observed (Figure 20):

Lowest growth rates consistently at:

7,000
10,000
13,000

Highest growth rates consistently at:

8-9000
11-12,000

Conflicting results at:

$\leq$ 5,000
14,000
16,000
20,000

The summary curve for the average of all three scans is given in Figure 21. The central portion of the frequency range (from 7000-13,000 Hz) and the upper region (18,000-20,000 Hz) show the greatest variation. The maximum difference in growth rate is 5 μm/min. For plants growing at mean daily rates of 25 μm/min, therefore, up to a 20% difference in growth rate was observed at different frequencies.

Noise vs. Growth

In the initial tests when the growth rate fluctuations first

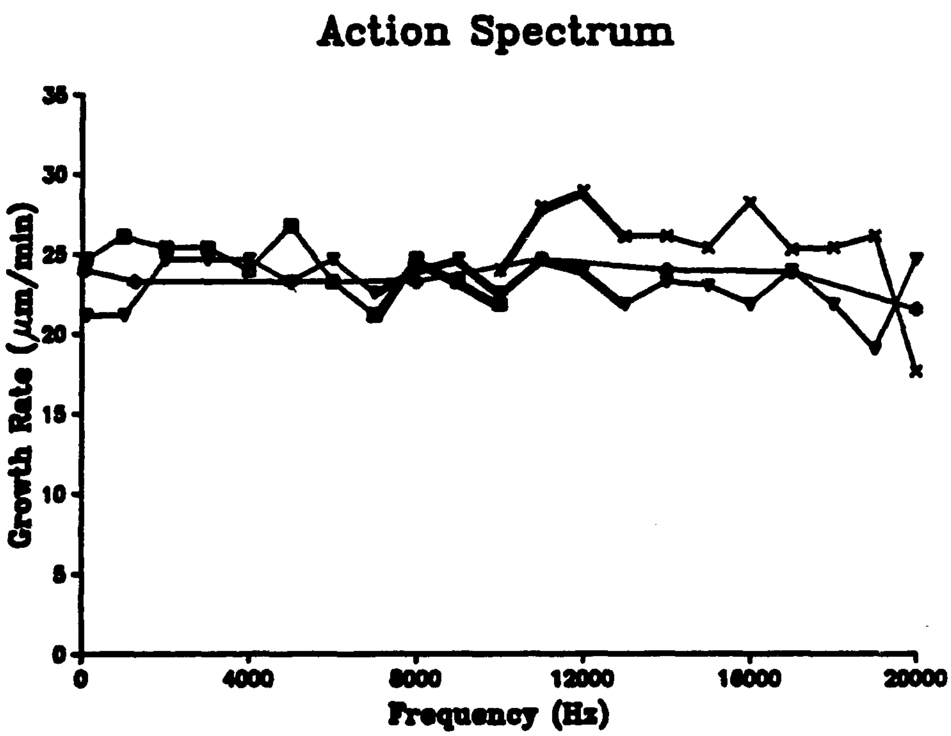

Figure 20. The three frequency scans of Figure 18 combined. The outlined central portion of the curve is the regular, oscillating pattern described in the text.

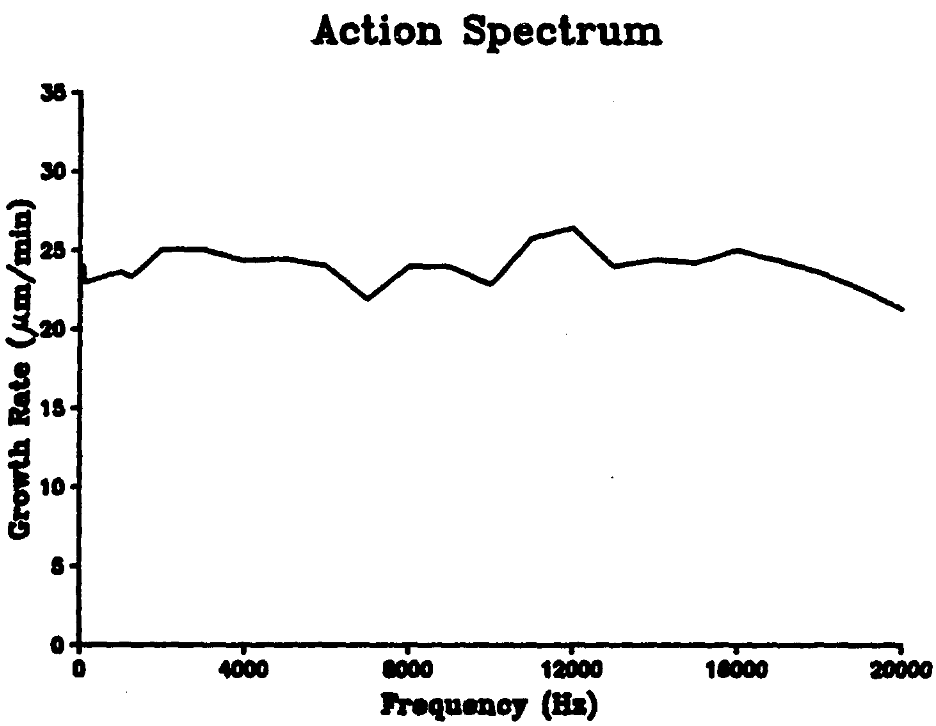

Figure 21. The summary action spectrum, obtained by averaging the three scans of Figure 20.

appeared, in the issue of how much to smooth out the curve, and later during the statistical analysis, the question which repeatedly arose was: how much of the fluctuation in the growth rate curves is real, and how much is due to random statistical variation or experimental error? It is the custom in most statistics to focus on the general trend. While variations from the trend are quantified as a total in such terms as standard deviation, variance, and sum of squares, they are usually not systematically analyzed beyond frequency distributions. These experiments, however, were designed to look at short time periods (15 minutes) within a curve, to determine whether a particular frequency affected plant growth. Further the photographic technique used to measure growth had never been extended to these limits of precision or accuracy before. The variations from the general trend, therefore, assume much greater importance here. Largely because plant physiologists are accustomed to viewing plant growth on a time scale of days, weeks and months, there is the general feeling that plant growth is constant over short time periods (minutes), and that any variations from a constant rate are due to experimental error or random variation. Yet some of the growth rate curves suggested patterns in the variation such as periodic oscillation (see Figure 18c) or a truncation of growth rate peaks (Figure 17), which seemed to warrant further investigation.

Two approaches were taken to attempt to distinguish between experimental noise and real growth within short time intervals:

1) to quantify the noise, a test was run with glass

micropipettes modified to simulate seedings -- i.e. a silicone "seed" and fine string "roots" were added;

2) to quantify the short-term growth rate variability, an analysis for possible oscillatory patterns was attempted using Fourier analysis. This technique is a curvilinear fit process that determines a set of harmonic sine waves which in combination, create a periodic pattern that best approximates the data. Since plants are known to have diurnal and seasonal rhythms on one end of the temporal spectrum, and metabolic periodicities[1] at the other end, it seemed reasonable to look for periodicities at an intermediate point in the temporal spectrum.

The Fourier analysis was not fruitful; more time and better understanding of its principles is needed than this thesis project allowed. The calibration with the micropipette "plants", however, was more informative. Figure 22 and Table 9 summarize the results. Figure 22 presents the "growth rate"[2] as a function of time, in the 3 different graph formats used for the regular tests. The same scaling dimensions were retained for this test, although the entire curve is shifted downward, reflecting the presumed zero net growth of the micropipettes. The unexpected variability suggests that either the simulated plants were not stable in height or more

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1. See Goto, 1985, for a discussion of possible roles of NAD^+, NAD^+ kinase, the mitochondrial Ca^{2+}-transport system, Ca^{2+}, calmodulin and $NADP^+$ phosphatase, for example, in circadian rhythms.

2. The *y*-axis in Figure 22 is labelled "Growth Rate" to maintain the comparison with the plant test results, not to suggest that the micropipettes actually grew.

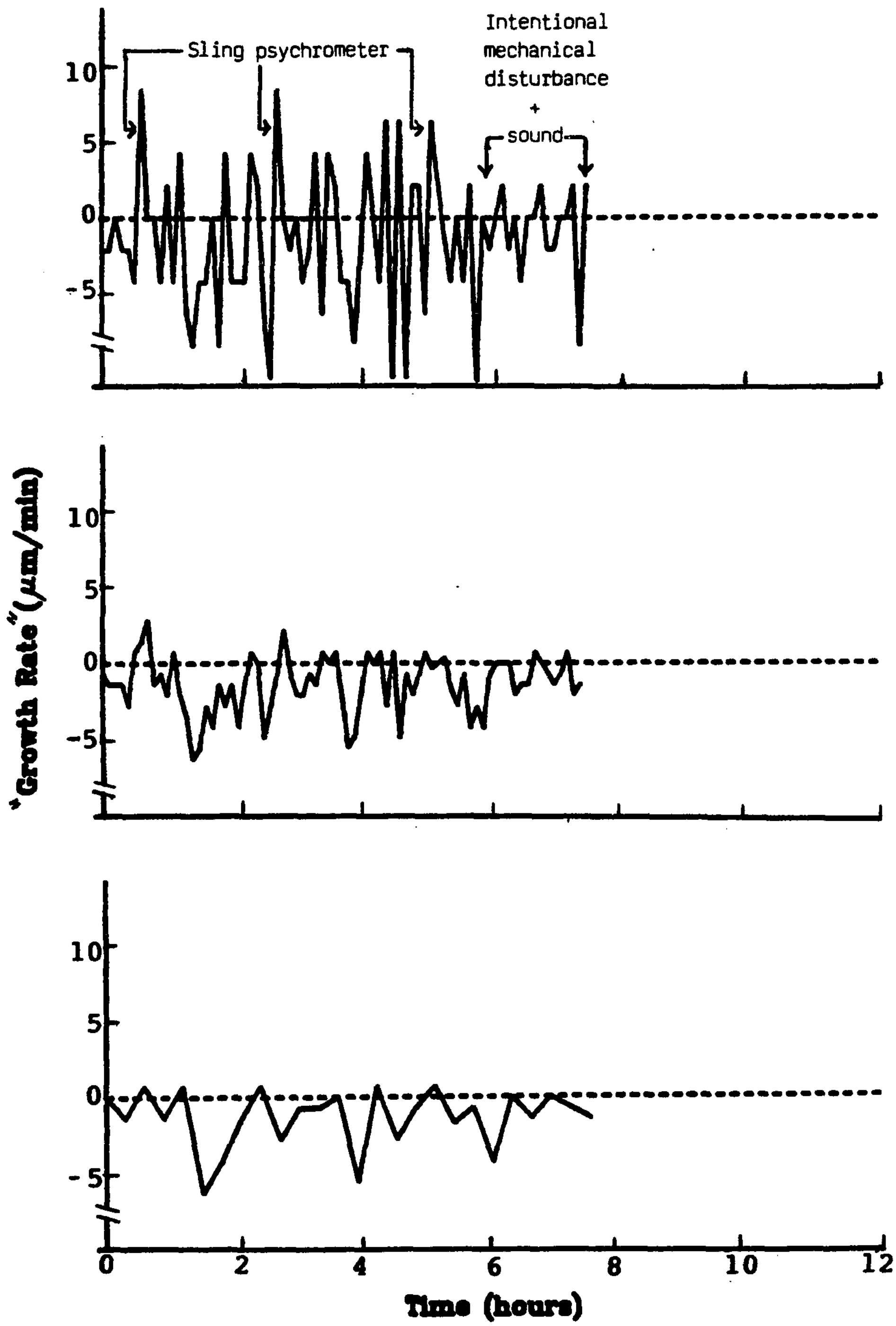

Figure 22. Micropipette calibration test results.

Table 9. Summary of Statistics for the Micropipette Calibration Test.

Statistic	Value
Constant growth model:	
Mean	-1.01
Standard deviation	1.9
n	57
Maximum value	4.3
Minimum value	-5.58
Linear model:	
Linear regression:	
y-intercept	2.136
slope	.00481
F	2.53
P	.1172
Polynomial model:	
Order of best fit	1
P of best fit	.1172

likely, that something in the experimental procedure caused uncontrolled variation. Up to 5 hours, there was no intentional disturbance of the set-up. From then on until the end of the test various potential disturbances to the system were tested. These disturbances included changing the film, knocking the camera, disturbing various other parts of the experimental set-up, and applying sound; wavelengths close to the dimensions of the leaves and of the Hoaglands solution container--2500, 3600, and 5700 Hz--were used to attempt to maximize any sound effects. None of these intentional disturbances induced changes in "growth rate" of the magnitude which occurred a few times during the supposedly undisturbed part of the test. To the contrary this period of sound and mechanical disturbance was actually the least fluctuating time of the test. The largest fluctuations were 6.35μm/min in the two 15-min interval graphs (moving averages and 15 min average) and 19 μm/min in the 5 minute interval graph. It suggests that deltas, or changes, in growth rate detected using this method must be greater than these amounts to be considered significant. Only a few times (in Control #1) were the changes larger than these.

The source of the error is puzzling. A relaxation and contraction of the hard netting used as part of the mounting system would likely produce a gradual trend in one direction. A relaxation may have occurred in the beginning of the test as the netting wicked up moisture from the Hoaglands container via the plants. This gradual relaxation certainly does not account for the rapid fluctuations throughout the test, however. There are at least three possible explanations:

1) The roughness of the camera shutter action may have disturbed the set-up; movement of the camera and transfer of the disturbance to the frame of the set-up had been observed earlier, but the effects were thought to be minimal and confined to the frame only.

2) Air disturbances may have jostled the "plants". The three times sling psychrometer readings were taken corresponded to three of the largest fluctuations - at 25-30, 130-135, and 225-230 minutes. For the psychrometer readings, the instrument was swung overhead at 3 cycles per second for 1.5 minutes at a distance of approximately 1.5 m from the plants. Although it is difficult to imagine glass micropipettes being affected by such air movements, the size of the largest fluctuations is still only micrometers. This test is the only one for which a sling psychrometer was used. For all other tests, temperature and relative humidity were determined using stationary wet and dry bulb thermometers. If the psychrometer was a significant source of error in this test, such error due to air disturbance was probably much less in the real plant tests.

3) The reading of the plant heights from the photographic projections may have introduced error. The lower edge of the ruler behind the "plants" is used as the zero marker for measuring plant heights. Inadvertently this ruler was left farther behind the micropipettes than usual, such that it was slightly out of focus in the slide projections. Although considerable effort and care were taken to ensure consistency in the zero mark for each plant, there

were several instances where it was difficult to exactly pinpoint the mark. Further, because of parallax any variations in height would tend to be magnified if the background ruler was farther back than usual.

The calibration should be repeated to pinpoint the source of the error. The test should eliminate the sling psychrometer, and have the background ruler closer to the micropipettes. A camera with a smoother shutter should be used, and it should be remounted to be independent of the frame. The plant mounting system can also be redesigned to eliminate the hard cloth netting and thus reduce possible water absorption effects. Finally, the measuring of the plant heights from the film projections should be repeated for at least one or two tests, to quantify the precision in this process. Unfortunately, this entire issue did not arise until the time of writing the thesis, so that these changes could not be tested in time for this report. The regularities in the fluctuations of some of the test curves, however--in the 7000-13,000 Hz range of the action spectrum (Figures 21 and 22) and in the experimental test of Series 4 (Figure 17), for example--seem too ordered to be due to chance alone. Thus, the author feels that all of the results are not necessarily nullified by the calibration test results. The question will not be finally resolved, however, until these changes to the system are tested and the source of the error is identified. Until that time, the use of at least two calibration sticks along with the plants is recommended during testing.

CONCLUSIONS

No consistent effect larger than the variation in controls was observed for any sound treatment, pure tone or variable. There were differences in the growth rate curves of the sound-treated vs. control tests, however, which were supported by the statistical analysis. Also, the action spectrum (growth rate as a function of frequency) displayed differences in growth rate of 10-20% relative to the mean, and a pattern of regular oscillation occurred between 7000 and 13,000 Hz. Growth during the early part of the test day (from 1.5 to 3 hours test time or roughly 10:00-noon real time) was higher in many of the sound-treated vs. control tests. Until further replications of the tests are conducted, however, any apparent trends must remain only suggestive, and not conclusive.

The purpose of the experiment was to test one possible quick-and-easy screening method which could be used to systematically select sound treatments for more comprehensive laboratory or field studies of sound effects. The task was made more difficult by the apparent variation in plant growth rate over short time periods (on the order of minutes), which made selecting a control period difficult. The questions of how much of this variation is real growth vs. experimental noise has not been answered but the micropipette calibration suggests that the noise contribution may be substantial.

In terms of plant growth, the dip at 7 hours test time (4-

5:00 p.m. real time) was common to all controls and some of the experimental tests. It is marked enough to warrant further investigation as perhaps a signal of a major metabolic shift in the plant's daily cycle.

Finally, important for both sound and growth research is the need for more tools to probe the curves from living systems for non-linear patterns and periodicities. The trend in statistical analysis is towards greater emphasis on "residuals" -- the statistical variation that remains when general trends such as linear or polynomial regressions are subtracted. Fourier analysis, because it is a means of characterizing periodic data, offers much promise in this area. Much insight into life's intricacies remains unaccessible to science until these subtler behaviors can be scrutinized.

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