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ABSTRACT

Measurements of temperature and light intensity were made at different locations within a growth chamber. These two factors were found to vary with position and may account for the growth chamber position effect noted for growth rates of corn, cucumber and wheat. This data provided the basis for regular rotation of plants grown in chambers for experimental purposes.

The effects of audible sound on germination were found to vary with species, temperature and sound frequency, intensity, and period of exposure. Exposure of Rideau wheat to 5KHz sound at 20C and 25°C had no effect on the rates of imbibition, but protrusion of the radicle was accelerated at the lower temperature. Germination of Marquis wheat at 20C was accelerated by exposure to either 5KHz or 12KHz frequencies, whereas that of Manitou wheat was inhibited by exposure to 5KHz sound. At 25°C, sound exposure had little effect on the germination of either variety. Sound exposure also had very little, or no, effect on germination of Genesee and Talbot wheats, oats, barley and millet seed at either temperature.

The early stages of growth of both Marquis and Rideau wheats appeared to be unaffected by exposure to audible sound. However, plants of both varieties were larger after eight weeks growth under controlled conditions following exposure to 5KHz at an intensity of 92 db in the case of both varieties and exposure to 300Hz sound in the case of Marquis wheat. Sound

exposure of Marquis wheat during a one week chilling period was insufficient to stimulate plant growth. Extension of the chilling period to four weeks accelerated the growth rate. Root growth, of Rideau wheat was slightly accelerated by exposure to 12KHz sound but shoot growth of both Marquis and Rideau wheats was unaffected by exposure to either 1250 or 12KHz frequencies. Rideau wheat exposed to random white noise during both the vernalization and growth periods, did not differ significantly from controls.

Floral initiation and development of Rideau wheat were unaffected by exposure to audible sound in both the laboratory studies and in the field trials. The increase in tiller number noted in the laboratory studies was no longer apparent when the plants were grown in the field.

Growth of Rideau wheat was inhibited by continuous exposure to 5KHz sound at intensities of 105 or 120 db. Exposure to the 105 db level during the vernalization period led to a slight increase in the dry weight of the shoots.

Although growth of Rideau wheat was accelerated by exposure to 5KHz sound at the 92 db level in the laboratory, leaf morphology, plant respiration, photosynthesis, and peroxidase activity were unaffected. Respiration rates of chilled grains were also unaffected but that of non-chilled grains was accelerated following 48 hr of sound exposure. Amino acid and RNA metabolism appeared to be modified following exposure during the chilling period. The total amino acid composition was lower, but the

RNA concentration higher in the sound-exposed series. When the alcohol-soluble amino acids are expressed on a percentage basis, glycine and aspartic acid were more abundant in the endosperm of the sound-exposed series, asparagine was more abundant in the endosperm of the control series and alanine was more abundant in the embryo but less abundant in the endosperm of the sound-exposed grains. DNA content and mitotic activity of the root tips appeared to be unaffected by exposure to 5KHz sound. P-32 uptake by both grains and roots was inhibited following exposure to audible sound.

Exposure to 5KHz audible sound appeared to impair the motility of P. fluorescens and E. coli. Motility decreased as the sound intensity was increased.

The growth of Marquis wheat was affected by exposure to magnetic fields. Weight of heads was reduced by field strengths between 1700 and 14,000 Gauss but increased by a field strength of 220 Gauss. However exposure periods of 7, 24 or 47 hours summed over all Gauss levels reduced the weight of heads, whereas that of 18 hours had no effect.

RESUME

La température et l'intensité lumineuse furent mesurées à divers emplacements dans un phytotron. Ces deux facteurs varient avec la position et ceci peut expliquer les différences observées dans le taux de croissance du maïs, du concombre et du blé selon leur position dans le phytotron. A cause de cet effet, nous avons donc régulièrement changé l'emplacement des plantes lors de nos expériences.

Les effets du son audible sur la germination varient selon l'espèce, la température, l'intensité et la fréquence du son ainsi que la durée de l'exposition. L'exposition des grains de blé Rideau à un son d'une fréquence de 5KHz à des températures de 2°C et de 25°C n'affecte pas le taux d'imbibition, mais le radicelle apparaît plus rapidement à 2°C. La germination du blé Marquis à 2°C est accélérée par une exposition à des fréquences de 5KHz et de 12KHz; par contre, celle du blé Manitou est arrêtée à une fréquence de 5KHz. A 25°C, l'exposition au son n'influe pas sur la germination de ces deux variétés. Une exposition au son à des températures de 2°C et de 25°C n'affecte presque pas, ou pas du tout, la germination du blé Genessee, du blé Talbot, de l'orge, de l'avoine et du millet.

Le son audible ne semble pas affecter les premières phases de la croissance des variétés de blé Marquis et Rideau. Par contre, après une période de croissance de huit semaines, les

plante provenant de grains traités au son à une fréquence de 5KHz et une intensité de 92 db sont plus grosses que les témoins pour les deux variétés. Le même effet est observé chez le blé Marquis avec une fréquence de 300Hz.

L'exposition du blé Marquis au son à une température de 2°C durant une semaine n'est pas suffisant pour en stimuler la croissance. L'extension de cette période à quatre semaines en stimule la croissance. La croissance de la racine du blé Marquis est légèrement accélérée à une fréquence de 12KHz; celle de la tige n'est pas affectée à des fréquences de 1250KHz et de 12KHz. Le blé Rideau soumis à du "random white noise" durant la période de vernalisation et de croissance ne diffère pas des témoins d'une façon significative.

Le début de la floraison et le développement du blé Rideau ne sont affectés par une exposition au son audible tant dans le laboratoire que sur le terrain. L'augmentation du nombre de rejetons observée lors des études en laboratoire ne s'est pas reproduite sur le terrain.

La croissance du blé Rideau est arrêtée par une exposition continuelle à une fréquence de 5KHz et des intensités de 105 et de 120 db. L'exposition à une intensité de 105 db durant la période de vernalisation résulte en une légère augmentation du poids sec des pousses. Même si la croissance du blé Rideau en laboratoire est accelérée par une exposition à un son de 5KHz et 92 db, la morphologie des feuilles, la respiration, la photosynthèse et l'activité de la peroxidase ne sont pas changées.

Après une période de 48 heures d'exposition au son, le taux de respiration des grains traités à 2°C n'est pas changé tandis que celui des grains traités à 25°C est accéléré. Le métabolisme de l'ARN et des acides aminés semble modifié durant une exposition au son à de basses températures. La concentration totale d'acides aminés est plus basse et la teneur en ARN est plus élevée chez les grains traités au son. Lorsque la concentration de chaque acide aminé est exprimé en pourcentage du total, la glycine et l'acide aspartique sont plus abondants dans l'endosperme des grains, soumis au son, l'asparagine dans l'endosperme des témoins et l'alanine dans l'embryon. Par contre, ce dernier composé est moins abondant dans l'endosperme des grains traités que dans celui des témoins. La teneur en ADN et l'activité metotique du bout de la racine ne semblent pas être changées par une exposition à des fréquences de 5KHz. Le son audible empêche l'assimilation du P-32 par les grains et la racine.

La mobilité de P. fluorescens et de E. coli semble être diminuée par une exposition à une fréquence de 5KHz. La mobilité décroit lorsque l'intensité du son augmente.

La croissance du blé Marquis est affectée par une exposition à un champs magnétique. Le poids des épis est moindre lorsque les grains sont soumis à des champs de 1700 et 14,000 Gauss mais est plus élevé lorsque le champs est de 220 Gauss. Des périodes d'exposition de 7, 24 et 47 heures résultent en des épis moins lourds, indépendemment de l'intensité du champs magnétique tandis qu'une période de 18 heures n'a pas d'effets.

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INTRODUCTION

The manifold effects of the environment on the physiology and development of plants have been well documented for many species (Evans 1963). Hence changes in rates of germination and growth can often be correlated to manipulations of the physical environment.

I Standardization of Growing Conditions

Plants utilized for growth studies are frequently grown in controlled environment units where physical factors such as temperature, light intensity, daylength and relative humidity can be regulated to some extent. Conditions within a greenhouse may vary considerably (Desjardins and Robertson 1968), hence growth chambers and growth rooms (Stoskopf et al 1970) are often preferentially used.

One of the major problems encountered during utilization of growth chambers involves the use of artificial lighting since light spectra produced by artificial lights are different from that of the sun (Salisbury and Ross 1969). Fluorescent lights are often mixed with incandescent lights in an attempt to balance the spectrum. The fluorescent lights are the more efficient in the production of visible light, but little red and no far-red radiations are produced. Incandescent lights provide these two ranges of wavelengths but produce a large amount of infra-red energy and hence add to the problem of temperature control. A proper balance must be maintained between incandescent and fluorescent lights in

order to ensure optimal conditions for plant growth and development (Friend 1961). However, even with a balanced spectrum, plant growth under artificial conditions may not approximate that in the field because of low day time light intensity in the chamber and variable light intensity in the field. In addition, fluorescent lights also produce ethylene (Wills and Patterson 1970) a compound which may affect plant growth (Pratt and Goeschl 1969).

Studies of the effects of the physical environment on crop yields under field conditions are much more difficult to assess than laboratory studies on individual plants because it is virtually impossible to standardize conditions. In addition, large numbers of plants, growing in close proximity, alter their immediate environment and hence influence the growth patterns of each other. Moreover measurements of temperature, humidity and wind velocity taken over a grass field show a vertical distribution of parameters different from that of a similar set of measurements taken over a millet stand (Mushkin 1963). Seeding rates (Pelton 1969) and distance between rows (Kilcher and Heinrichs 1969) also affect crop yield. Plants growing close together compete with one another for nutrients and moisture, among other things, whereas plants growing too far apart do not make maximal use of the available space.

II Growth and Development of the Cereal plant

During the early stages of germination, the proteins, cellulose and pectic substances in the grains become hydrated (Mayer and Poljakoff-Mayher 1963). Cells of the embryo begin to divide and elongate and the primary root and coleorhiza break through the pericarp-testa. After protrusion of the primary root, the seminal roots and the stem and leaves begin to expand and develop.

The apical meristem of the stem forms ridges of tissue which develop into leaves. An axillary bud, with the potential of developing into a tiller, or branch, develops in the axil of each leaf (Sharman 1945). As the cereal plant matures, the apical meristem ceases production of leaf primordia and begins production of double ridges of tissue. The appearance of the double ridges marks the beginning of the reproductive phase (Friend et al 1963). The lower ridge does not develop further, but the upper ridge grows and divides to form the spikelets of the inflorescence. As the inflorescence develops, the stem elongates resulting in the emergence of the head above the leaves.

The number of spikelets per head depends on the temperature and light intensity during growth of the plant (Friend 1965b), partly because of spikelet competition for carbohydrates (Walpole and Morgan 1970). Head development proceeds from about the centre of the spike toward both the base and the tip (Sunenson and Cox 1964). The size of the grains produced

is one of the factors controlling the yield of the next generation of plants (Frey and Huang 1969, Austenson and Walton 1970).

Nutritive value depends on both variety of plant and environmental conditions during growth (Peterson 1965, Williams 1966). A single period of stress at any stage of crop growth will affect the yield and composition of the grain, even if the stress period is followed by optimal growing conditions (Hutcheon and Rennie 1960). Protein content of wheat grains depends largely on the availability of such major nutrients as nitrogen and phosphorus in the soil during plant growth and seed maturation, the moisture and organic content of the soil, the presence or absence of rusts, insects and weeds and the use of weed-killers (Hutcheon and Rennie 1960, Williams 1966, Harper and Paulsen 1969). The protein content of grain harvested at an early stage of maturity, when kernel moisture is decreased to 35%, does not differ significantly from that of fully ripe (< 14.5% moisture) grain (Dodds and Warder 1966). However, the protein content of kernels from the top third of the spike is significantly lower than that from the bottom two-thirds (McNeal et al 1954, 1966b). McNeal et al (1966a) found total kernel nitrogen to be highly correlated (r = 0.95) with plant dry weight.

III Some Important Factors Affecting Seed Germination and Plant Growth, with Special Reference to Cereals

A) Mineral Nutrition

i) Uptake by plants

In most plants the minimal nutrient elements essential for normal growth are nitrogen, phosphorus, sulphur, calcium, magnesium, potassium, iron, manganese, copper, zinc, boron, molybdenum and chlorine (Hewitt 1963). Uptake is influenced by several environmental factors which affect both plant metabolism and soil chemistry.

Ions are absorbed more readily by nutrient-deficient plants than by non-deficient plants (Salisbury and Ross 1969). Dicotyledonous plants appear to absorb some elements, such as calcium, more quickly than most monocotyledonous plants (Ioneragan 1968). Most plants appear to extract nutrients more readily from soil solution than from culture solution (Steward and Sutcliffe 1959). Changes in environmental factors such as temperature, light intensity and aeration, which enhance respiration and photosynthesis also enhance nutrient uptake (Hopkins 1956, Sutcliffe 1962, Devlin 1969, Salisbury and Ross 1969).

Ion absorption is also affected by the presence of other ions either in the nutrient solution or within the plant. Monovalent ions are more readily absorbed than divalent ions and the presence of large quantities of monovalent ions may inhibit uptake of the divalent ions (Hewitt 1963). Calcium

is absorbed in preference to magnesium in barley seedlings (Lazaroff and Pitman 1966) and affects the uptake of both anions and other cations possibly by changing membrane permeability and ion selectivity (Leggett 1968). Phosphorus uptake appears to be correlated with nitrogen metabolism since phosphorus uptake by corn plants can be increased to a greater extent by pretreatment with nitrogen than by the addition of higher concentrations of phosphorus to the nutrient medium (Cole et al 1963).

ii) Availability of Nutrients from Soils

In a moist, but not water-logged, soil, water forms a film around the soil particles, leaving adequate spaces between the particles for air diffusion (Black 1957). Nutrients must move from the solid phase into the soil solution and move to the vicinity of a root before they can be absorbed by growing plants (Fried and Shapiro 1960). Olsen et al (1962) made observations over a 100 day period and noted that is was the phosphate within 5 mm of the root surface that was extracted from the soil by corn roots.

PH affects both nutrient absorption and nutrient availability, since it has important effects on soil chemistry. Soil pH varies with the soil: water ratio and with the salt content of the soil (Bould and Hewitt 1963). At low pH levels the solubility of aluminium, manganese, iron and heavy metals increases and these elements may reach concentrations that are toxic to plant growth (Black 1957). In addition,

phosphate concentration may become a limiting factor since phosphate is readily precipitated by iron and aluminium under conditions of low pH (Chakrawarti 1964).

The assessment of nutrients available to plants grown in soil is very difficult because of equilibria between the soil solid and liquid phases. Water-soluble compounds, such as ammonium phosphates, are readily available, whereas calcium phosphate is slowly available and iron, aluminium and organic phosphates are still less readily available (Buckman and Brady 1969). Ions often enter the soil solution by anion or cation exchange with ions adsorbed onto soil colloids (Black 1957).

The soil nutrients most often in limiting supply are nitrogen, phosphorus and potassium. Fertilizers must be applied in the correct proportions to balance the availability of all essential nutrients and to maintain an optimal pH range. Some fertilizer salts, such as phosphorus, do not move substantial distances through the soil and hence must be placed close to the zone of root development (Buckman and Brady 1969). The majority of fertilizer phosphorus is precipitated or adsorbed by the soil and as little as 10-15% may be available for plant growth (Chakravarti 1964). Other elements such as potassium and nitrogen move vertically through the soil as water moves up and down. Hence time and placement of fertilizer are important if nutrients are to be available in the proportions needed to maintain normal plant growth.

iii) Usefulness of Culture Solution for Plant Growth Studies

Plants used in botanical studies are often grown in nutrient solutions since the chemical composition of such media is more easily controlled than that of soils. Solutions must be changed every few days to replenish the oxygen supply and overcome nutrient gradients and pH changes induced by the preferential absorption of ions (Salisbury and Ross 1969).

B) Aeration

A majority of higher plants require an oxygen level close to that of the atmosphere for normal germination and growth (Burke 1930, Mayer and Poljakoff-Mayher 1963, Salisbury and Ross 1969). Many plants have a continuous system of gas spaces which allow gaseous diffusion throughout the plant (Barber et al 1962). Air spaces occupy 5-30% of the total volume of rice plants, compared with less than 1% of the total volume of barley plants, and hence may partially account for the ability of rice plants to grow in water-logged soils (Barber et al 1962).

Roots must receive an adequate oxygen supply in order to absorb and accumulate many plant nutrients since metabolic energy is required for active transport and retention of nutrients (Hopkins 1956). Most soils, when not water-logged, are aerated by gaseous diffusion between the soil particles.

Wheat plants grown in poorly aerated soils exhibit malformed stamens (Campbell et al 1969a). This leads to poor seed

set and hence lower grain yield. Campbell and Ferguson (1969b) found yields of Chinook wheat grown in well aerated soils to be 2.3 times the yields of plants grown in poorly aerated soils.

C) Light

Visible light is one of the factors limiting photosynthesis and hence carbon fixation. Therefore light intensity and daylength are major factors controlling crop yield.

When other factors are not limiting, the rate of photosynthesis is increased with increasing light intensity. Several varieties of wheat do not appear to reach saturation during growth in the field because of shading of the lower leaves (Lupton 1969), thus indicating that under field conditions light intensity may be a limiting factor in dry weight production. Yields of wheat can be increased by extension of the daylength, even with intensities as low as 50 ft-c (Hofstra et al 1969). Wheat grown at high light intensities transpires more moisture per day than wheat grown at low light intensities, but utilizes less water in toto because of the shorter time period required for maturation (Campbell and Ferguson 1969b). Shaded plants are more efficient utilizers of solar radiation, as reflected in grain production, than non-shaded plants (Campbell et al 1969c).

Initiation of floral induction and head development of wheat plants can be hastened by increased light intensity (Friend et al 1963), far-red radiation (Friend 1964) and day

length (Rawson 1970). However, rapid head development may result in lower yields since few potential spikelet sites are differentiated after the inflorescence begins to develop (Rawson 1970). Hence fewer grains are produced per plant.

Although diurnal light and dark regimes are very important factors controlling the floral initiation of most species, several plants, such as Nishimura winter wheat, can initiate flower primordia in total darkness (Inouye 1965, Inouye and Katayama 1964b).

B) Temperature

i) Germination and Growth

Temperature is one of the crucial factors influencing the germination of many seeds. Optimal germination temperatures vary widely, even between species and varieties of the same genus. While some species germinate equally well over a wide range of temperatures, others have a more restricted temperature range for germination (Toy and Willmingham 1966).

Growth, as well as germination, is affected by temperature since temperature regulates many physical and chemical reactions. Temperature affects shoot: root ratios (Friend et al 1965), the quantity of water utilized during plant development (Campbell and Ferguson 1969b) and percent grain protein (Campbell and Read 1968) as well as the rate of development (Peterson 1965) of wheat.

ii) Interaction of Temperature and Available Moisture During Storage and Germination

During early stages of germination, the rate of water uptake increases with temperature over the range of 5 to 35°C (Schull 1920). Pollock and Toole (1966) and Christiansen (1967) found the imbibition period to be the temperature sensitive period during the germination of lima bean seeds and cotton seeds, respectively. Plants of both species were malformed when seeds were imbibed and germinated at low temperatures, but showed little or no injury when the seeds were allowed to imbibe water at a high temperature for a few hours before chilling. The length of the imbibition period before chilling is also an important factor in determining the early phases of embryo development and root growth of wheat varieties requiring a vernalization period (Weinberger and Ku 1966, Jones 1969).

Some species germinate more readily if subjected to low temperatures during imbibition, and warmer temperatures during protrusion of the radicle (Mayer and Poljakoff-Mayher 1963). Guitton (1965) found that pine seeds germinate most readily if imbibed at 4°C for 48 hr before transfer to a 22°C room. The time required for germination was increased when the chilling period was either extended or shortened.

Many seeds become less viable if stored for long periods of time (Mayer and Poljakoff-Mayher 1963). Viability of most seeds is retained for longer periods when storage conditions

are such that metabolic activity is low. Therefore viability varies with temperature and moisture levels. Viability of wheat grains is reduced when grains are exposed to high temperatures. Heat damage is more severe in moist grains than in dry ones (Watson 1970).

The combination of warm temperatures and high moisture levels may also lead to fungal growth in stored wheat since both internal spores and spores present on the seed coat germinate readily under these conditions (Ampratwum and McQuitty 1970).

iii) Vernalization

The grains and seedlings of many cereals must be chilled during early stages of development in order that the plants flower and produce grain in one summer (Leonard and Martin 1967). The morphogenetic change to double ridges, indicating the changeover to reproductive growth, takes place after the differentiation of 5 to 7 leaves, rather than after approximately 14 leaves are formed. Hence plants grown from vernalized grains have a lower leaf number at flowering than plants grown from non-vernalized grains (Inouye and Katayama 1964a, Silsbury 1964). The chilling period must exceed a minimal length of time for vernalization to be completed (Silsbury 1964, Weinberger and Ku 1966) and partially vernalized plants can be devernalized by high temperatures and by short days (Purvis and Gregory 1952, Chintraruck and Katellapper 1969). The effect can be enhanced in rye if grains germinated at low temperatures are exposed to red radiation (Friend 1965d).

Vernalization of the grains of winter wheat alters the amino acid and protein content of the leaves (Pauli and Mitchell 1960, Weinberger and Godin 1966, Trione 1966, 1967). During the vernalization process, the protein pattern of winter wheat embryos changes, leading to a pattern very similar to that of spring wheat when vernalization is complete (Teraoka 1967). The total amino acid content of vernalized winter wheat grains is higher than that of non-vernalized grains (Jones 1969). In addition, there is a synthesis or turnover of RNA as vernalization proceeds. It has been suggested that vernalization is correlated with nucleic acid metabolism since response to vernalization is exhibited only in tissues containing actively dividing cells (Wellensiek 1964, 1965) and since winter barley has been induced to flower, without cold treatment, by the application of RNA (Suge and Yamada 1965).

E) Interaction of Temperature and Light

The optimal combination of temperature and light varies with species and with the growth parameter studied. For example, Marquis wheat grown over a range of temperatures and light intensities maintains its maximum relative growth rate at 15-20°C and 2500 ft-c, but maintains its maximum absolute growth rate at 20-25°C and 2500 ft-c (Friend et al 1962a).

The production of leaves by young plants is very important since leaves are the major sites of photosynthesis. In Marquis

wheat rates of leaf initiation, emergence and expansion and their final length, breadth and thickness vary with temperature and light intensity (Friend et al 1962b). The leaf area ratio, the ratio of leaf area to total plant dry weight, increases as temperature increases and decreases as light intensity increases, partly because of changes in leaf thickness (Friend et al 1965). Temperature and light intensity also influence the total number of leaves per plant. Both the rate of tillering and the rate of leaf emergence on individual axes are stimulated by increases up to 25°C and 2500 ft-c (Friend 1965c).

Interactions of light intensity and temperature are also important factors affecting the length of time required for floral initiation, development of the inflorescence (Friend et al 1963), and the final ear length and spikelet number of Marquis wheat (Friend 1965b).

F) Soil Moisture Stress

The yield of alfalfa crops varies greatly with the width of the space between rows (Kilcher and Heinrichs 1969). Row spacing appears to have its greatest effect during years of low rainfall, when moisture stress is highest. The time-space distribution of available soil moisture is very important since it affects factors such as nutrient absorption, disease, pests and the effectiveness of herbicides, as well as the water balance of the plant (Mack and Ferguson 1968). The yield of snap beans is lowered when plants are subjected to a moisture

stress (Maurer et al 1969), but that of peas is unaffected if plants grown under severe stress prior to blossom receive ample water during the development of the fruit, (Maurer et al 1968). The effect of soil moisture stress on the yield of wheat also depends on the stage of development at the time of the stress, (Campbell 1968, Campbell and Read 1968).

More tillers appear in plants grown at low moisture stress but the difference disappears by the time of heading, (Campbell and Read 1968). Productive tillers of wheat plants grow rapidly after the death of unproductive tillers and mature at the same time as those of plants that have been subjected to much less inter-tiller competition (Bremner 1969).

Changes in yield are mainly due to poor seed set and not to the number of florets per head or the weight of the grains. When water stress is low, high relative humidity also depresses seed set while increased soil aeration stimulates it (Campbell et al 1969a). Leaves of wheat, cotton and corn often exhibit an increased density of stomata and a decreased leaf area when grown under conditions of high soil moisture stress (Gindel 1969).

G) Clipping

The date and frequency of clipping influence the yield of forage crops. Highest yields of wheatgrass, bromegrass and reed canarygrass are obtained when the crop is harvested during

the seed set stage of development (Lawrence and Ashford 1969a). Yields of alfalfa are high if plants are cut three times during the growing season, providing that the third cut is not made until late October when the amount of available carbohydrate and nitrogen in the roots is highest. If the third cut is made early in September, when the roots are low in assimilates, yields are low the following year (Gasser and Lachance 1969).

Although the yield of alfalfa crops is highest when only three cuts are made each year, protein content is highest when crops are cut more often (Gasser and Lachance 1969).

H) Electromagnetic Radiation

Electromagnetic radiation is emitted following the acceleration of charged particles which are in random motion (Marshall and Pounder 1957). The wavelengths of this radiation range from 10^{9} to 10^{-4} cm and are used to classify the forms of radiation. These forms include radio and television waves, infra red heat, visible light (Section III C), ultraviolet light, X-rays, γ-rays and cosmic rays (Ferris 1968). Where the ranges of two types of radiation overlap, such as in the case of X-rays and γ-rays the two types of radiation may be regarded as identical, except for their origin.

Many small-seeded legumes, such as clover and alfalfa, produce a large percentage of hard seeds which require a long time to germinate because of an impervious seed coat (Works and Erickson 1963). Germination rates of such seeds can be accele-

rated by exposure to either infra red or radiofrequency radiation (Works and Erickson 1963, Nelson and Wolf 1964a, 1964b). The direction of the field of force may influence the effects of such radiation.

Stimulation and inhibition of germination and growth by treatment with γ-radiation have also been investigated recently. Woodstock and Justice (1967) found that seedling growth of corn, wheat, sorghum and radish is stimulated by low doses of γ-radiation and inhibited by high doses. Respiration rates of corn, wheat and sorghum, but not of radish, were also stimulated by low doses of γ-radiation.

I) Electromagnetic Fields

Seeds of several species germinate earlier and grow more rapidly when oriented longitudinally parallel to the lines of force of a geomagnetic or an introduced magnetic field, than when oriented in any other direction (Pittman, 1963). This stimulatory effect is exhibited in some species when dry seeds are exposed to the magnetic field before planting (Pittman and Anstey 1967, Pittman 1968) and is not lost when seeds are stored for up to eighteen months before germination (Pittman 1967). The effect is greatest in some species if seeds are planted with the embryo pointed toward the earth's north magnetic pole (Pittman 1968). Roots of some species will grow in a north-south direction with respect to the earth's magnetic field (Pittman 1968), but will grow away from the area of

maximum field strength when placed in a strong introduced field (Audus 1969). The yield of snap beans has been increased by subjecting dry seed to a magnetic field and then planting the seeds with the micropylar end toward the geomagnetic north pole (Pittman and Anstey 1967). Stimulatory effects are largest when seeds are exposed to the magnetic fields at the optimum growing temperature for a given crop (Strekova et al 1965).

Germination rates of seeds of sorghum, corn, wax beans and bush beans are unaffected by the presence of either an electrostatic or an electrokinetic field (Murr 1965b, 1966a). However, Sidaway (1966) has shown the germination rates of lettuce seeds to be decreased in the presence of a conventional electrostatic field where the air is positively charged with respect to the ground, when compared with controls, but to be unaffected when the polarity is reversed. Potato tubers in a low intensity electrokinetic field germinate up to twenty-five days earlier than tubers not exposed to an electric field or those exposed to a high intensity field (Lazarev et al 1965).

Growth of corn and bush beans is reduced when plants are grown in an electrostatic field (Murr 1963). The degree of inhibition increases as the field strength increases (Murr 1965b), but is greater in a reversed field, where the air is negatively charged with respect to the soil, than in a conventional field (Murr 1966c). Growth is also inhibited when plants are grown in a continuous electrokinetic field (Murr 1965b,

1966a, 1966c), but can be stimulated when plants are exposed to a low intensity field for a short period of time (Murr 1966c). Extensive cell and tissue damage have been noted on the tips of leaves of plants exposed to electric fields (Murr 1964, 1965a, 1965b). Damage occurs on all plants, including those whose growth rate has been stimulated (Murr 1966a), and is more severe on plants exposed to a reversed field than on plants exposed to a conventional field of the same potential difference. Electric storms set up a natural reversed electrostatic field, but the potential difference appears to be insufficient to cause tissue damage and to inhibit growth (Murr 1966a).

J) Sound waves

Exposure of seeds of several plant species to ultrasonic waves has been reported to stimulate both their germination and growth rates (Obolensky 1953, 1957; Johnson and Obolensky 1954, Timonin 1966, Rubtsova 1967). The optimal frequency and intensity of the vibrations and the length of the exposure period vary with species (Obolensky 1953, Timonin 1966). Germination and growth can be inhibited by increasing the intensity level or exposure period above a critical point, below which germination and growth are enhanced (Obolensky 1953, Timonin 1966, Rubtsova 1967). Exposure of seeds to ultrasound, before planting, has resulted in increased yields of fodder beans and wheat (Ausländer et al 1967, Rubtsova 1967) and earlier heading of barley (Johnson and Obolensky 1954). Stimulation of germination and growth is accom-

panied by an increase in the respiration rate, the action of hydrolytic and oxidative enzymes, and the concentration of sugars (Kozubov and Gamyushkina 1964, Ausländer et al 1966). Ste. Marie, (1970), reports an increase in the permeability of artificial membranes during exposure to ultrasound.

Very few studies concerning the effects of audible sound have been made to date. Singh (1965), Singh and Gnanam(1965) and Smith (Hicks 1963), report stimulation of the growth of rice and corn plants subjected to daily exposure periods of music. However, the effects of single-frequency audible sound on seed germination and plant growth have not been extensively investigated.

iv) Purpose of Present Studies

The main purpose of this study was to investigate the effects of various environmental factors on the germination and growth of several plant species. The homogenity of environmental conditions inside a growth chamber was investigated to ascertain the validity of growth chamber studies. The effects of audible sound were studied since very few reports have appeared in the literature to date. An experiment where seeds were subjected to magnetic fields of very high gauss levels was also undertaken since earlier studies report only the effects of low guass levels. Both audible sound and electromagnetism may be considered as potential environmental pollutants and as such, the interactions, if any, between these factors and actively growing cells was of interest.

MATERIALS AND METHODS

I Standardization of Growth Conditions for Laboratory Studies

A) Description of Growth Chamber

The growth chamber used in this study was a Sherer-Gillett, model CEL 255-6. Internal dimensions were 93 cm x 60 cm x 83 cm. The rack supporting the plants was placed 28 cm from the bottom of the chamber. The temperature controls and the light controls were wired through two 24 hr time-clocks to allow the chamber to be set for a day and night regime. Light during the 16 hr daytime period was provided by four 25 watt incandescent and eight fluorescent (Sylvania F48-T13cwHO) light bulbs. The light intensity, measured at the level of the rack, in the centre of the chamber was 940 ft-c. Temperature was maintained at 25 ± 2°C. Air was circulated by a fan located in an end wall of the chamber.

B) Growth of Plants, as Affected by Positions within Chamber

Seeds of cucumber, corn and oats were germinated in petri dishes, in excess water, at 25°C. After forty-eight hours the seeds were planted in soil in 2" x 2" peat pots, which were then placed in a growth chamber. The pots were carefully arranged so that ten rows of 24 pots of each species completely filled the chamber. The pots were watered daily, but were otherwise undisturbed throughout the experiment. Height, number of leaves, fresh and dry weights of the shoot system and the

number of emergent buds and flowers were assessed after eight weeks growth. The experiment was repeated in three separate chambers at the same time.

In a second experiment, vernalized Rideau wheat grains were planted in a mixture of soil and vermiculite in three 23 x 28 cm flats. The flats were placed in a growth chamber and left undisturbed for one week. Plant heights were measured at 32 regularly spaced loci in each flat. The plants were watered daily throughout the experiment.

C) Measurements within the Chamber

i) Temperature

Twenty-one thermocouples were arranged inside the chamber as shown in Fig. 1. Numbers 1-6 were placed on the bottom, numbers 7-15 were placed on the rack, and numbers 16-21 were suspended from the top of the chamber. The twenty-second thermocouple was placed outside the chamber, directly in front of the air intake opening. The thermocouples were attached to a Honeywell recorder Model 153x89-(c) - II - III - 16. Temperature was recorded continuously for a three day period, both when the chamber was empty and when the rack was completely filled with 8 week old plants growing in four inch pots. The leaf temperatures of those plants in positions 7, 9, 10, 11, 12, 13 and 15 were also recorded for three days.

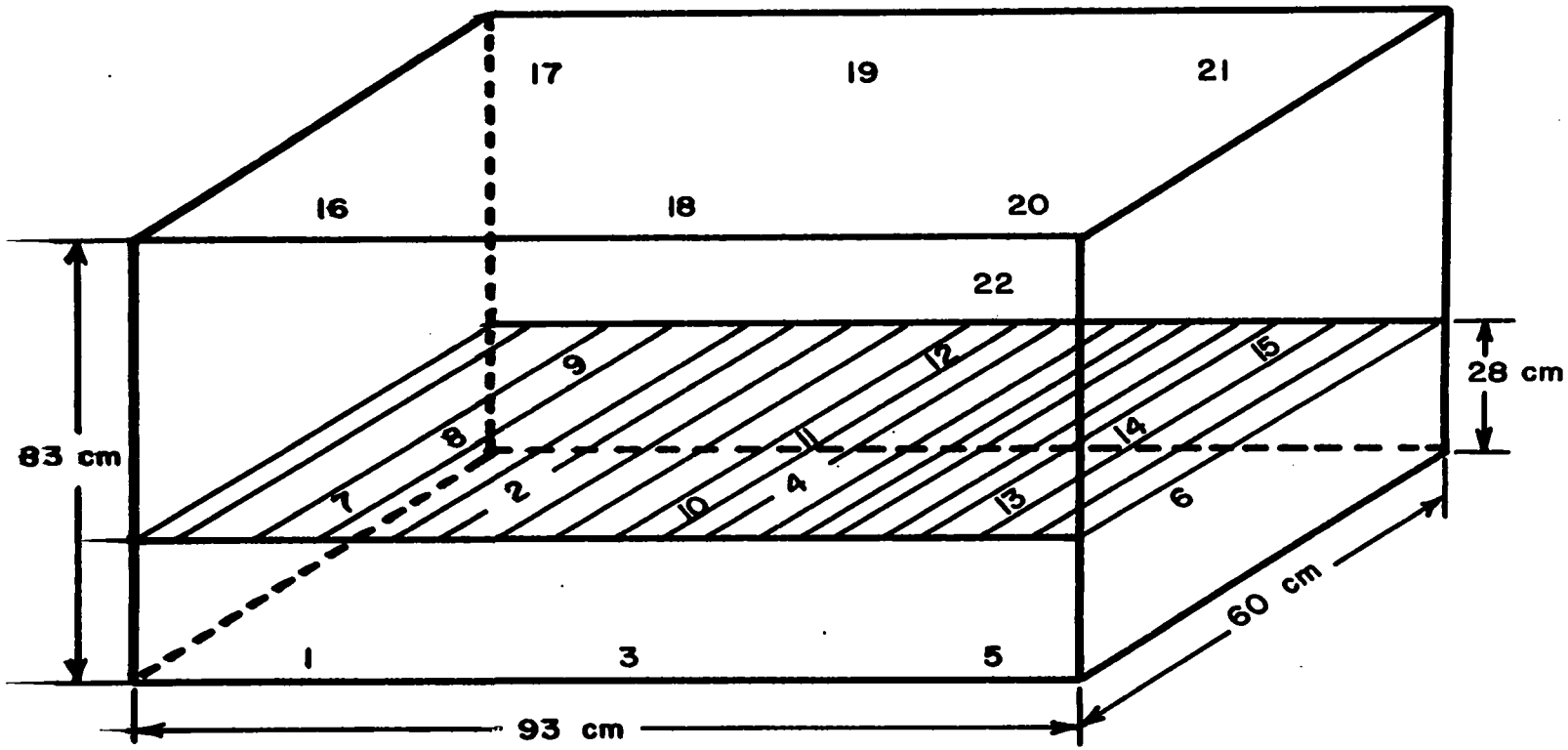


Fig. 1. Arrangement of thermocouples inside growth chamber.

ii) Light Intensity

Light intensity was measured at the level of the rack at fifteen equally spaced locations. Foot candle illumination was measured with a Weston illumination meter, Model 756, when there were no plants in the chamber.

II Environmental Effects on Plant Growth, with Specific Reference to Audible Sound

A) Exposure of Seeds and Plants to Audible Sound

Four audible sound frequencies, 300 Hz, 1250 Hz, 5KHz and 12KHz and one array of random white noise were used in the following experiments.

Hewlett - Packard model 200 CD audio-oscillators were used to define each single sound frequency. The 300 Hz and 1250 Hz frequencies were obtained through 9 in. and 9.5 x 13 in. woofer speakers, respectively, attached to the audio-oscillators. Frequencies of 5KHz and 12KHz were obtained through four inch tweeter speakers. The sound pressure level, monitored with a precision sound pressure level meter (Bruel Kjaer Type 2203/1613), was 93 db re 0.0002 dyne / cm^2 at 300 Hz, 89 db at 1250 Hz, 92 db at 5KHz and 95 db at 12KHz.

The four inch speakers were substituted by a horn (University Model H-600) and driver (University Model T-30) attached to an amplifier (Dynakit Stereo 70) to obtain 105 db and 120 db levels of the 5KHz sound.

White sound was obtained through an 8 inch speaker attached to a random noise source (Allison Model 650). A flat shaping of the sound spectrum was maintained; i.e., energy gain was never greater than 3 db per octave with increasing frequency. The sound pressure level varied between 78 db and 92 db over the range of 63Hz to 8KHz, dropping to 72 db at 16KHz and 56 db at 31.5KHz.

In each experiment, only the speaker or horn and driver was placed inside the germination or growth chamber. General background noise (from machinery) was 35 ± 3 db in the refrigerators and 45 ± 3 db in the growth chambers.

B) Laboratory Studies

i) Imbibition of Grains and Seeds

In all studies, except seedling growth studies, grains or seeds were placed in 8.5 cm, blackened, plastic petri dishes lined with two pieces of Whatman No. 1 filter paper. Excess distilled water, 10 ml per 5 g grains (approximately 150) was added to each petri dish. Where only 10 grains or seeds were placed in each dish, 7.5 ml of distilled water was added.

In the seedling growth studies, ten grains were placed in 5 ml pyrex glass beakers, lined with two circles of Whatman No. 1 filter paper. Distilled water, equivalent to 60% (Set α) or to 100% (Set β) of the dry weight of the grains was added to the beakers. The beakers were sealed with squares of parafilm.

Unless otherwise stated, grains or seeds were imbibed in growth chambers maintained at 25 ± 2°C, with no addition of audible sound above background levels.

ii) Germination as Affected by Temperature and Audible Sound

a) Initial Stages of Imbibition

Four petri dishes containing 5 g of dry Rideau wheat grains were placed in refrigerators maintained at 2 ± 2°C immediately after the addition of water to the dishes. The refrigerators were housed in a constant temperature room (10 ± 2°C) to ensure that the daily opening of the refrigerators to aerate the grains in no way added a variant temperature change. Two of the dishes of grains were exposed to continuous 5KHz sound at the 92 db level (5K) while the other two dishes were not exposed to any sound above the background level (C).

After one hour, the grains were removed from the petri dishes, blotted, weighed and then returned to the dishes with a fresh supply of water. The dishes were then returned to the appropriate refrigerator. All operations were carried out in the cold. The procedure was rapid in order that the grains would remain chilled when removed from the refrigerators.

Weights were recorded hourly for the first 6 hr, every 2 hr for the next 6 hr and then every 12 hr as the rate of water uptake decreased. Weights were recorded up to 192 hr after the initial addition of water.

In a second experiment, the increasing weight of Rideau wheat grains during the initial stages of imbibition was measured at 25 ± 2°C. Two dishes of grains were placed in a growth chamber exposed to continuous 5KHz sound at 92 db and two were placed in a control growth chamber. Weights were recorded every half hour for the first 3½ hr and then every 2 hr for the next 8 hr. The final weight was recorded 24 hr after the initial addition of water.

The above procedures were followed at least twice and the weights of a minimal total of 300 seeds were recorded for each set of conditions.

b) Protrusion of the Radicle

The germination rates of Russell oats, York barley, Crown millet seed and Marquis (spring), Manitou (spring), Genesee (winter), Talbot (winter) and Rideau (winter) wheats were studied. In each experiment, five lots of 10 seeds were germinated in refrigerators at 2 ± 2°C or in growth chambers at 25 ± 2°C. Control seeds, designated (C), were not exposed to sound above the chamber background level. The other batches of seeds were exposed to 5KHz (5K) sound at an intensity of 92 db, or to 12KHz (12K) sound at an intensity of 95 db. The number of germinated seeds were counted every 2 hr. Germination was assessed to be complete when the radicle first pierced the seed coat.

The effect of exposure to 5KHz sound at an intensity of 120 db on the germination potential of Rideau and Marquis wheats was also tested.

Each experiment was repeated at least three times so that rates of germination of a minimal total of 150 control seeds and 150 sound exposed seeds were examined for each species.

iii) Growth of Seedlings as Affected by Temperature, Available Moisture and Audible Sound

Thirty batches of ten medium-sized Rideau wheat grains were imbibed as outlined on page 25 to form set α and thirty batches were imbibed to form set β.

Each set containing a total of 300 seeds was further subdivided into two series. The 150 grains of one series were allowed to imbibe the added water at 25 ± 2°C for 3 hours while the 150 grains of the second series were allowed an imbibition period of 6 hours. Following completion of the imbibition periods, the grains were placed in refrigerators maintained at 2 ± 2°C.

Each series was further subdivided into three groups. Each group consisted of 5 beakers with 10 grains per beaker. One group was continually exposed to 5KHz sound, at an intensity of 92 db, throughout the chilling period. The second group was continually exposed to 12KHz sound. The third group was the control, which was not exposed to any sound above the background level of the refrigerator.

The grains were aerated daily by removing the parafilm from each beaker for a few seconds. Weights were checked at weekly intervals and adjusted with distilled water when necessary, in order to maintain the level of the 60 or 100% added water.

The lengths of the roots of each grain were measured after 2, 3, and 4 weeks of treatment. Measurements were made in a petri dish, sitting in an ice bath, in order to maintain the grains at a low temperature.

The whole experiment was repeated three times so that germination data was obtained on a total of 150 seedlings per experiment.

iv) Growth of Plants

a) Vernalization and Growth Procedures

Marquis and Rideau wheats provided the experimental material for this study.

5g lots of grains of each variety were imbibed for 6 hr at 25 ± 2°C. Following this, the grains were placed in refrigerators maintained at 2 ± 2°C, until vernalization was complete (one week in the case of Marquis and four weeks, in the case of Rideau) (Weinberger et al 1966). Chloramphenicol (20 µg/ml) was added to the water to inhibit bacterial and fungal growth (Jones and Varner 1967).

The petri dishes were then transferred to growth chambers where a sixteen hour day at 25 ± 2°C and 1500 ft-c and an 8 hr night at 18 ± 2°C were maintained. Unless stated otherwise seedlings were transferred to blackened polyethylene bottles containing ¼ strength Hoagland solution when their roots reached 3 cm in length. The culture solution in each bottle was changed at least twice weekly. The bottles were rotated within the chamber when the solution was changed.

Plant height, number of roots, and fresh and dry weights of both the root and shoot systems were measured after four, six and eight weeks growth. Replicates of at least ten plants were taken to a minimal total of thirty for each species and each treatment.

b) Response to Audible Sound

The effects of 5KHz and 12KHz sound frequencies on the growth of Marquis and Rideau wheats were studied (Weinberger and Measures 1968). Three series of grains of each variety were imbibed and subsequently chilled. During chilling one series was not exposed to sound, the other two were exposed to 5KHz and 12KHz respectively. After vernalization, the sound-treated series were each divided into two sets. With one of the sets the same treatment was continued during the subsequent growth period, while the other was transferred to control chambers and not exposed to sound. Using a two symbol notation to describe the conditions during vernalization and growth respectively, the four resultant sets are represented by 5K,5K; 5K,C; 12K,12K; and 12K,C. The vernalized, but sound-unexposed group was split into three sets, two of which were sound treated during the growth period, giving the sets represented by C,C; C,5K; and C,12K.

c) Frequency Response

The responses of Marquis and Rideau wheats to two frequencies in addition to 5KHz and 12KHz were studied in order to obtain

a frequency-response curve (Measures and Weinberger 1970). Two low frequencies, 300Hz and 1250Hz, were chosen. 1250Hz is a low resonance frequency of 5KHz.

The chilling period of the Marquis grains was extended to 4 wk; i.e., beyond the vernalization requirement, to ascertain whether the length of the chilling period was critical for plant response to audible sound. Sound was added only during the four week chilling period at 2 $\pm$ 2°C, hence the two additional series are represented by the notations 300,C and 1250,C.

d) Intensity Response

Rideau wheat grains and plants were exposed to 5KHz sound at intensities of 105 and 120 db, in addition to the 92 db treatment. The 105,105 and 120,120 series were sound treated both during the four week vernalization period and the subsequent eight week growth period. The 105,C and 120,C series were only exposed to sound during the vernalization period, whereas the C,105 and C,120 series were exposed to sound only during the growth period following vernalization.

e) Leaf Morphology

The number of stomata and the number of trichomes were counted in leaves of 5 wk old Rideau wheat plants. Counts were made on a mature (sixth) leaf in a region where mitosis and elongation were complete (8 cm from tip).

Both the stomata and trichomes of Rideau wheat leaves are arranged in numerous parallel rows along the leaf axis. A ruler was placed under a stereomicroscope beside the leaf to be examined. The number of stomata and the number of trichosomes in 5 mm of one row were counted. Three rows of stomata on both the upper and lower surfaces of each leaf were counted. Trichomes were only clearly discernable along the edge of the leaf and hence were counted in this area.

Counts were made on ten plants in each series. This was repeated once to a total of 20 plants in each series.

f) Morphogenesis

Three series, one control and two sound exposed, were studied. The experimental series were continously exposed to either 5KHz at 92 db or random white noise, during both the vernalization and growth periods.

Rideau wheat grains were vernalized for four weeks and then planted in vermiculite in two inch peat pots. The pots were placed in growth chambers and the seedlings watered daily with $\frac{1}{4}$ strength Hoagland solution.

Apices of the seedlings were dissected daily and examined under a stereomicroscope. Stage 14, where the double ridges are well established and beginning to swell (Friend et al 1963) was used as the criterion for floral initiation. The time required for floral initiation was noted.

At least two replicates, of twelve plants each, were examined for each treatment.

v) Rates of Respiration and Photosynthesis as Affected by Temperature and Audible Sound

a) Grains

a') Warburg Analysis

Five g of both Marquis and Rideau wheat grains were chilled for either two weeks or four weeks in refrigerators, in which there was no added sound above the background level (C) or to which 5KHz sound at 92 db (5K) was added throughout the chilling period. Two sets of fifteen grains were taken from each dish of each variety. Respiration rates were measured using a Warburg apparatus, maintained at 30 ± 1°C (Kurtz and Nellor 1966). Respiration rates one hour and twenty-four hours after grains were removed from the cold were noted. Carbon dioxide production and oxygen uptake were both measured on each set of grains. The experiment was repeated on three different dates to a combined total of 90 grains per treatment.

In a second experiment, grains were placed in growth chambers immediately after addition of water to the petri dishes. The growth chambers were maintained on a sixteen hour day (25 ± 2°C) and eight hour night (18 ± 2°C) regime. The grains were exposed continuously to either 5KHz sound at 92 db or to control conditions. Respiration rates were measured, using the Warburg apparatus 24 and 48 hr later.

b') Infra-Red Gas Analysis

The accuracy of the Warburg method of measuring respiration rates was checked by comparison with measurements made on a more

sensitive instrument.

Fifty morphologically similar grains were selected from each of the C and 5K series of 4 wk vernalized Rideau wheat grains. Carbon dioxide production of each series was measured by an infrared (Beckman) gas analyzer 10 hr after the grains were moved from the cold. Ten hr is intermediate between the 1 hr and 24 hr periods utilized in the Warburg measurements.

The experiment was repeated once at a later date.

b) Plants

Rideau grains were vernalized for four weeks in refrigerators exposed to either no sound above background level or to 5KHz sound. The grains were then planted in flats containing a mixture of soil and vermiculite and placed in a growth chamber. Plants were watered daily.

After two weeks growth, carbon dioxide uptake at a light intensity of 2200 ft-c and carbon dioxide production in the dark, were measured by means of an infrared (Beckman) gas analyzer. Measurements were taken on twenty-five second leaves of plants from each of the two series. The leaves were placed in tap water in a 10 ml beaker attached to the side of a glass chamber. The bottom of the chamber was sealed with saran wrap and a four inch speaker, attached to an audio-oscillator, was placed three inches below the saran wrap membrane. An air pump was added to the system to ensure a continuous flow of

air from the chamber into the gas analyzer.

The audio-oscillator was turned on at full amplitude, during respiration and photosynthesis measurements of the control series and the frequency altered through 300, 1250, 5K, 12K and 17K frequencies. Respiration was measured by flushing the carbon dioxide out of the system with nitrogen and recording the rate of increase of carbon dioxide concentration. Net photosynthesis was measured by filling the chamber with a known amount of carbon dioxide, (approximately 375 ppm) and recording the rate of decrease of carbon dioxide concentration.

The experiment was repeated once at a later date.

vi) Amino Acid Analysis

a) Preparation of Grains

Grains of Rideau wheat were exposed to 5KHz sound at 92 db or to control conditions during a four week vernalization period. The grains were then removed from the cold and their pericarps removed. Embryos were carefully dissected out and separated from the endosperm.

b) Free (Alcohol-Soluble) Amino Acids

Free amino acids were extracted in 70% ethanol. 0.5 ml. ethanol was used for each sample of 15 embryos and 2.0 ml for each sample of 15 endosperms. The tissue was homogenized, let stand for half an hour at 60 $\pm$ 2°C and then centri-

fuged for 15 minutes at 6700 r.p.m. The debris was further extracted three times, the final extraction lasting overnight. The combined supernatants were dried in a warm air current and picked up in 1.0 ml of 12.5% sucrose solution. Determinations of amino acids were made on a Technicon amino acid analyzer. The determination of asparagine and glutamine was obtained from samples hydrolyzed for 3 hr in 1N HCl in sealed tubes at 110°C (Boulter 1966).

c) Total Amino Acids

Samples of 15 embryos or 15 endosperms were hydrolyzed for 18 hr in 6N HCl in sealed tubes at 110°C (Boulter 1966). The HCl was then evaporated over a hot plate and the residue taken up in 12.5% sucrose solution and analyzed for amino acids on a Technicon amino acid analyzer.

vii) Nucleic Acid Content

a) Extraction of RNA

Grains of two lots of ten 2-and4-wk chilled control and 5K-exposed Rideau wheat were dissected into embryo and endosperm fractions. Embryos and endosperms were separately homogenized, mixed with 10% trichloracetic acid and centrifuged at 6700 rpm. Resuspension and centrifugation was repeated once with 10% trichloracetic acid, once with 70% ethanol, once with 95% ethanol and three times with a 3:1 alcohol: ether mixture. The extracts were discarded.

The residues were then resuspended in 1N KOH and placed in a 37°C oven for 20 hr. This treatment resulted in the solution of the tissue. The solution was then neutralized with 6N HCl and the protein precipitated in 5% trichloracetic acid. The centrifuged precipitate was washed once with 5% trichloracetic acid and centrifuged.

The combined extracts were mixed with orcinol reagent and heated in a boiling water bath for 20 min. The solutions were cooled and read in a Beckman D.U. spectrophotometer at 660 mμ (Volkin et al 1954). The concentration of RNA was calculated from a standard curve.

b) Extraction of DNA

The residue left following extraction of RNA was resuspended in 5% trichloracetic acid, heated for 15 min at 90°C, cooled and centrifuged. The residue was washed once with 5% trichloracetic acid.

The combined extracts were mixed with diphenylamine reagent and heated in a boiling water bath for 5 min. The solutions were cooled and read in a Beckman D.U. spectrophotometer at 540 mμ (Volkin et al 1954). The concentration of DNA was calculated from a standard curve.

viii) Mitosis in the Root Apical Meristem

Ten morphologically similar grains were selected from petri dishes of both control and 5K-exposed 4-week vernalized

Rideau wheat grains. The grains were placed in a growth chamber (25 ± 2°C) for twenty-four hours. After this time interval, the primary roots were approximately 1 cm in length. The primary roots were dissected from the grains, fixed with FAA, embedded in parafin and serially sectioned, mounted and stained by the Feulgen method (Sass 1958). The sections were made into permanent mounts which were then examined under the microscope. The number of metaphases, the cell size and the length of the apical meristem were noted for the three median sections of each root. These central sections were identified by the presence of large pith cells in the middle of each root.

ix) Peroxidase Activity

a) Grains

a') Preparation of Extract

Five grains were randomly selected from each of 4 petri dishes of fully vernalized C and 5K Rideau wheat grains. Each set was homogenized in 2.0 ml of 10 mM phosphate buffer, pH 7.0 and the homogenate centrifuged at 19,000 r.p.m. for 20 minutes. Following centrifugation, the volume of each enzyme extract was brought up to 25 ml with phosphate buffer.

b') Test for Peroxidase Activity

The activity of the peroxidase was determined by the guaiacol method outlined by Kaehly (1963).

3 ml of enzyme extract were placed in a test tube. 1 ml of 20 mM aqueous guaiacol solution was added and the optical density read at 470 mu in a Beckman D.U. spectrophotometer. The spectrophotometer was then set 0.050 optical density units above the reading. The cuvette was removed from the spectrophotometer and 20 µl of 10 mM H_2O_2 were added to the top of the cuvette. It was then inverted twice to ensure mixing of the solutions, and quickly returned to the spectrophotometer. A stop-watch was started as the cuvette was being inverted and the time required to reach the preset optical density was noted.

b) Seedlings

a') Preparation of Extract

Whole seedlings with one primary and 2 seminal roots, approximately 1 cm in length, were homogenized singly in 2.0 ml of 10 mM phosphate buffer, pH 7.0. The homogenates were centrifuged, as outlined above, and the enzyme extracts brought up to a volume of 10 ml.

b') Test for Peroxidase Activity

0.5 ml of extract were placed in a test tube. 2.5 ml of the buffer and 1.0 ml of the guaiacol solution were added. Peroxidase activity was measured by the method outlined above.

Duplicates of both control and 5K-exposed seedlings were assayed for each of five batches of grains.

x) Artificial Membranes

a) Treatment of Dialysis Membrane

A dialysis membrane of seamless regenerated cellulose, average pore radius 24A° was tied at one end, and filled with water. It was then suspended in a waterproof plastic bag filled with water in a growth chamber maintained at 25 ± 2°C. The dialysis membrane was exposed to 5KHz sound via two 8 in. speakers placed on either side of the plastic bag. A control, non-exposed membrane was treated in another growth chamber at the same time. Membranes were treated for 24, 48, 96, 144 and 192 hr.

b) Sugar Dialysis

3 ml of 50% glucose solution were placed in a 15 ml centrifuge tube. The open end of the tube was covered with a piece of the dialysis membrane, sonicated as described above. The tube was inverted and partially immersed in a beaker containing one l distilled water. The water was stirred with a magnetic stirrer. A 1 ml aliquot of the solution external to the centrifuge tube was removed after 10 min for sugar analysis.

c) Sugar Analysis

The sugar concentration of the samples were measured using the phenol method of Dubois et al (1956).

One ml of 5% phenol and 5 ml concentration sulphuric acid were added to the 1 ml sample of sugar solution. The optical

density was read at 490 mu in a Beckman D.U. spectrophotometer 30 min after the reagents were added. The sugar concentration was then read off a standard curve.

xi) P-32 Uptake Studies

a) Preparation of Solution

The P-32 solution was prepared by adding 1 ul of stock solution (1 mci P-32 in 1 ml of HCl, specific activity 84) per 50 ml distilled water or culture solution. A standard was prepared by drying 0.5 ml of solution on planchet. This planchet was counted with each experiment in order to calculate a correction factor for the decay of the isotope (half-life = 14.2 days; Wang and Willis 1965).

b) Grains

Both control and 5K-exposed grains were chilled for only two weeks so they would be fully imbibed, but not sprouted. The seed coats were removed from four sets of four grains which were then placed in a 10 ml glass beaker and covered with 2 ml of aqueous P-32 solution. After 4 hr the grains were removed from the solution, rinsed for a few seconds in running water and blotted with cheese cloth. The embryos were separated from the endosperms and the two parts ashed separately at 550°C for 2 hr. Approximately 40 ug of ash were spread onto each planchet with 0.5 ml of detergent solution. The planchets

were dried and counted in a Nuclear Chicago gasflow counter, model D47. The experiment was done in duplicate.

c) Seedlings

Fully vernalized control and 5K-exposed grains were grown in plastic bottles in a growth chamber for four days, until the young plants each had five roots and the shoots were approximately 5 cm high. Twenty µg/ml chloramphenicol was added to the culture solution of one series to surface-sterilize the roots. The remains of the endosperm were removed from five similar seedlings from each series. The seedlings were then attached by a piece of masking tape to the side of a 50 ml beaker containing 25 ml of the P-32 solution. The plants were arranged so that only the roots and the very bottom of the shoot system were immersed.

After 3 hr the roots were rinsed, blotted and separated from the shoots. Both the root and shoot systems were ashed and counted. The experiment was repeated twice.

xii) Effect of Audible Sound on Contaminants of Rideau Wheat Grains

a) Growth of Contaminants

Approximately one g of Rideau wheat grains were imbibed in a petri dish. The dish was left at room temperature for three days. By that time, the grains had spouted and become covered with a white mycelium.

b) Isolation and Growth of a Phycomycete

Strands of mycelium were plated on sterile petri dishes containing 0.8% Difco nutrient broth mixed with 1.5% agar. The petri dishes were placed upside down until the fungal colonies almost covered the dishes and were covered with black sporangia (approximately 48 hr). The culture was kept in a growth chamber maintained at 25 ± 2°C.

As the colonies grew, the mycelium grew down and attached itself to the lid of the petri dish. Consequently, several stands of mycelium and several sporangia adhered to the lid of the dish when the dish was opened. These sporangia were used to innoculate other plates.

Successive innoculations were made until the fungus no longer appeared to be contaminated by bacteria.

Sporangia from non-contaminated petri dishes were used to innoculate test petri dishes. One innoculation was made at the centre of each plate. Five plates were placed in both control and 5K-exposed growth chambers. The size of the colonies was measured over a 48 hr period, and the time necessary for the formation of sporangia was noted.

The fungus was tentatively identified as Mucor rouxianus.

c) Isolation and Growth of Bacteria and a Fungus Imperfectus

Water left in the petri dish after the imbibition and germination of the grains was smeared across the agar in several petri dishes with the tip of a small sterilized spatula. The dishes were left at 25°C for three days. Two bacteria and one

member of the fungus imperfectus were isolated by successive transfers under sterile conditions. The bacteria were tentatively identified as Pseudomonas fluorescens and Bacillus sp. and the fungus imperfectus was tentatively identified as Fusarium solani.

Samples of each pure culture were used to innoculate a culture solution consisting of 250 ml 0.8% Difco nutrient broth. The cultures were shaken at 30°C for 24 hr for Bacillus sp., 18 hr for P. fluorescens and 48 hr for F. solani. The cultures were diluted to 10^4 for Bacillus sp. 10^6 for P. fluorescens and 10^2 for F. solani. 0.1 ml of diluted solution was spread on sterile agar plates, colonies were counted and measured 24 hr later.

xiii) Effect of Audible Sound on a Motile Bacterium

E. coli was grown in culture in 0.8% Difco nutrient broth for 18 hr at 30°C. One loopful of culture was placed in the centre of each of nine petri dishes containing soft agar (0.8% nutrient broth and 0.375% agar). Three plates were placed in each of the following; a control chamber, a chamber exposed to 5KHz sound at an intensity of 92 db and a chamber exposed to 5KHz sound at an intensity of 105 db. The size of the colonies was noted as the bacteria swam out across the soft agar.

C) Field Studies

i) Wheat

a) First Generation

Thirty petri dishes containing approximately 10 g of imbibed Rideau wheat grains were placed in refrigerators where the grains were vernalized at 2 ± 2°C for six weeks (Gefeller 1968). Ten petri dishes were placed in a control refrigerator, receiving no sound above the background level and a further ten dishes were placed in each of the two sound-exposed refrigerators, receiving either 5KHz sound at an intensity of 92 db or 12KHz sound.

After six weeks the grains were planted in sterile loam in two inch peat pots. The pots were then chilled for an additional two weeks in a temperature - controlled room, maintained at 2 ± 2°C in order to cold harden to enable early outdoor planting (Gefeller 1968). The pots were watered twice weekly. Thus three sets of grains were obtained which could be represented by the notation FC,C; F5K,C and F12K,C. The notation is preceded by the letter "F" to differentiate those seeds treated for field trials from those grown under controlled conditions.

In a second experiment, dry grains were cold treated at 2 ± 2°C and sound-exposed, under control, 5KHz and 12KHz sound conditions for six weeks. The grains were chilled for an additional two weeks after removal from the sound.

The grains were planted in May 1968, in a fertilized (8-10-10) field at the Central Experimental Farm, Ottawa, Ontario. The field was divided into five blocks. Six rows of each of the FC,C; F5K,C and F12K,C grains were randomly arranged within the blocks. Three blocks of grains which had been cold- and sound-treated in the presence of excess water, and two blocks of grains which has been treated dry, were planted. Rows were planted ten feet long and spaced eighteen inches apart. The rate of seeding was five grains per foot.

Rain was the only source of water during the experiment.

The wheat was left in the field until the grain had ripened. The number of tillers per plant and the yield per row were noted at maturity.

b) Second Generation

Grains harvested from the 1968 field experiment were vernalized and grown in culture solution under control conditions. After eight weeks in the growth chamber, plants were sampled and the growth parameters of plants whose first generation had been treated with 5KHz sound were compared with those of plants whose first generation had been controls.

ii) Alfalfa and Grasses

a) 1968 Sowing

Batches of 500 g of seeds of vernal alfalfa, frontier

reed canary grass and Chinook orchard grass were placed into large, 50 cm x 66 cm, plastic bags. The seeds were sprayed with a fine mist of distilled water equivalent to 12% of the dry weight of the seeds. The bags were closed and the seeds allowed to imbibe the water at 25°C for 6 hr. The bags were then placed in either control refrigerators or in refrigerators to which either 5KHz or 12KHz sound was added. The bags were carefully placed so the seeds formed as thin a layer as possible. During the four week chilling period, the seeds were aerated daily by opening the bags and sifting through the seeds. Weights were checked weekly and adjusted to the 12% added water level by the addition of distilled water in the form of a mist.

When the chilling period was completed, the seeds were shipped to Steeples Ranch, Bull River, B.C. They were sown via a John Deere cc 247A Press drill at the commercial density. Each treatment of each species was sown in five rows, 500 ft in length and 7 in. apart. Water was applied, as required, throughout the growing season. Five rows of each treatment were hand clipped for twenty feet, after a two month growing period. The clippings were carefully collected and weighed.

The following spring, the 500 ft rows were subdivided to form three blocks. Two of the blocks of alfalfa were fertilized at a rate of 250 lb/acre of 10-30-10 fertilizer. The third block was left unfertilized. The plants in each block were hand-clipped three times during the growing season and the clippings weighed separately.

b) 1969 Sowing

Batches of seeds of vernal alfalfa, Frontier reed canary grass and Kentucky bluegrass were imbibed and vernalized by the method outlined on page 47. One batch was exposed to 5KHz audible sound, at an intensity of 92 db and the second at an intensity of 105 db during the chilling period. A third series, the control series, did not receive any sound treatment. A fourth series, a non-imbibed, non-chilled control was also added.

Following completion of the chilling period, half of the alfalfa seeds were treated for 3 sec with infra red light from a 500 watt bulb, at a distance of 1 cm (Nelson and Wolf 1964b). Subsequently, seeds were allowed to air dry, at room temperature, for 24 hr.

One half the seeds of each series was sown in a fertilized field (400 lb/acre 8-16-16) at the Central Experimental Farm, Ottawa Ontario. Six randomized blocks of four twenty ft rows, spaced 12" apart, were sown with seed of each treatment of each species, at a seeding rate of 10 lb/acre, or 2.1g/row. The alfalfa was clipped two months after sowing and again two months later. The grass was clipped once, at the end of the growing season and again in June of the following year. The centre 18 ft of the middle two rows of each treatment was clipped and weighed.

The second half of the seeds was sent to Cardston, Alberta where they were sown in the manner described on page 47. The plants were cut at the end of the growing season and the weight of the clippings noted.

III Environmental Effects on Plant Growth with Specific Reference to Electromagnetic Fields

A) Treatment of Seeds

40 Marquis wheat grains were treated in electromagnetic fields of either 220, 1750, 3500, 7000 or 14,000 gauss in both an NMR and an ESR field generator. The field of force of the generator was in a north-south direction. Seeds were aligned so their embryos pointed in four different directions in relation to the applied field. Two series were aligned parallel to the field of force; i.e., their embryos pointed either north (N) or south (S). Two series were aligned perpendicular to the field of force, so their embryos pointed either east (E) or west (W). Control series were aligned with reference to the earth's magnetic field. Seeds were placed on a tape and held in position in a clear plastic container and exposed to the magnetic field for 7, 18, 24 or 47 hr. Immediately prior to positioning in the magnetic field, excess glass distilled water was added to the seeds.

B) Growth of Plants

Control and sound-treated seeds were separately planted in a mixture of soil and vermiculite in 23 x 28 cm flats. Seedlings were separately transplanted into 6 in. plastic pots containing loan soil when they were approximately 20 cm high.

The pots were then placed in an open field and watered daily until grain was produced. Weights of the heads and number of grains per plant were noted when the grain was mature.

IV Replication of Data and Statistical Analysis

When germination and growth experiments were carried out in refrigerators and growth chambers, the sound-treatment and control chambers were systematically interchanged with each replicate to ensure that the observations recorded could not be attributed to any variation between chambers.

Sound-treatments of seeds used in the field trials were designated A,B,C,.. The growers were not told which symbol represented sound treatment and which represented control treatment, in order to eliminate any bias on their part.

Data, comparing three or more means, were subjected to an analysis of variance, followed by a Scheffé's test of multiple means. Data, comparing only two means, were analyzed with a student's t-test. A 5% level of significance was chosen for all analyses. Data obtained from seed subjected to electromagnetism was processed by an APL / 360 computer using an ANOVA program. Sample size, for seeds or plants was usually determined by the coefficient of variation for any treatment.

RESULTS

I Standardization of Growth Conditions for Laboratory Studies

A) Measurements within the chamber

The temperatures and the location of each thermocouple, averaged over ten twenty-four hour periods are presented in Fig. 2. Maximum variation between points within the chamber was 2.5C°. The average temperature outside the chamber was 28.0°C.

At the bottom of the chamber, the two most extreme temperatures were on the left hand side, the side away from the fans. The presence of plants had little effect on the temperatures of this region.

Temperature of the empty chamber was lower on the left-hand side than at the middle or on the right hand side on both the shelf, and at the top, near the lights.

The average leaf temperature ranged from 23.1°C to 25.9°C, but there appeared to be no pattern of distribution dependent on the position of the plants in the chamber. The temperature range of an individual leaf was quite high since the leaf temperature dropped between 1.0 and 4.2C° when the plants were watered.

Room temperature fluctuated between 23.9°C and 30.4°C. Growth chamber temperature was negatively correlated with room temperature (Fig. 3). The calculated correlation coefficient

Top

23.7 (24.5)	24.6 (25.0)	24.5 (25.6)
23.8 (25.2)	24.4 (24.5)	25.2 (25.6)

Fan

Shelf

24.1 (24.1) [25.9]	25.2 (24.1) [24.8]	24.8 (24.7) [23.5]
24.2 (24.6)	24.5 (23.9) [23.1]	24.9 (24.5)
24.0 (24.3) [24.9]	24.2 (24.2) [24.7]	25.0 (24.5) [24.9]

Bottom

25.0 (25.3)	24.5 (24.5)	24.2 (24.4)
23.5 (23.1)	24.0 (23.9)	24.3 (24.4)

Front (Door)

T Empty Chamber Temperature

(T) Filled Chamber Temperature

[T] Leaf Temperature

Fig. 2. Mean temperature(°C)over a 24 hr period (s.d. ± 1.3)

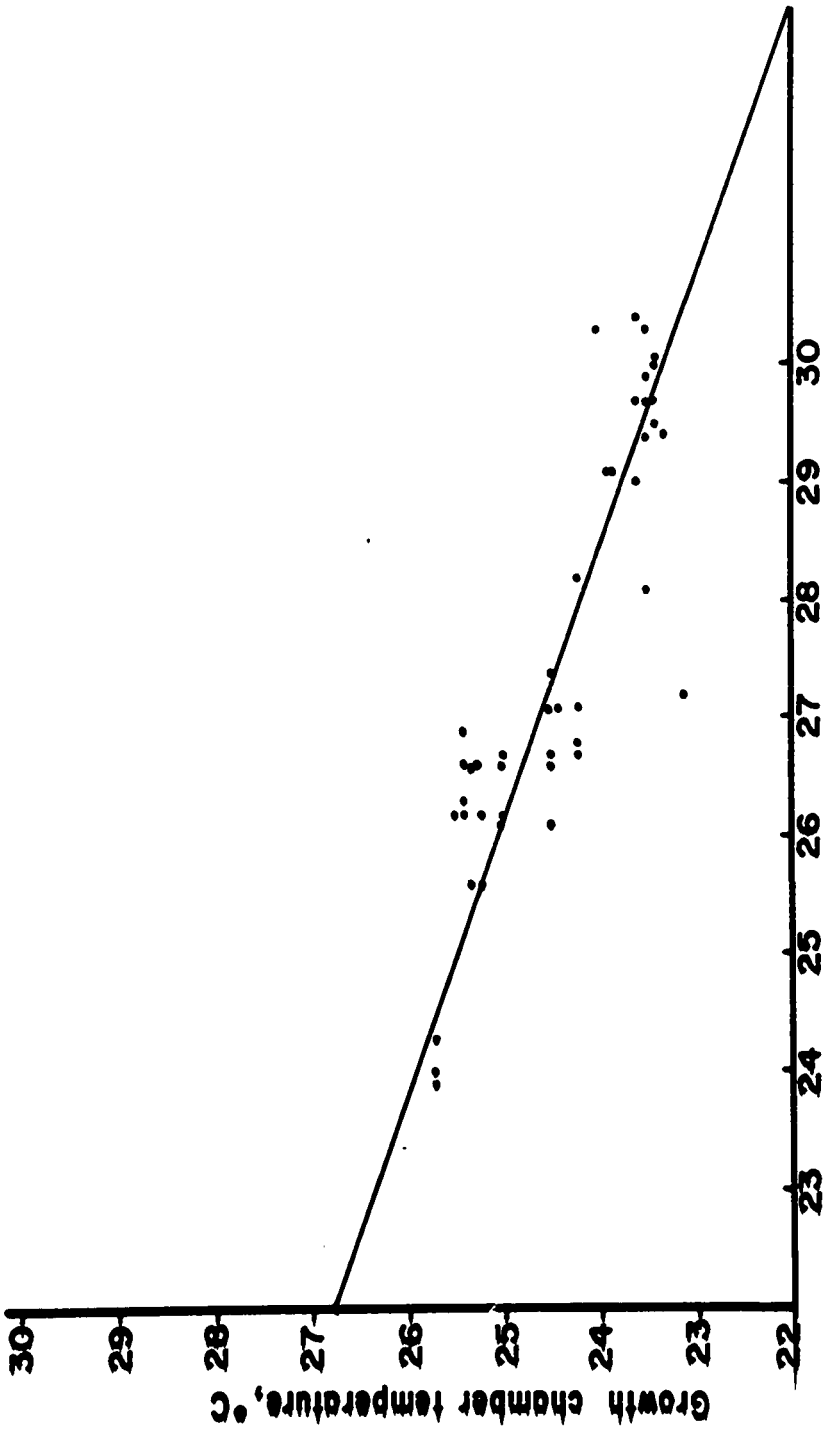


Fig. 3. Correlation of growth chamber temperature with room temperature (r = -0.89)

of r = -0.89 was highly significant. Thus as the room temperature increased, the temperature in the growth chamber decreased.

Fluctuations of the light intensity in the growth chamber are illustrated in Fig. 4. Measurements were taken at regularly spaced intervals on the shelf. The values shown in brackets were noted two months after the original measurements were taken.

Light intensity was highest in the centre of the chamber, decreasing toward the sides and toward the front and back. The rate of decrease was greater toward the front of the chamber than toward the back. Hence the light intensity was higher at the back than at the front.

Light intensity in the empty chamber was reduced by an average of 200 ft-c over a two month period.

B) Growth of Plants as Affected by Positions within the Chamber

The positioning of plants in the growth chamber appears to affect their subsequent growth. This is illustrated in Figs. 5-11 for oats, corn and cucumber. Not all plants were equally affected. In the case of oats, plant position in the growth chamber had very little effect on overall plant growth. There was only a 6% increase in the height of plants at the back, when compared with that of the plants in the front. Differences between dry weights were also very small. Row 1 represents the plants at the front of the chamber, whereas row 10 represents the plants at the back of the chamber.

730 (570)	840 (600)	900 (660)	850 (610)	750 (580)
770 (570)	855 (680)	940 (720)	870 (660)	790 (580)
710 (520)	790 (600)	870 (680)	795 (610)	730 (550)

Front

Fig. 4. Light intensities (ft-c) at regularly spaced intervals on shelf in growth cabinet. Bracketed values represent light intensities after a two months interval.

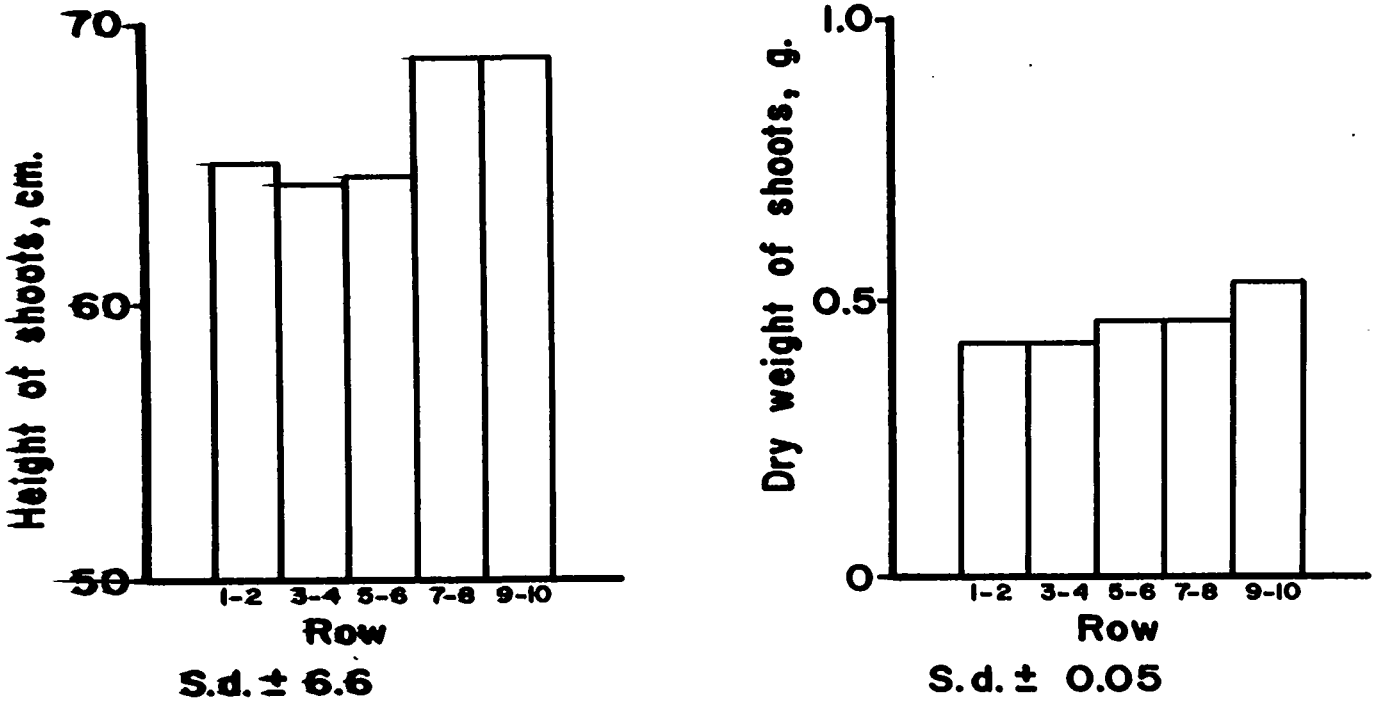
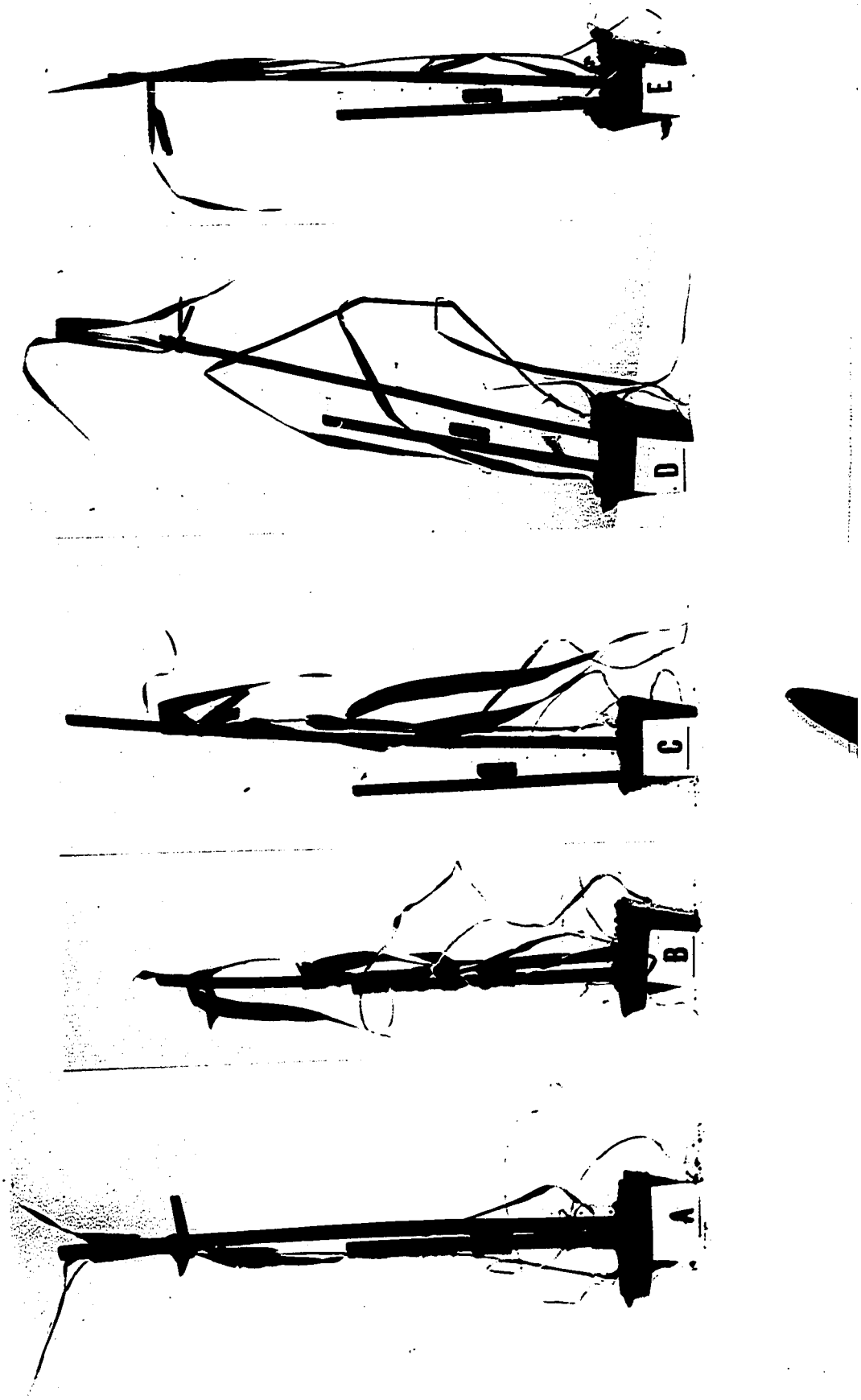


Fig. 5. Oats: Effect of position in growth chamber on height and dry weight after seven weeks growth.

Fig. 6, A-E: Effect of position in growth chamber on growth of oats. (A) Rows 1-2. (B) Rows 3-4. (C) Rows 5-6. (D) Rows 7-8. (E) Rows 9-10.


E
D
C
B
A

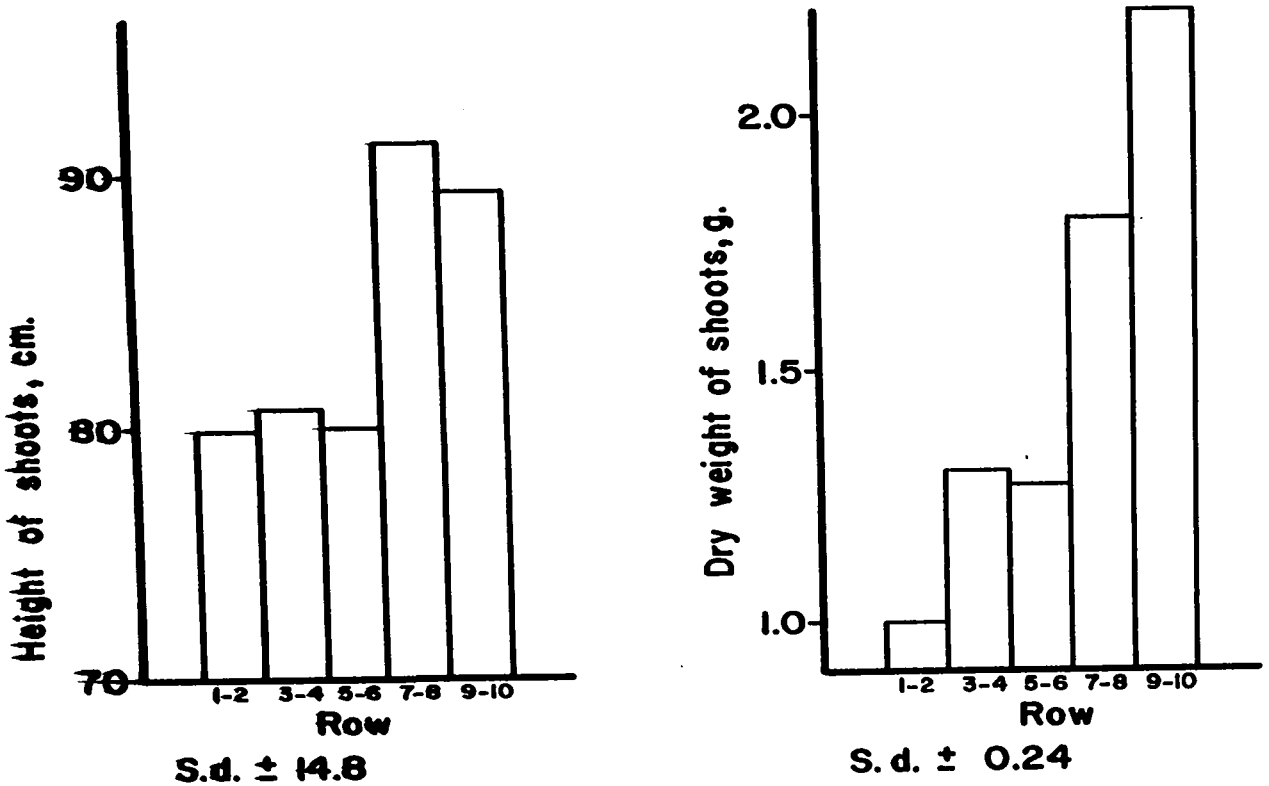
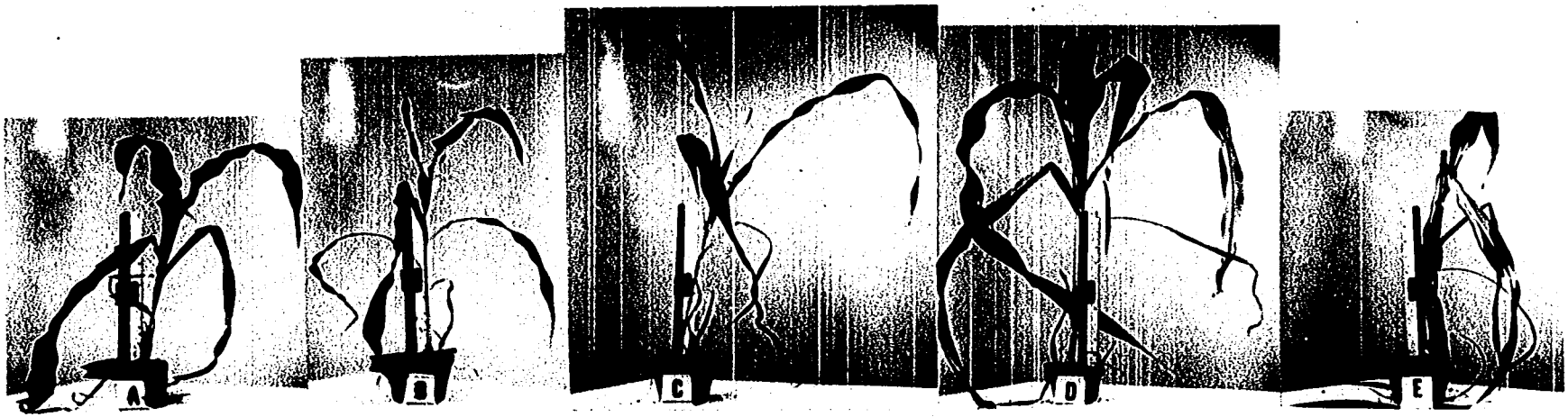


Fig. 7. Corn: Effect of position in growth chamber on height and dry weight after seven weeks growth.

Fig. 8, A-E: Effect of position in growth chamber on growth of corn. (A) Rows 1-2. (B) Rows 3-4. (C) Rows 5-6. (D) Rows 7-8. (E) Rows 9-10.


A
B
C
D
E

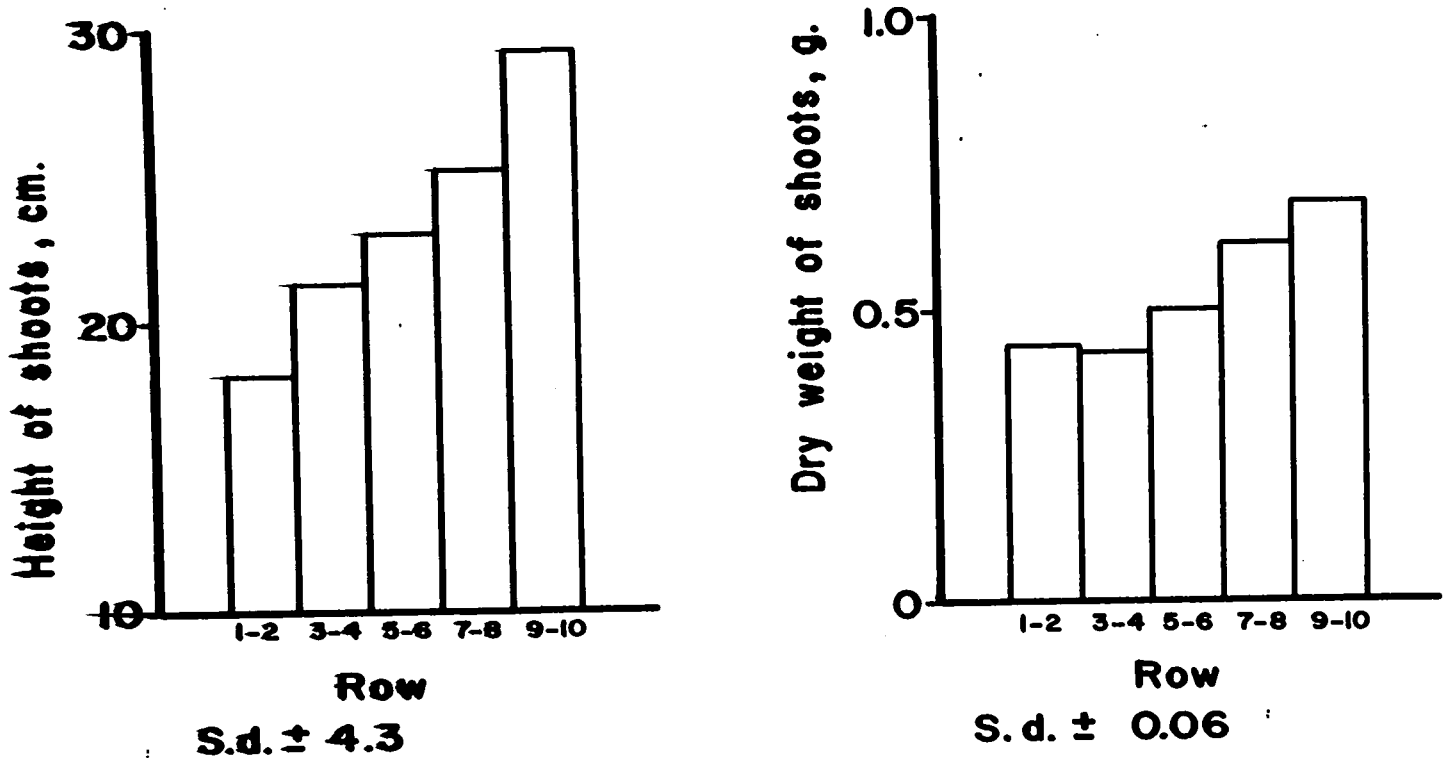


Fig. 9. Cucumber : Effect of position in growth chamber on height and dry weight after seven weeks growth.

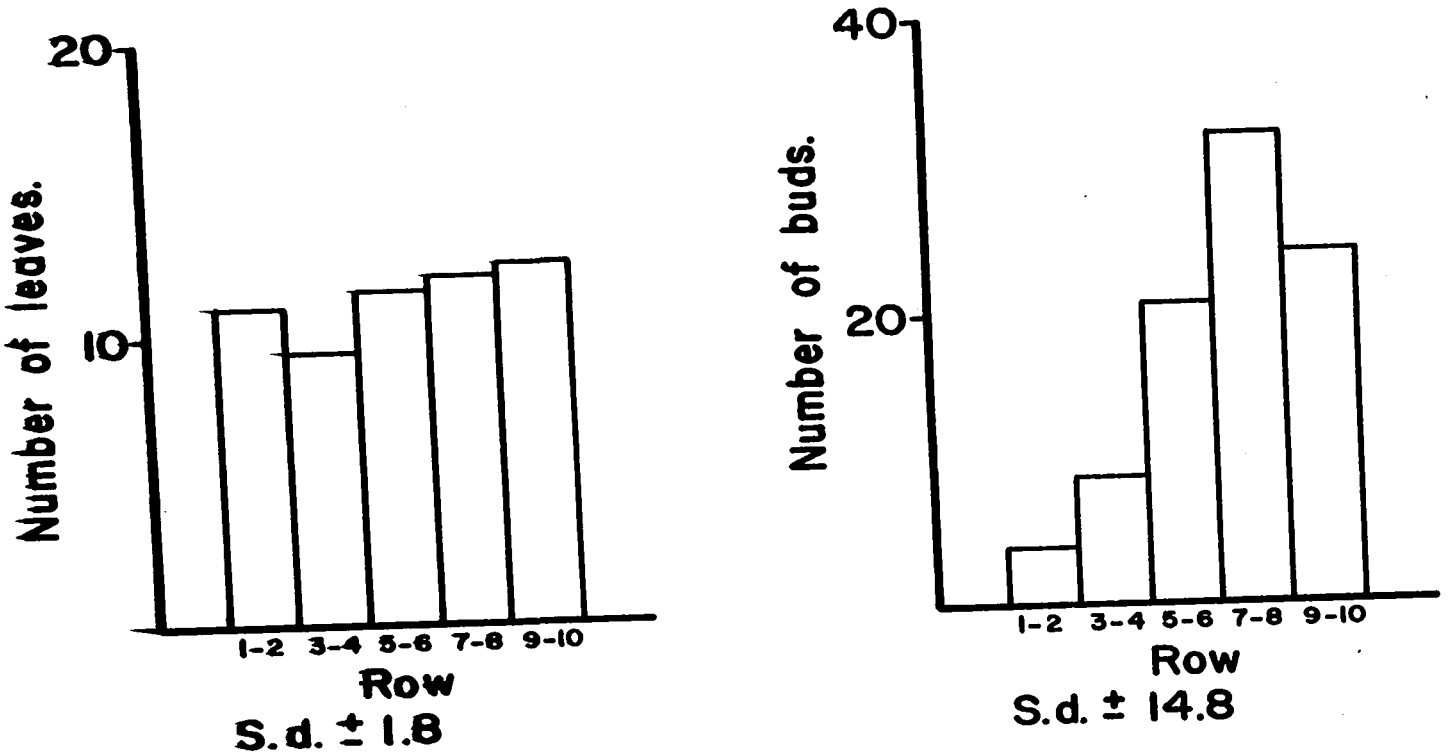
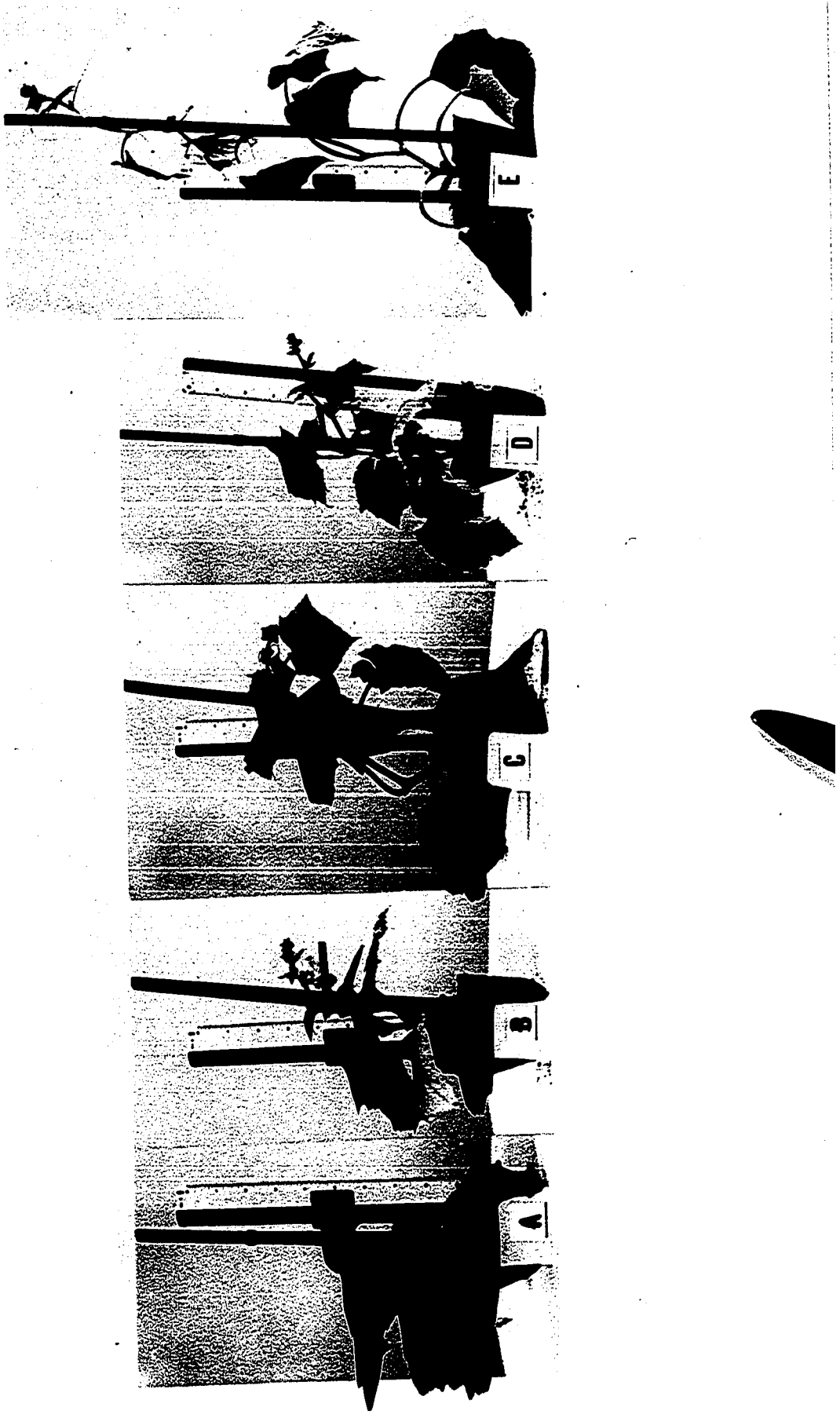


Fig. 10. Cucumber : Effect of position in growth chamber on numbers of leaves and buds after seven weeks growth.

Fig. 11, A-E: Effect of position in growth chamber on growth of cucumbers. (A) Rows 1-2. (B) Rows 3-4. (C) Rows 5-6. (D) Rows 7-8. (E) Rows 9-10.


E
D
C
B
A

The height and dry weight of corn, however, was very much affected by position within the chamber. Plants in the back two rows were 10 cm taller than plants in the front six rows. The dry weight of plants in rows 3 to 6 was 30% larger than that of plants in the first two rows. Dry weights of plants in rows 7 - 8 and 9 - 10 were 80% and 120%, respectively, larger than those of plants in the first two rows.

The height and dry weight of cucumber plants increased with change in position from front to back of the growth chamber. Height of plants in rows 9 and 10 was 50% larger that of plants in rows 1 and 2. Variation in the numbers of leaves was small but the number of buds changed drastically with the position of the plants in the chamber. The number increased with changes in position from the front of the chamber to rows 7 and 8 and then decreased again in rows 9 and 10. The number of buds per plant in rows 7 and 8 was eight times the number per plant in rows 1 and 2.

The effect of positioning in the growth chamber on the growth of Rideau wheat seedlings are shown in Figs. 12-14.

The average height per row was highest at the front of the chamber, decreasing toward the back (Fig. 12). In left-right rows, the average height was higher in the centre tray than in the two side trays, but was lower in the centre of each tray than at the edges. This is further illustrated in Fig. 13.

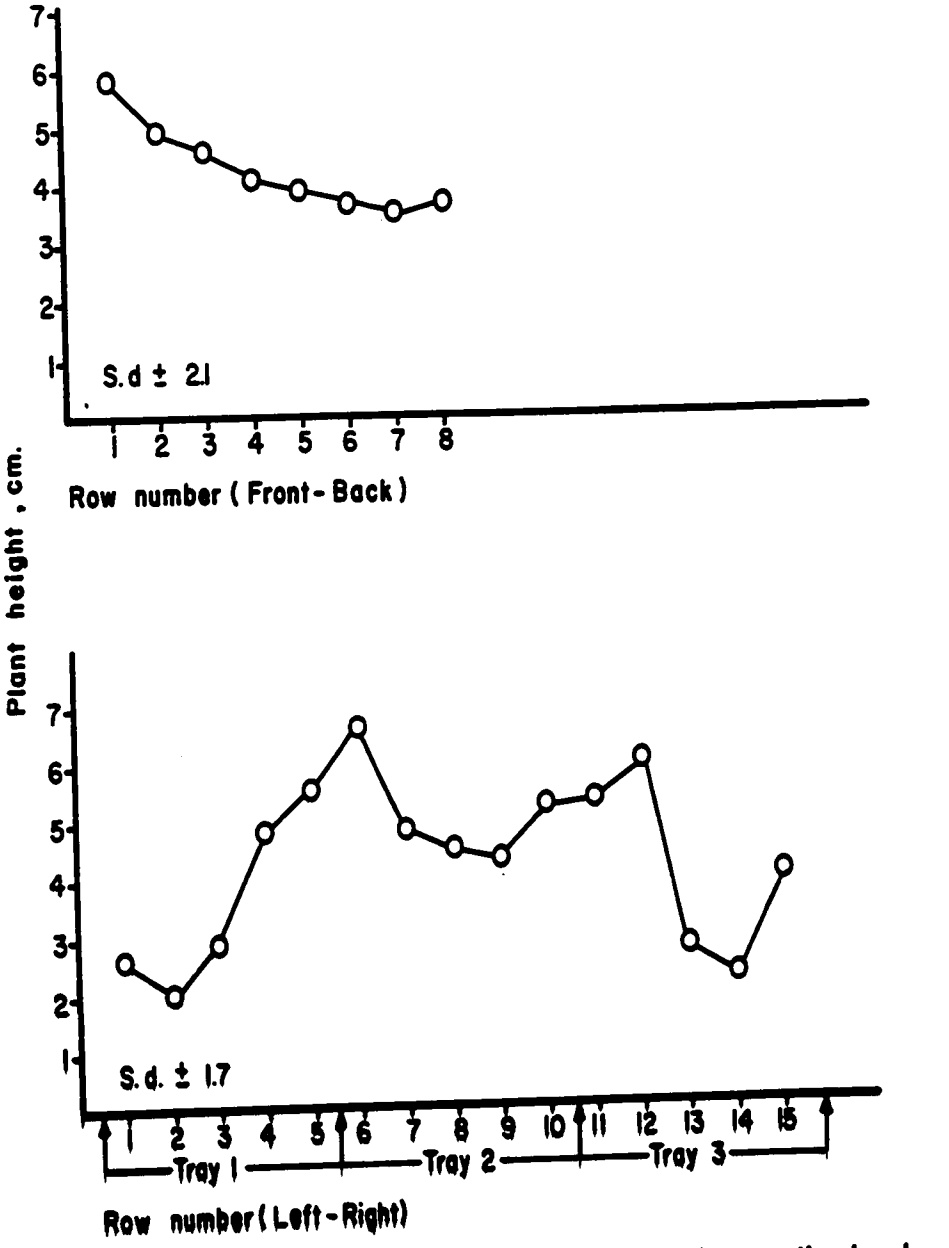


Fig. 12. Rideau wheat seedlings: Effect of position in growth chamber on average height per row after one week of growth.

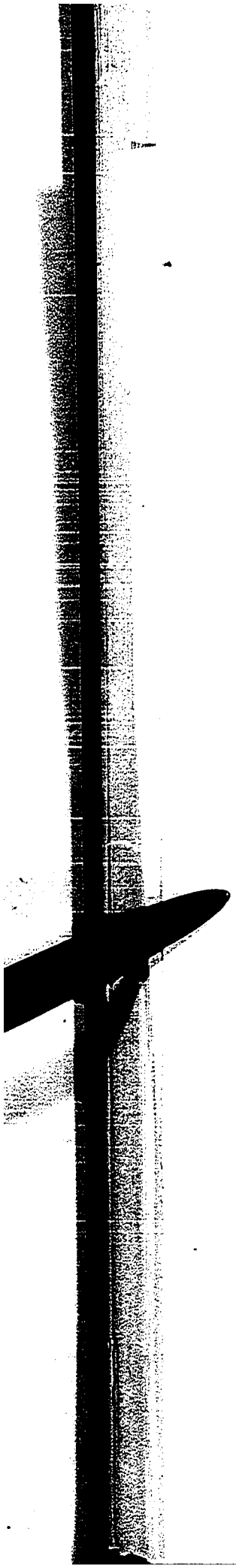


Fig. 13. Effect of position in growth chamber on growth of Rideau wheat seedlings. (View from front of chamber as illustrated in Fig. 14).

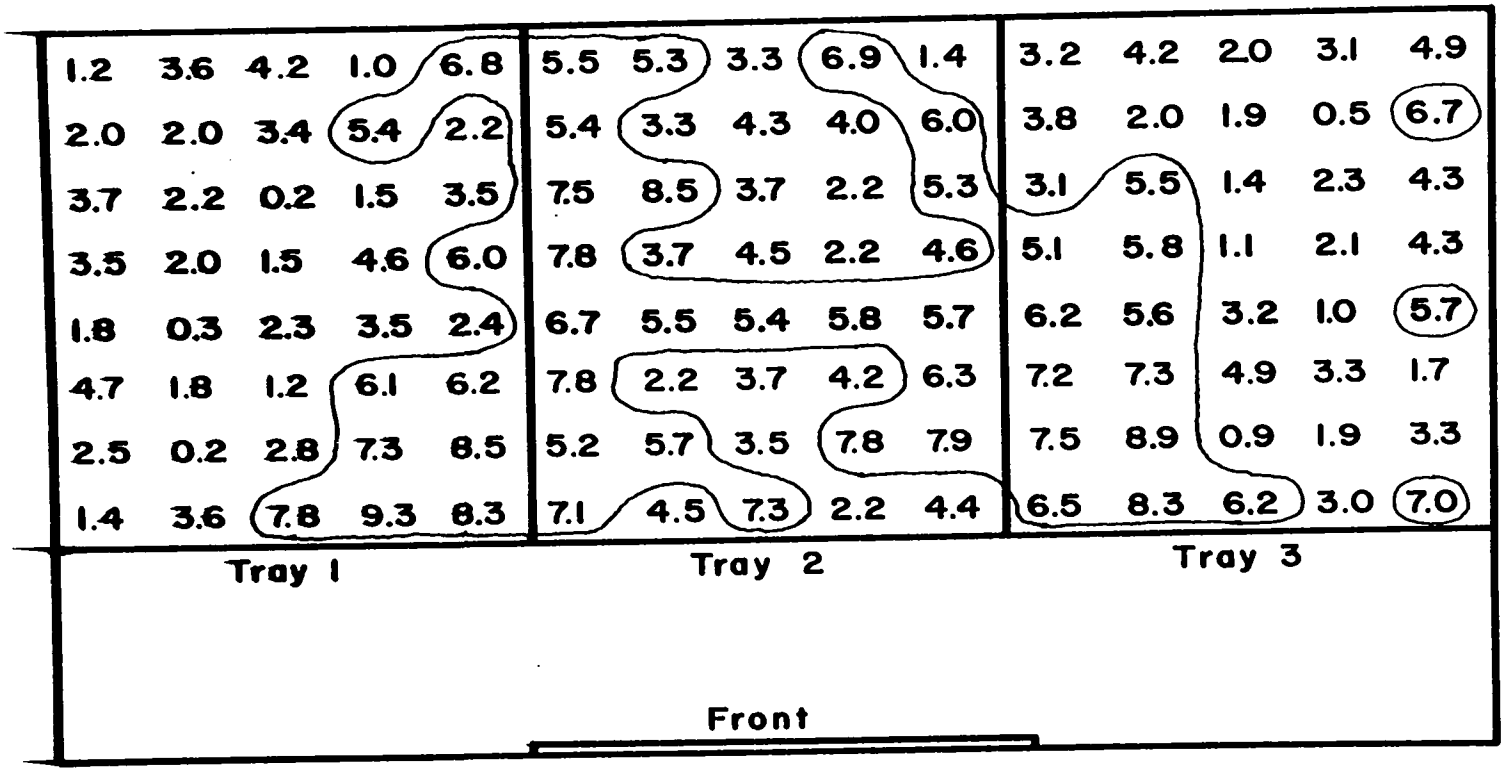


Fig. 14. Effects of position in growth chamber on height of Rideau wheat seedlings, cm.

The plant height at each specific location within the chamber is shown in Fig. 14. An isometric line has been drawn separating plants with a height of 5.0 cm or more from smaller plants. This value was chosen since the heights of nearly all the plants lie in the range of 1.0 - 9.0 cm. Five cm is the mid-point of this range. The isometric line shows that the majority of plants grow larger at the right edge of tray 1, the two edges of tray 2, and the left edge of tray 3.

C) Attempts to Overcome Positioning Effects of Plants within Growth Chamber

Both back-to-front and left-to-right positioning within the growth chamber appear to affect the growth of plants. Therefore in all subsequent growth and development studies, plants were systematically rotated twice weekly within the chambers. Plants for each treatment were placed in back-to-front rows and both the plants within each row and the rows within the chamber were rotated. In addition, replicates were grown in separate chambers.

II Environmental Effects on Plant Growth, with Specific Reference to Audible Sound

A) Laboratory Studies

i) Germination as Affected by Temperature and Audible Sound

a) Imbibition

The rates of uptake of water by Rideau wheat grains maintained at 2°C and at 25°C are illustrated in Figs. 15 and 16. 5KHz sound appears to have no significant effect on this initial stage of germination at either temperature.

b) Protrusion of Radicle

The effect of sound exposure on the rate of germination of each species is illustrated in Figs. 17-20. Standard deviations are included in order to indicate where divergence between curves is significant.

Germination of barley was unaffected by exposure to 5KHz audible sound at 2°C, whereas that of Manitou wheat was inhibited (Fig. 17). After 32 days, only 42% of the sound-exposed grains had germinated compared with 84% of the control grains. Neither Genesee nor Talbot wheat germinated well at 2°C. After thirty days, less than 40% of both control and sound-exposed grains had germinated. The rest appeared to be rotting.

When Rideau wheat grains were maintained at 2°C for three days, a significantly larger number of the grains exposed to 5KHz sound had germinated when compared with controls or with those exposed to 12KHz sound (Fig. 18). By the fourth day, 73% of the 5K-exposed grains had germinated, compared with 26% of both

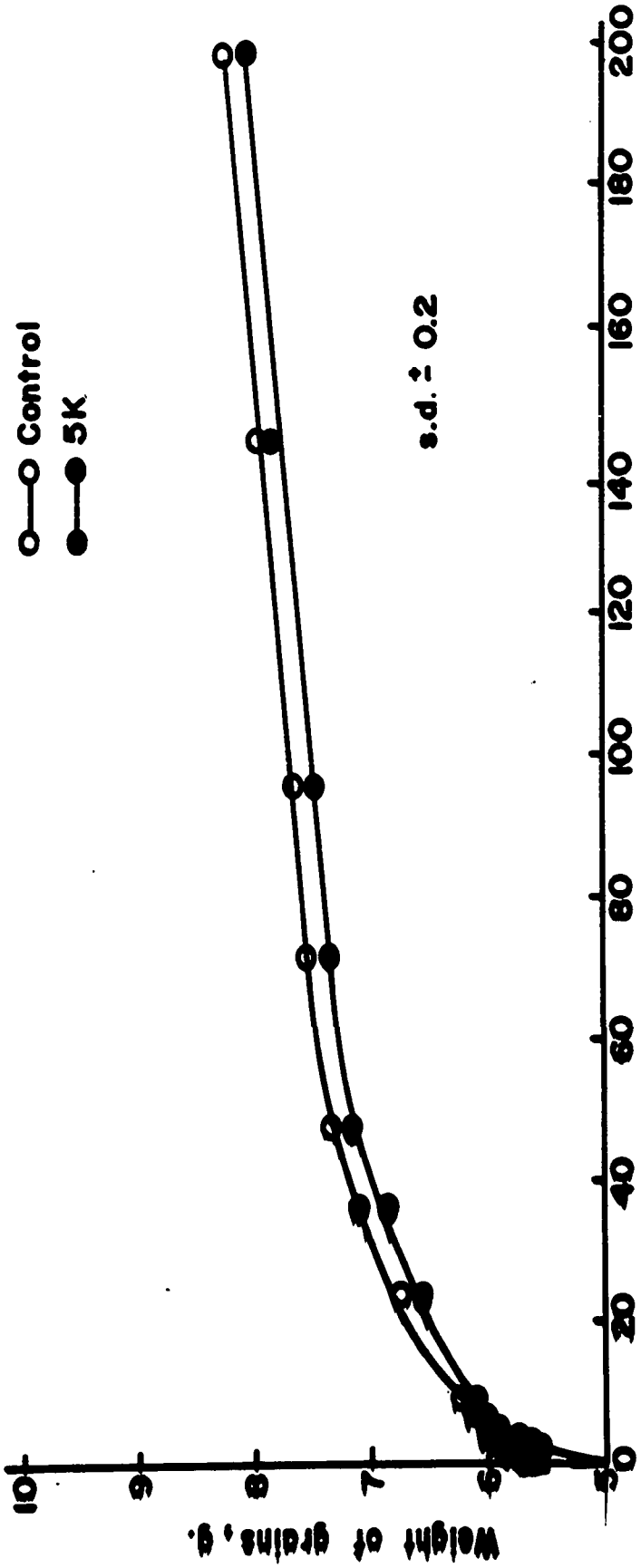


Fig. 15. Uptake of water by Rideau wheat grains at 2°C

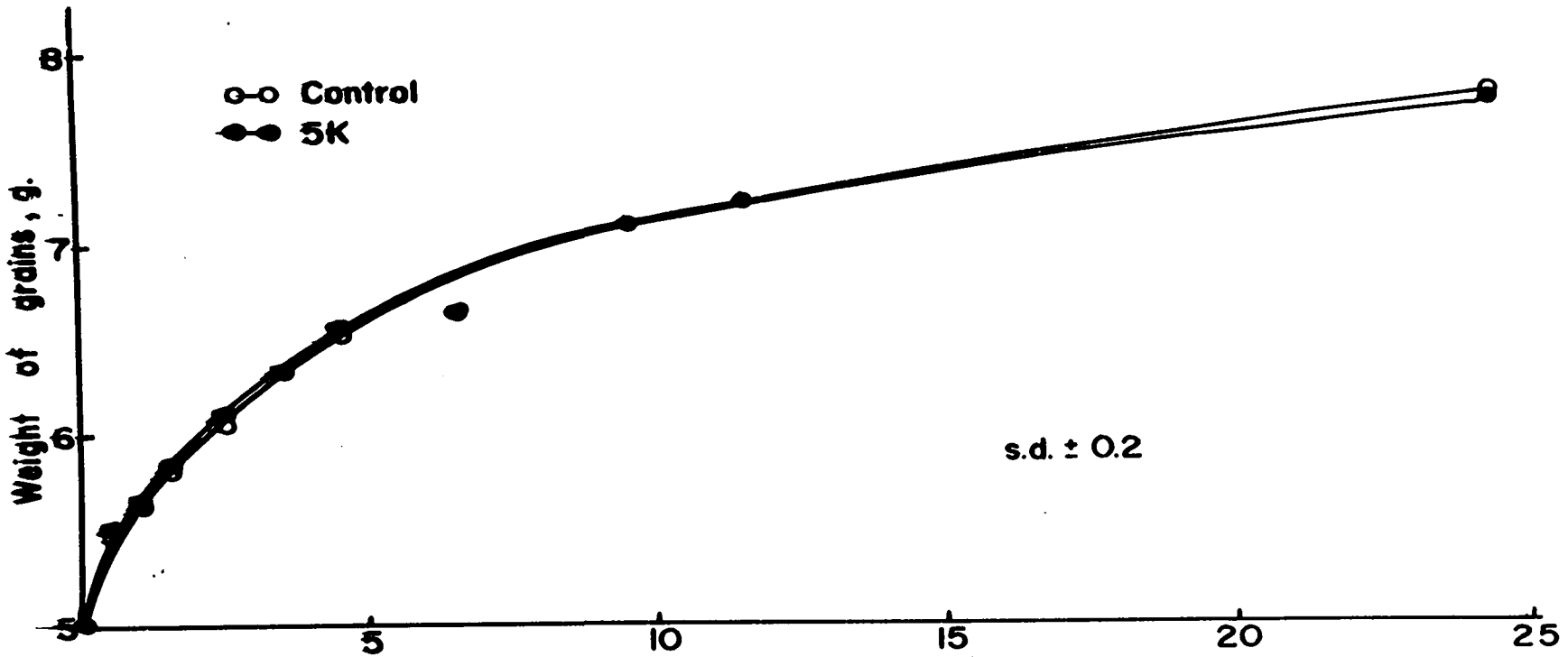


Fig. 16. Uptake of water by Rideau wheat grains at 25°C.

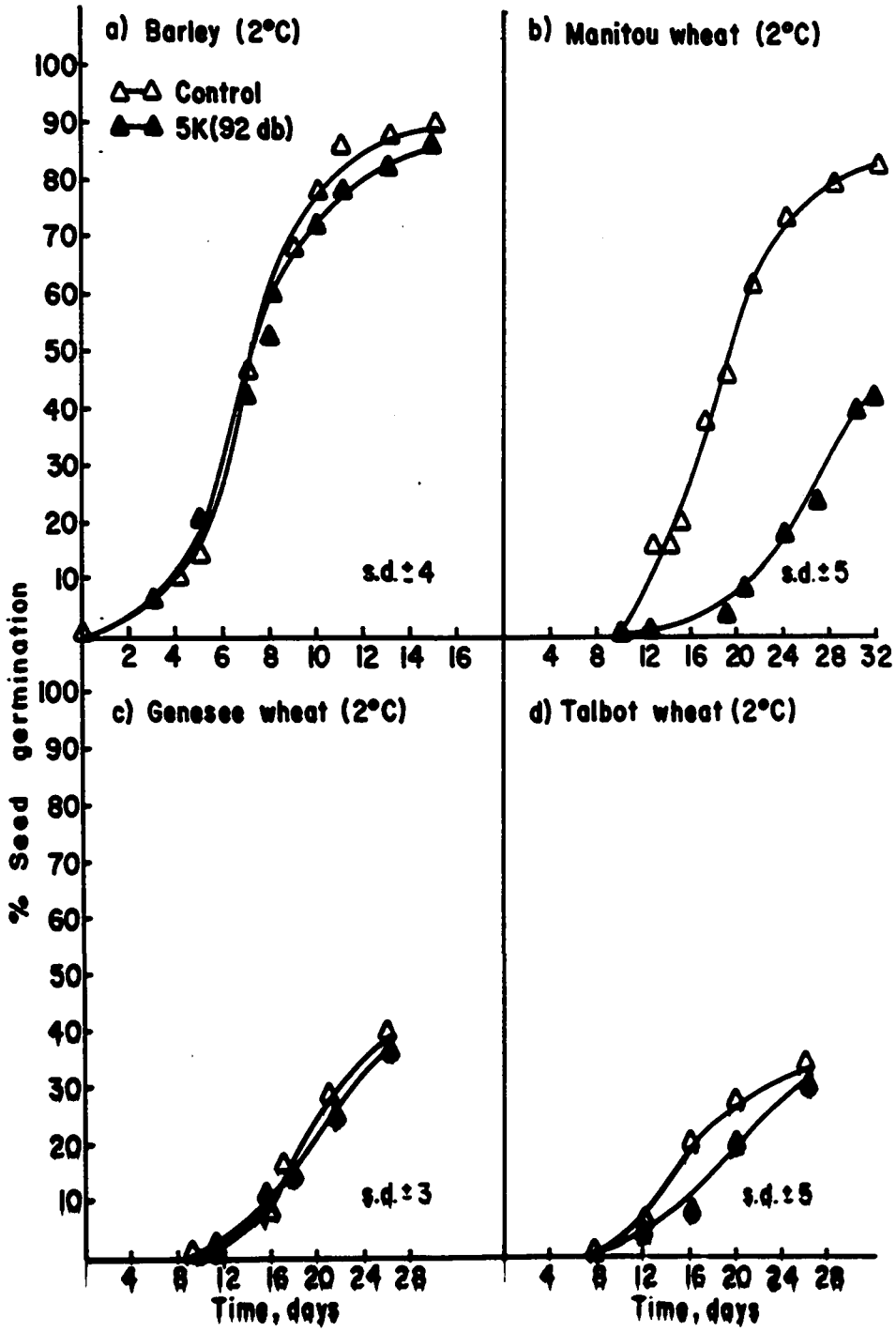


Fig. 17. Effect of sound exposure on rate of germination.

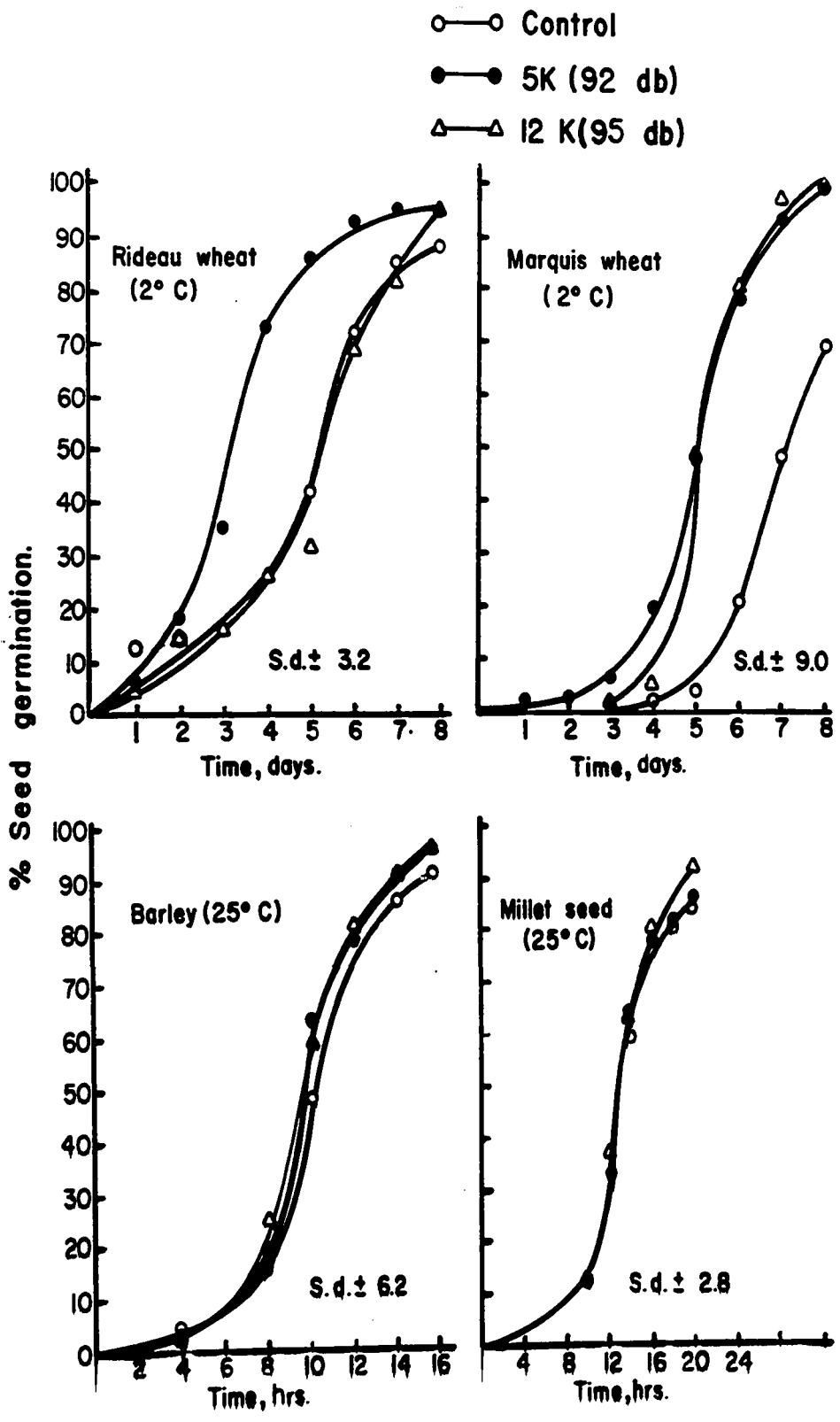


Fig. 18. Effect of sound exposure on rate of germination.

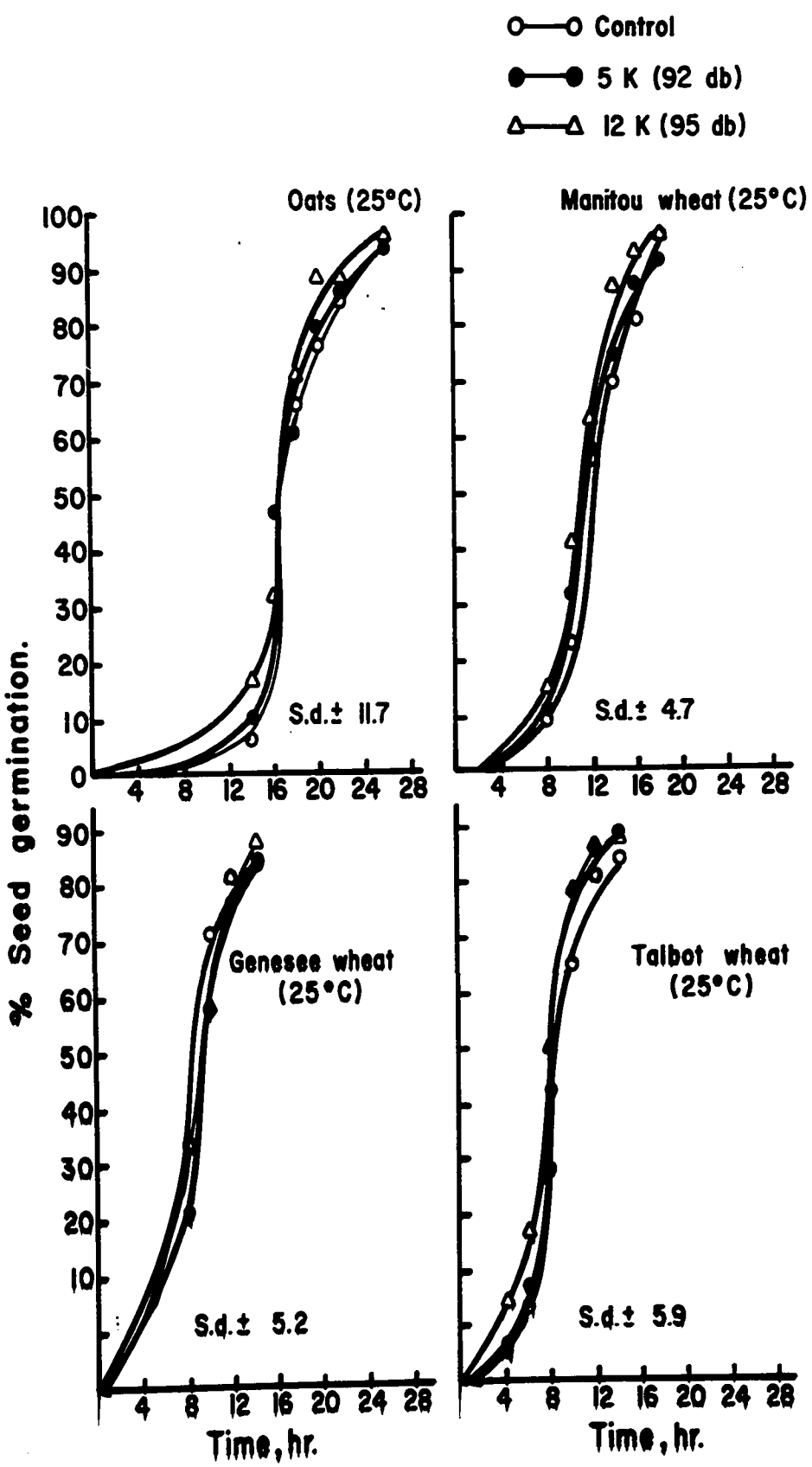


Fig.19. Effect of sound exposure on rate of germination.

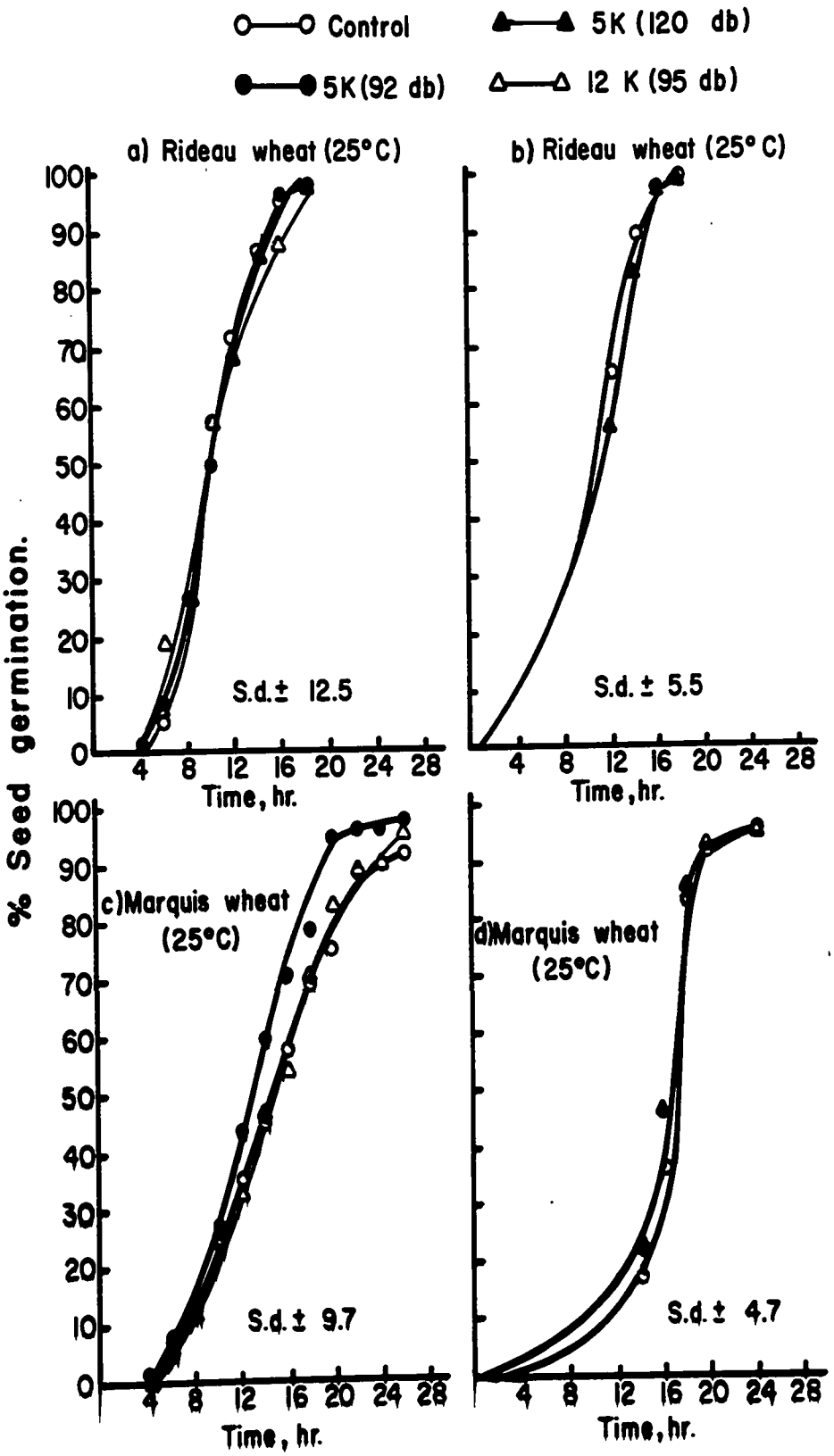


Fig.20. Effect of sound exposure on rate of germination.

the control and 12K-exposed grains. The divergence between curves decreased with time until the eighth day when approximately 90% of all grains had germinated.

5K-exposed Marquis wheat grains, maintained at 2°C, also began to germinate earlier than either 12K-exposed grains or control grains. By the fifth day, the 12K series no longer lagged behind the 5K series. By the eighth day, 100% of the grains of both sound-treated series had germinated, in contrast to only 70% of the controls.

Sound exposure at 25°C, had no effect on the germination rates of Rideau, Marquis and Manitou wheats, oats, barley and millet seed (Figs. 18-20). Germination of Genesee wheat was slightly inhibited after eight hours, but differences were no longer significant after twelve hours sound exposure. Germination of Talbot wheat was slightly accelerated by both 5KHz and 12KHz sound treatments following ten hours of sound exposure.

The germination rates of both Marquis and Rideau wheats were unaffected by exposure to 5KHz sound at an intensity of 120 db at 25°C. However, a comparison of the control curves of Marquis wheat observed in Figs. 20c and 20d, shows the effect of storage on the germination rates of this wheat. Grains harvested in 1967 were used for both studies. The data represented by Fig. 20c were compiled in the autumn of 1967, whereas that represented by Fig. 20d were compiled in the spring of 1969. In the 1967 experiment 45% of the control grain had germinated after 14 hours and 58%, after 16 hours.

In the experiment carried out approximately 18 months later, only 17% of the grains had germinated after 14 hours and only 36% after 16 hours. The grain was at least 95% viable in both studies. An 18 month storage period does not appear to affect the germination rates of Rideau wheat (Fig. 20 a,b).

ii) Growth of Seedlings as Affected by Temperature, Available Moisture and Audible Sound

Sound exposure during early stages of growth of Rideau wheat appeared to have no effect on the number of roots. Approximately 90% of all seedlings had three roots during the second, third and fourth weeks of growth. The remaining 10% had either one or two roots. The average totalled root length of the three roots per seedling is illustrated in Fig. 21.

Average root length was unaffected by either sound exposure or length of the imbibition period within each water regime but was doubled when grains were imbibed with a quantity of water equivalent to 100%, rather than 60% of their dry weight.

iii) Growth of Plants

A) Response to Audible Sound

The effects of sound exposure on the growth of Marquis and Rideau wheats are shown in Tables I - VI.

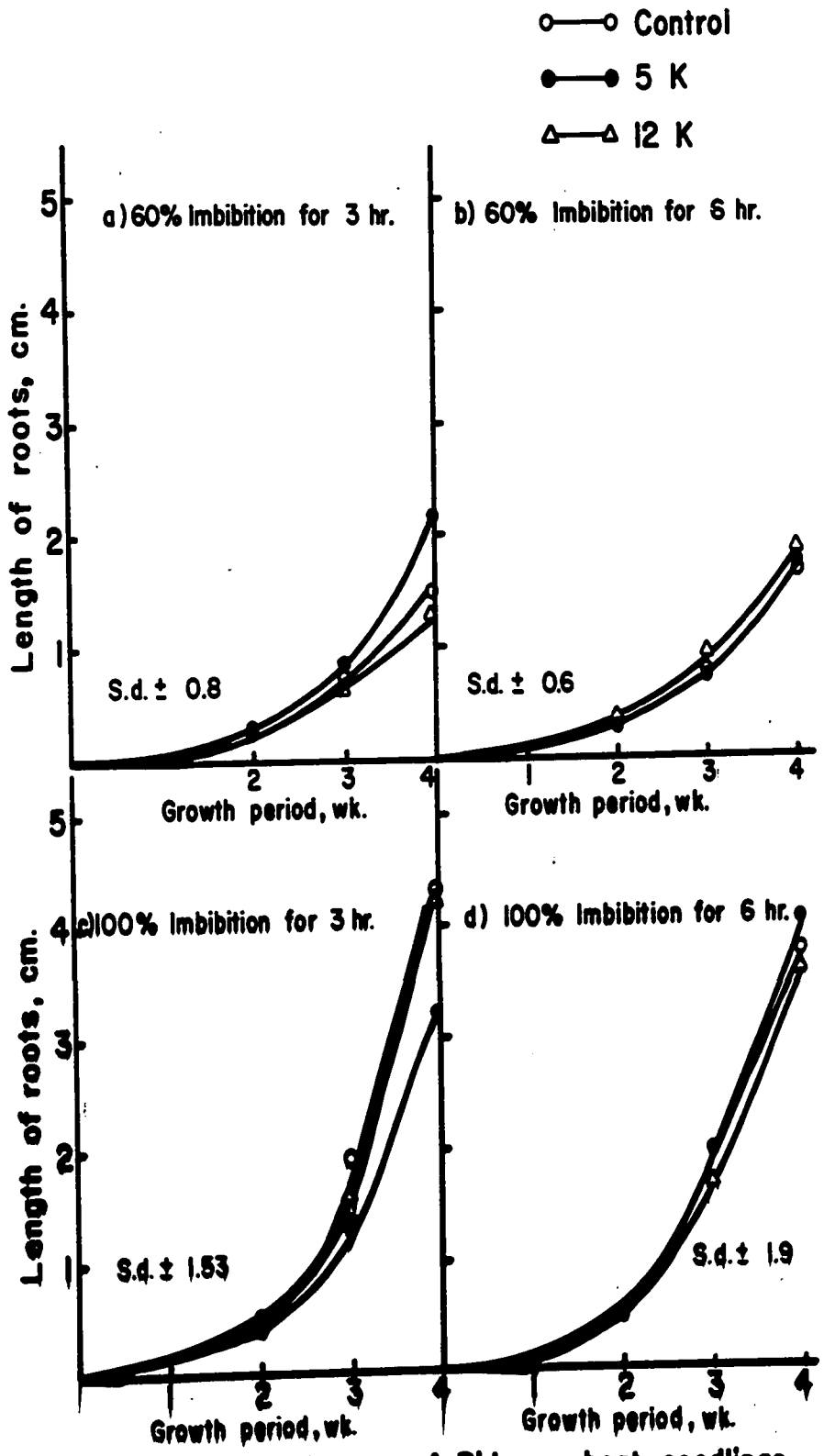


Fig.21. Total length of roots of Rideau wheat seedlings grown at 2°C.

Table I. Marquis wheat after 4 weeks growth (Scheffé's test at the 5% level)

Height cm						
12K,12K*	C,5K	5K,C	5K,5K	12K,C	C,C	C,12K
48.8	46.9	46.0	45.2	42.1	41.8	41.6
Number of roots						
5K,5K	12K,12K	C,5K	5K,C	C,C	12K,C	C,12K
28	27	25	24	23	20	16
Fresh wt. of roots, g						
12K,12K	C,5K	5K,5K	5K,C	C,C	12K,C	C,12K
2.88	2.85	2.70	2.62	2.45	2.28	1.24
Dry wt. of roots, g						
12K,12K	5K,5K	C,5K	C,C	12K,C	5K,C	C,12K
0.21	0.18	0.18	0.17	0.15	0.15	0.08
Fresh wt. shoots, g						
12K,12K	5K,5K	C,5K	5K,C	12K,C	C,C	C,12K
6.49	5.61	5.60	4.96	4.43	3.90	3.48
Dry wt. shoots, g						
12K,12K	5K,5K	C,5K	5K,C	12K,C	C,C	C,12K
0.76	0.68	0.65	0.59	0.54	0.49	0.41

*Treatment.

Table II . Marquis wheat after 6 weeks growth (Scheffé's test at the 5% level)

Height, cm						
5K,5K*	C,5K	5K,C	C,C	12K,12K	12K,C	C,12K
60.1	56.8	55.3	51.6	50.3	47.0	46.1
Number of roots						
5K,5K	C,C	C,5K	5K,C	C,12K	12K,12K	12K,C
63	50	49	48	48	47	45
Fresh wt. roots, g						
5K,5K	C,C	5K,C	C,5K	C,12K	12K,12K	12K,C
5.20	4.83	4.53	3.91	3.89	3.72	3.62
Dry wt. roots, g						
5K,5K	C,C	5K,C	C,5K	C,12K	12K,C	12K,12K
0.48	0.42	0.34	0.33	0.32	0.29	0.27
Fresh wt. shoots, g						
5K,5K	C,C	5K,C	C,5K	C,12K	12K,12K	12K,C
15.75	13.31	13.17	10.05	8.42	8.42	7.75
Dry wt. shoots, g						
5K,5K	C,C	5K,C	C,5K	12K,12K	C,12K	12K,C
2.35	1.79	1.56	1.50	1.25	1.22	1.08

*Treatment.

Table III. Marquis wheat after 8 weeks growth (Scheffé's test at the 5% level)

Height, cm						
5K,5K*	C,12K	5K,C	C,5K	C,C	12K,12K	12K,C
72.8	70.8	67.7	67.1	62.7	62.0	61.3
Number of roots						
5K,C	12K,C	5K,5K	C,C	12K,12K	C,12K	C,5K
106	101	99	90	89	85	85
Fresh wt. roots, g						
12K,C	5K,C	5K,5K	C,C	C,5K	12K,12K	C,12K
14.43	12.67	10.21	8.60	8.04	7.79	7.50
Dry wt. roots, g						
5K,C	12K,C	5K,5K	C,C	C,5K	12K,12K	C,12K
1.27	1.21	1.07	0.98	0.84	0.76	0.57
Fresh wt. shoots, g						
5K,5K	5K,C	12K,C	C,5K	C,C	C,12K	12K,12K
31.55	30.08	29.36	25.73	25.18	23.13	18.68
Dry wt. shoots, g						
5K,5K	5K,C	12K,C	C,12K	C,5K	C,C	12K,12K
5.90	5.30	4.90	4.37	4.13	4.03	3.45

*Treatment.

Table IV. Rideau wheat after 4 weeks growth (Scheffé's test at the 5% level)

Height, cm

5K,5K*	5K,C	12K,12K	C,5K	12K,C	C,C
42.2	4.00	39.7	34.6	33.1	30.2

Number of roots

5K,5K	5K,C	12K,C	12K,12K	C,5K	C,C
16	15	15	13	12	11

Fresh wt. roots, g

5K,C	5K,5K	12K,C	12K,12K	C,5K	C,C
1.40	1.27	1.05	0.88	0.69	0.44

Dry wt. roots, g

5K,C	5K,5K	12K,12K	12K,C	C,5K	C,C
0.10	0.08	0.07	0.07	0.04	0.03

Fresh wt. shoots, g

5K,5K	5K,C	12K,12K	12K,C	C,5K	C,C
3.17	2.80	1.67	1.62	1.38	0.90

Dry wt. shoots, g

5K,5K	5K,C	12K,12K	12K,C	C,5K	C,C
0.41	0.36	0.22	0.22	0.16	0.13

*Treatment.

Note: C,12K data not included because of wide spread of values for all parameters.

Table V. Rideau wheat after 6 weeks growth (Scheffé's test at the 5% level)

Height, cm					
5K,5K*	5K,C	C,5K	C,C	12K,12K	C,12K
54.3	51.5	51.5	47.4	44.5	42.4
Number of roots					
5K,5K	5K,C	12K,12K	C,5K	C,12K	C,C
35	32	30	30	22	21
Fresh wt. roots, g					
5K,5K	5K,C	C,5K	5K,C	C,12K	C,C
3.90	3.32	2.94	2.54	1.69	1.44
Dry wt. roots, g					
5K,5K	5K,C	C,5K	12K,12K	C,12K	C,C
0.28	0.24	0.20	0.20	0.13	0.09
Fresh wt. shoots, g					
5K,5K	5K,C	C,5K	12K,12K	C,C	C,12K
12.43	10.71	9.12	7.28	4.90	4.01
Dry wt. shoots, g					
5K,5K	5K,C	C,5K	12K,12K	C,C	C,12K
1.51	1.32	1.07	0.92	0.64	0.60

*Treatment.

Table VI. Rideau wheat after 8 weeks growth (Scheffé's test at the 5% level)

Height, cm

C,5K*	5K,5K	5K,C	C,C	C,12K	12K,12K	12K,C
61.0	60.3	57.2	57.1	55.4	55.1	55.0

Number of roots

5K,5K	12K,12K	5K,C	C,5K	C,12K	12K,C	C,C
71	68	67	55	54	53	36

Fresh wt. roots, g

C,12K	5K,5K	C,5K	12K,12K	5K,C	12K,C	C,C
9.64	8.31	7.26	6.76	6.60	6.26	4.79

Dry wt. roots, g

C,12K	5K,5K	12K,12K	5K,C	C,5K	12K,C	C,C
0.96	0.77	0.69	0.64	0.59	0.50	0.35

Fresh wt. shoots, g

5K,5K	C,5K	5K,C	12K,12K	C,12K	12K,C	C,C
29.24	23.79	23.72	21.11	21.04	18.68	15.27

Dry wt. shoots, g

5K,5K	5K,C	12K,12K	C,12K	C,5K	12K,C	C,C
4.50	3.73	3.31	3.14	3.03	2.60	1.85

*Treatment.

a) Marquis Wheat

After four weeks growth, the number of roots and the fresh and dry weights of both the root and shoot systems of Marquis wheat were depressed in the C,12K series, when compared with controls. Only the fresh and dry weights of the tops of plants in the 12K,12K series were significantly larger than those of the controls (Table I).

Depressed growth of root and shoot systems was also evidenced after six weeks of growth during exposure to 12KHz sound. However, the height and dry weight of the tops of plants of the 5K,5K series were significantly larger than those of the control series (Table II).

After an eight week growth period, (Table III) only the fresh weight of the roots of the 12K,C series of Marquis wheat was significantly larger than controls. There is an indication that treatment with 5KHz sound may have slightly stimulated the growth of the shoots, but this is not significant at the 5% level (Fig. 22).

b) Rideau Wheat

After a four week growth period, all measured parameters of the 5K,5K and 5K,C Rideau wheat series were significantly larger than those of controls. Root growth was also stimulated in the 12K,12K and 12K,C series. The height of plants in the 12K,12K series was significantly larger than that of the controls. There was no evidence of a depressed growth response to any of the treatments (Table IV).

Fig. 22, A-B. Effect of sound exposure on relative size of Marquis wheat plants after 8 weeks growth (A) 5K,5K. (B) C,C.


A
B

After six weeks growth, all measured parameters of the 5K,5K series of Rideau wheat were still significantly larger than those of the controls (Table V).

Following eight weeks growth the 5K,5K series of Rideau plants exhibited a doubling of the number of roots, the fresh and dry weights of the roots and the fresh and dry weights of the shoots, when compared with controls (Table VI). This stimulation is further illustrated in Fig. 23. The numbers of roots of the 12K,12K and 5K,C series were also significantly larger than controls. Dry weight of the shoots of the 5K,C series was double that of the controls, but was just on the border-line of significance owing to the high degree of variability between the Rideau plants.

B) Frequency Response

a) Marquis Wheat

The response of four week chilled Marquis wheat to audible sound is shown in Table VII. After eight weeks growth, plants treated with 5KHz sound were significantly larger than controls for all parameters examined. The height, dry weight of the roots, and fresh and dry weight of the shoots of plants treated with 300Hz sound were also significantly larger than control plants. Growth parameters of plants treated with 1250Hz and 12KHz sound were not different from those of the controls.

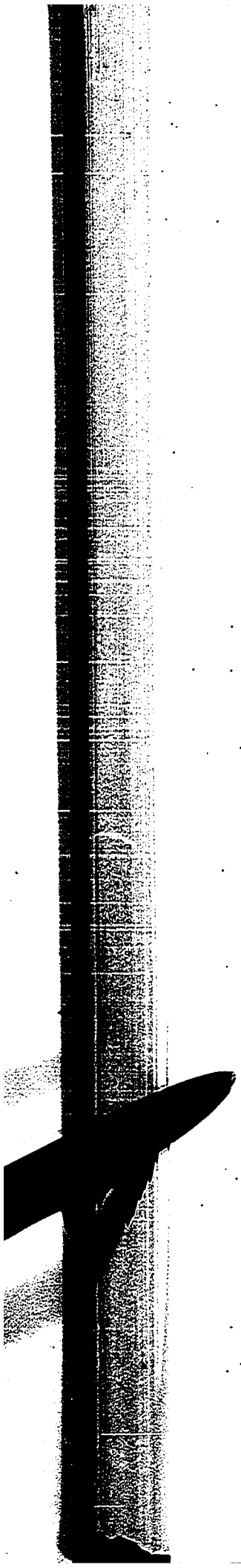


Fig. 23, A-B. Effect of sound exposure on relative size of Rideau wheat plants after 8 weeks growth. (A) 5K,5K. (B) C,C.

ize

rowth.

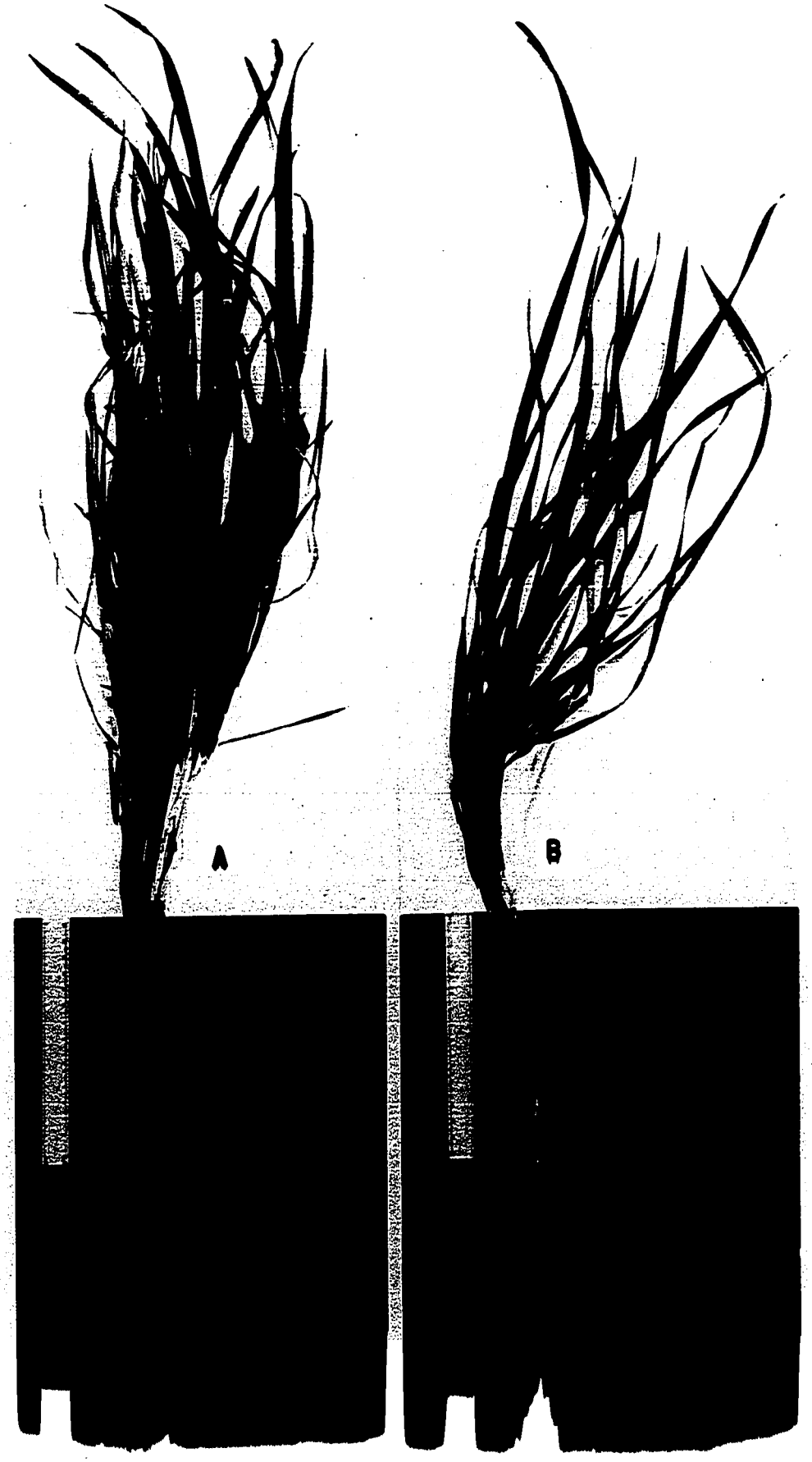


Table VII. Frequency response: Marquis wheat after 4 weeks chilling and 8 weeks growth (Scheffé's test at the 5% level)

Height, cm				
5K,C*	300,C	1250,C	12K,C	C,C
57.0	55.5	50.9	49.5	49.2
Number of roots				
5K,C	300,C	1250,C	12K,C	C,C
44	34	32	26	23
Number of tillers				
5K,C	300,C	1250,C	12K,C	C,C
10.1	7.6	7.5	6.5	5.5
Fresh wt. roots, g				
5K,C	300,C	1250,C	12K,C	C,C
4.83	3.44	2.70	2.49	2.10
Dry wt. roots, g				
5K,C	300,C	1250,C	12K,C	C,C
0.44	0.36	0.30	0.25	0.16
Fresh wt. shoots, g				
5K,C	300,C	1250,C	12K,C	C,C
11.91	9.24	7.89	6.01	4.93
Dry wt. shoots, g				
5K,C	300,C	1250,C	12K,C	C,C
1.94	1.53	1.27	0.94	0.84

*Treatment.

b) Rideau Wheat

The response of vernalized Rideau wheat to 300Hz, 1250Hz, 5KHz and 12KHz audible sound frequencies is shown in Table VIII. The dry weights of both the root and shoot systems of plants exposed to 5KHz sound were double those of controls, but were only on the border-line of statistical significance at the 5% level owing to a high degree of variability amongst the Rideau plants. Plants grown from grains exposed to 300Hz, 1250Hz and 12KHz were not statistically different from controls.

C) Intensity Response

The responses of Rideau wheat to 5KHz audible sound at intensities of 92, 105 and 120 db either during vernalization or during the entire growth period are shown in Table IX and Fig. 24.

Plant height was unaffected by any of the treatments. The number of roots was increased by continuous exposure to 92 db level and decreased by continuous exposure to 105 db level, when compared with controls. The dry weight of roots of plants treated with 92 db sound during the chilling period was double that of the controls, but this was not significant at the 5% level because of a high degree of variability between plants. The dry weights of the roots of plants exposed to continuous 105 or 120 db sound were not different from the controls but were significantly lower (2 fold) than the 92,92 db series. The dry weight of the shoots was significantly

Table VIII. Frequency response: Rideau wheat after 4 weeks chilling and 8 weeks growth (Scheffé's test at the 5% level)

Height, cm*				
300,C	5K,C	C,C	1250,C	12K,C
61.1	57.2	57.1	55.4	55.0

Number of roots				
5K,C	12K,C	1250,C	300,C	C,C
67	53	41	36	36

Dry wt. roots, g				
5K,C	12K,C	300,C	C,C	1250,C
0.64	0.50	0.36	0.35	0.32

Dry wt. shoots, g				
5K,C	12K,C	1250,C	300,C	C,C
3.73	2.60	2.06	1.85	1.85

*Treatment.

Table IX. Intensity response: Rideau wheat after 4 weeks chilling and 8 weeks growth (Scheffé's test at 5% level)

Height, cm*						
92,92	105,C	120,120	92,C	120,C	105,105	C,C
60.3	59.9	57.8	57.2	53.4	52.9	51.5

Number of roots						
92,92	92,C	105,C	C,C	120,C	120,120	105,105
71	67	56	46	45	41	34

Dry wt. roots, g						
92,92	92,C	105,C	C,C	120,C	120,120	105,105
0.77	0.64	0.38	0.34	0.31	0.27	0.26

Dry wt. shoots, g						
92,92	92,C	105,C	C,C	120,C	120,120	105,105
4.50	3.73	3.13	2.4	2.0	1.9	1.4

*Treatment.

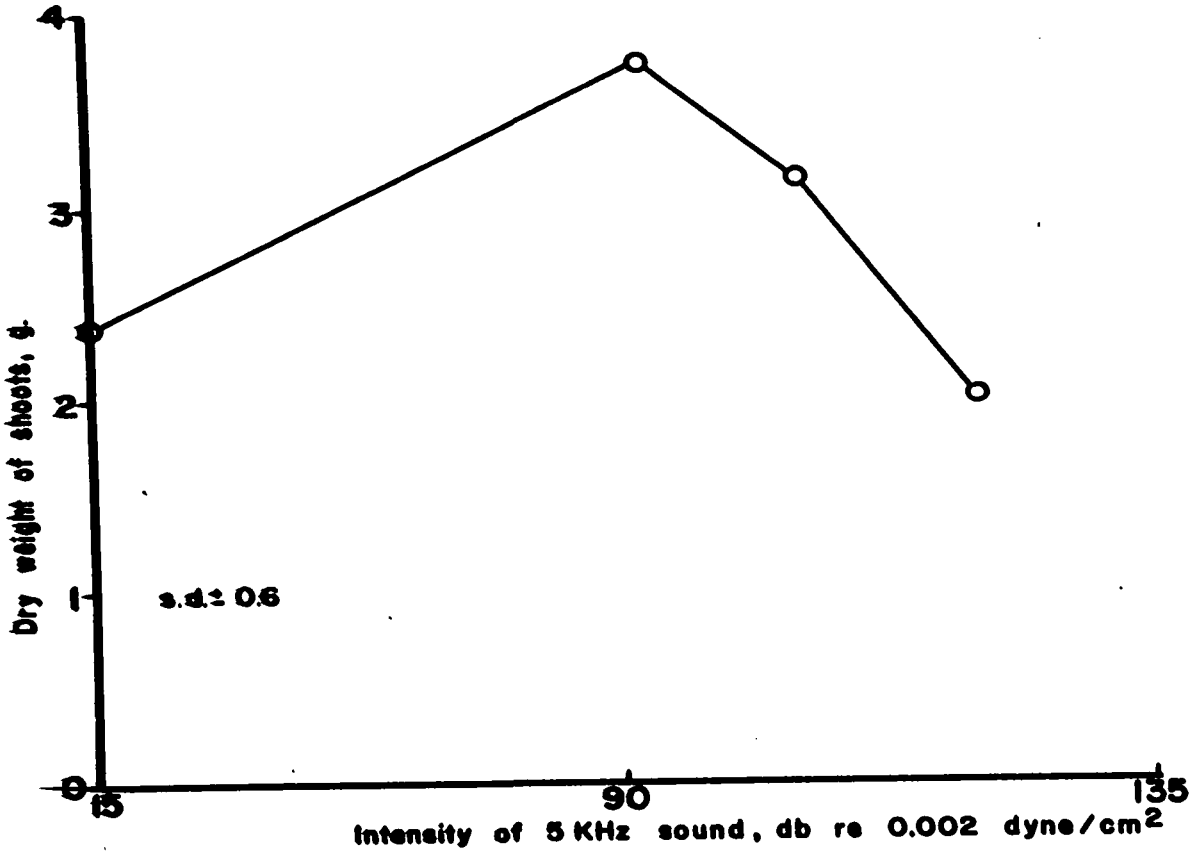


Fig. 24. Intensity response: Dry weight of shoots following 4 weeks grain vernalisation during exposure to 5 KHz sound and eight weeks growth under control conditions. (Rideau wheat)

increased, (2 fold), by exposure to the 92 db level. A decrease was noted after continuous exposure to the 105 or 120 db level but this was not significant. The dry weight of shoots of plants exposed to 105 db sound during the chilling period is only on the borderline of significance at the 5% level when compared with controls.

D) Leaf Morphology

The number of stomata and number of trichomes 8 cm from the tip of the sixth leaf of 35-day old control and 5K,C plants are shown in Table X. The number of stomata on the upper surface of the leaf was greater than that of the under surface of the leaf in all cases. There appeared to be no significant differences between control and sound-exposed plants.

Table X. Number of stomata and trichomes 8 cm from tip of sixth leaf of 35 day old Rideau wheat plants

Treatment	Stomata		Trichomes
	upper	lower	
C,C	23.6±2.7	19.7±3.8	14.0 ± 2.7
5K,C	25.1±4.4	20.6±2.6	12.5 ± 1.9

E) Morphogenesis

The time required by Rideau wheat plants to initiate floral induction as evidenced by stage 14 (Friend et al, 1963) is shown in Table XI. Plants vernalized and grown in control chambers are compared with those vernalized and grown in the presence of either continuous 5KHz sound or continuous random white noise (RWN).

All three series required twenty-nine days to reach stage 14. Differences in flag leaf number were insignificant.

Table XI. Morphogenetic studies of Rideau wheat

Treatment	Stage	Growth period	No. of Tillers	No. of Emerged Leaves	Flag Leaf Number
C,C	14	29 days	2.6	10.5	10.2
5K,5K	14	29 days	4.0	13.5	10.6
RWN	14	29 days	2.7	10.9	10.0

The number of tillers and number of emerged leaves of plants in the 5KHz series were significantly larger than those of both the control and random white noise series. Differences

between control plants and those treated with random white noise were insignificant.

iv) Respiration Rates

a) Grains

Oxygen uptake and carbon dioxide output of fully imbibed Marquis and Rideau wheat grains are presented in Tables XII and XIII. There was a direct relationship between respiration rates and the length of the chilling period and/or length of time in the growth chamber.

Oxygen was taken up more rapidly by non-chilled Marquis grains exposed continuously to 5KHz sound for 48 hr than by control grains. Differences in carbon dioxide production were on the borderline of significance. Neither oxygen uptake nor carbon dioxide production of the chilled grains were significantly affected when sound-exposed grains were compared with controls.

Both oxygen uptake and carbon dioxide production of Rideau grains were enhanced in non-chilled grains exposed to 5KHz sound for 48 hr, and unaffected in chilled and sound-exposed grains, when compared with controls.

The volume of carbon dioxide given off by four-week chilled Rideau grains as measured in the Warburg apparatus was only 30% that measured in the infra-red gas analyzer. Differences between control grains and sound-exposed grains were insignificant.

Table XII. Respiration rates of Marquis wheat grains (t-test at 5% level)

Treatment	O_2 Uptake (µl / g / hr)		CO_2 Production (µl / g / hr)	
24 hr in g chamber	5K	C	C	5K
	104	104	111	107
48 hr in g chamber	5K	C	5K	C
	270	202	263	203
2 wk at 2°C	C	5K	C	5K
	163	142	184	160
2 wk at 2°C plus 24 hr in g chamber	C	5K	5K	C
	264	247	297	281
4 wk at 2°C	C	5K	C	5K
	346	306	379	344
4 wk at 2°C plus 24 hr in g chamber	C	5K	C	5K
	516	460	516	419

Table XIII. Respiration rates of Rideau wheat grains

(t-test at 5% level)

Treatment	O_2 Uptake (µl / g / hr)		CO_2 Production (µl / g / hr)	
24 hr in g chamber	5K	C	5K	C
	222	163	248	179
48 hr in g chamber	5K	C	5K	C
	503	357	548	381
2 wk at 2°C	C	5K	C	5K
	143	119	159	145
2 wk at 2°C plus 24 hr in g chamber	5K	C	5K	C
	217	193	237	184
4 wk at 2°C	C	5K	5K	C
	256	240	300	201
4 wk at 2°C plus 24 hr in g chamber	5K	C	C	5K
	484	476	482	473
4 wk at 2°C plus 10 hr at room temperature (IRGA analysis)			C	5K
			1230	1266

Respiration rates of non-chilled Rideau grains were double that of non-chilled Marquis grains, but rates of four-week chilled grains were only 75% those of the Marquis grains (Table XIV). Differences between respiration rates of two week chilled grains were insignificant.

b) Plants

Net respiration and photosynthesis of two-week old Rideau wheat leaves are shown in Table XV. There were no significant differences in the rate of carbon dioxide production or uptake when either plants exposed to sound while respiration and photosynthesis measurements were being made, or plants grown from grains vernalized in the presence of 5KHz sound, were compared with controls.

v) Amino acid analysis

a) Free amino acids

The concentrations of alcohol-extractable amino acids and amides of the embryo and endosperm of vernalized control and 5K-exposed grains are presented in Table XVI.

The amino acids were, on the average, 18 times more concentrated in the embryo than in the endosperm. The most abundant amino acids in the embryo were serine, proline and alanine. The concentrations of serine and proline were 21% and 26%, respectively, higher in the control series than in

Table XIV. Comparison of oxygen uptake of Marquis (M) and Rideau (R) wheat grains (t-test at 5% level)

Treatment	O_2 Uptake (µl / g / hr)	
24 hr in g chamber	R	M
	163	104
48 hr in g chamber	R	M
	357	202
2 wk at 2°C	M	R
	163	143
2 wk at 2°C plus 24 hr in g chamber	M	R
	264	193
4 wk at 2°C	M	R
	346	256
4 wk at 2°C plus 24 hr in g chamber	M	R
	516	421

Table XV. Net rates of respiration and photosynthesis of control and sound-exposed two-week old Rideau wheat leaves

Sound exposure	Rate of CO_2 Production (µl / g / min (± 6)	Rate of CO_2 Uptake (µl/g/min (±51)
C,C,C	81	976
C,C,300	83	976
C,C,1250	84	980
C,C,5K	84	958
C,C,12K	81	991
C,C,17K	86	984
5K,C,C	83	1005

Table XVI. Free amino acid composition (μM/10g dry weight) of vernalized control and sound-exposed Rideau wheat grains

	Embryo		Endosperm	
	C	5K	C	5K
Aspartic Acid	42	43	5	7
Serine	84	61	4	4
Glutamic Acid	59	55	6	5
Proline	244	193	4	4
Glycine	46	36	1	4
Alanine	121	196	5	2
Valine	35	27	2	2
Methionine	+	+	+	+
Isoleucine	17	14	2	1
Leucine	19	9	1	1
Tyrosine	-	-	1	1
Phenylalanine	11	13	2	2
Lysine	+	+	+	1
Histidine	14	14	+	1
Arginine	22	14	1	1
Asparagine	304	254	16	10
Glutamine	598	528	13	11

the sound-exposed series. Alanine, however, was 62% more concentrated in the 5K exposed series.

Differences in the concentrations of aspartic acid and glutamic acid between the control and sound-exposed embryos were insignificant. Concentrations of these two amino acids were lower than those of serine, proline and alanine, but the amides, asparagine and glutamine, were much more abundant. There was 20% more asparagine and 13% more glutamine in the control embryos, than in the sound-exposed embryos. The asparagine content of the endosperm was also higher (60%) in the control series than in the sound-exposed series.

Although the free amino acids and amides were more concentrated in the embryos of the control series than in those of the 5K series, the relative proportions of most amino acids were the same for the two series (Table XVII). The amino acids showing the greatest differences were alanine, which was twice as abundant in the embryo and half as abundant in the endosperm of the 5K series when compared with the control series; glycine, which was more than four times as abundant in the endosperm of the 5K series; and aspartic acid, which was approximately 1.5 times as abundant in the endosperm of the 5K series while its amide, asparagine, was approximately 1.5 times as abundant in that of the control series.

Table XVII. Relative proportions of free amino acids (%) of vernalized control and sound-exposed Rideau wheat grains

	Embryo		Endosperm	
	C	5K	C	5K
Aspartic Acid	2.6	3.0	7.9	12.7
Serine	5.2	4.2	6.3	7.3
Glutamic Acid	3.7	3.8	9.5	9.1
Proline	15.1	13.2	6.3	7.3
Glycine	2.8	2.5	1.6	7.3
Alanine	7.5	13.5	7.9	3.6
Valine	2.2	1.9	3.1	3.6
Methionine	+	+	+	+
Isoleucine	1.1	1.0	3.1	1.8
Leucine	1.2	0.6	1.6	1.8
Tyrosine	+	+	1.6	1.8
Phenylalanine	0.7	0.9	3.1	3.6
Lysine	+	+	+	+
Histidine	0.9	1.0	+	+
Arginine	1.4	1.0	1.6	1.8
Asparagine	18.8	17.4	25.4	18.1
Glutamine	37.0	36.2	20.6	20.1

b) Total Amino Acids

The total amino acid composition of the embryo and endosperm of vernalized control and 5K-exposed grains are shown in Table XVIII. Concentrations in the embryo were, on the average, 1.5 times higher than in the endosperm.

In all cases except methionine, the amino acid concentrations were higher in embryos from the control series than those from the sound-exposed series. The greatest differences appeared in proline (66%) and glycine (75%). Methionine was only present in trace amounts in both series.

Asparagine and glutamine concentrations were included with aspartic acid and glutamic acid concentrations since the amides were converted to their respective amino acids by acid hydrolysis during the preparation of the sample (Boutler 1966). There was 26% more aspartic acid and 41% more glutamic acid in the embryos from the control grains than those from the sound-exposed grains.

Amino acids were also more concentrated in the endosperm from the control series than in that from the sound-exposed series, except for threonine, valine and isoleucine, which were more concentrated in the sound-exposed series and methionine, histidine, lysine and arginine, which were about the same for the two series. The most abundant amino acids were aspartic acid, serine, glutamic acid, proline and glycine. These were respectively, 94, 34, 41, 56 and 68% more abundant in the control series than in the 5K-exposed series.

Table XVIII. Amino acid composition (μM / g dry wt) of vernalized control and sound-exposed Rideau wheat grains

	Embryo		Endosperm	
	C	5K	C	5K
Aspartic Acid	155	114	62	33
Threonine	77	52	114	37
Serine	120	101	79	59
Glutamic Acid	207	147	328	231
Proline	68	41	109	70
Glycine	163	93	96	57
Alanine	132	90	62	43
Valine	42	35	22	33
Methionine	+	+	+	+
Isoleucine	23	20	13	27
Leucine	97	63	52	47
Tyrosine	17	13	37	27
Phenylalanine	43	37	36	27
Lysine	52	37	+	12
Histidine	38	28	17	18
Arginine	63	47	12	14

Percentage composition was almost the same for the amino acids in both the embryo and the endosperm of the two series (Table XIX).

vi) Nucleic Acid Content

The RNA and DNA content of the embryos and endosperms of both control and sound-exposed grains following two and four weeks chilling at 2°C, are shown in Table XX.

After both 2 wk and 4 wk there was a significantly larger amount of RNA in the embryos of the 5K series when compared with that of the embryos of the control series, when the data were expressed either on a fresh weight or per 10 grains basis. Differences between the two series in RNA content of the endosperms and DNA content of both the embryos and endosperms were insignificant.

vii) Mitosis in the Root Apical Meristem

The effect of audible sound on the number of metaphases, the average cell length and the length of the apical meristem of roots of four week chilled Rideau wheat is shown in Table XXI. There were no significant differences between the control and sound-exposed series for any of the parameters examined.

Table XIX. Relative proportions of amino acids (%) of vernalized control and sound-exposed Rideau wheat grains

	Embryo		Endosperm	
	C	5K	C	5K
Aspartic Acid	11.9	12.4	6.6	4.5
Threonine	5.9	5.6	1.2	5.0
Serine	9.2	11.0	8.4	8.0
Glutamic Acid	15.9	16.0	35.0	31.4
Proline	5.2	4.5	11.6	9.5
Glycine	12.5	10.1	10.3	7.8
Alanine	10.1	9.8	6.6	5.9
Valine	3.5	3.8	2.4	4.5
Methionine	+	+	+	+
Isoleucine	1.8	2.2	1.4	3.7
Leucine	7.5	6.9	5.6	6.4
Tyrosine	1.3	1.4	4.0	3.7
Phenylalanine	3.3	4.0	3.8	3.7
Lysine	4.0	4.0	+	1.6
Histidine	2.9	3.1	1.8	2.4
Arginine	4.8	5.1	1.3	1.9

Table XX. RNA and DNA content of Rideau wheat grains

Chilling Period Wk	Treatment	RNA Content (mg/g fresh wt)		RNA Content (mg/10 grains)		DNA Content (mg/10 grains)	
		Embryo	Endosperm	Embryo	Endosperm	Embryo	Endosperm
2	C	54.9±8.5	7.8±1.2	1.2±0.3	4.4±0.6	0.2±0.1	0.3±0.2
	5K	79.0±1.6	7.4±0.9	1.8±0.2	4.3±0.5	0.2±0.1	0.3±0.2
4	C	175.6±4.9	13.0±0.8	5.9±0.3	7.5±0.5	1.2±0.3	0.3±0.2
	5K	199.0±4.0	13.4±0.5	6.3±0.2	7.6±0.5	1.2±0.4	0.3±0.2

Table XXI. The effect of audible sound on mitosis in the apical meristem of Rideau wheat seedlings

Parameter	Treatment of Grains	
	Control	5K-exposed
Number of metaphases	45 ± 10	46 ± 10
Length of cell, mm	0.016 ± 0.003	0.016 ± 0.003
Length of apical meristem, mm	0.36 ± 0.03	0.37 ± 0.03

viii) Peroxidase Activity

The activity of peroxidase in control and 5K-exposed grains and seedlings are shown in Table XXII. The enzyme activity in both the grains and the seedlings was unaffected by sound exposure. The enzyme was approximately 2.5 times more active per unit fresh weight in grains that had been allowed to sprout at 25°C than in the grains just removed from the cold.

x) Artificial Membranes

The effect of 5KHz audible sound on the dialysis of sugar is shown in Table XXIII. The audible sound appeared to have no effect on the rate of dialysis. The passage of sugar through both the sound-exposed and the control membranes appears to be slower after 96, 144 or 192 hours treatment than after 24 or 48 hours treatment.

Table XXII. Effect of sound-exposure on peroxidase activity of Rideau wheat grains and seedlings

Treatment		Peroxidase Activity (Change in Absorbance per 100 sec per mg fresh weight)
Grains	C	0.97 ± 0.06
	5K	0.92 ± 0.04
Seedlings	C,C	2.35 ± 0.53
	5K,C	2.43 ± 0.28

Table XXIII. Effect of 5KHz sound on rate of dialysis through cellophane membranes

	Sugar Concentration, µg/l	
Treatment Time, hr	C	5K
24	43	43
48	55	51
96	29	30
144	23	23
192	26	29

x) P-32 Uptake

P-32 uptake by control and sound-exposed wheat grains is shown in Table XXIV. Uptake into the endosperm and transport to the embryo appeared to be slightly inhibited in the sound-exposed series when compared with controls.

Table XXIV. P-32 uptake by control and sound-exposed Rideau wheat grains

	cpm / mg ash(±20)	
Grain Part	C	5K
Embryo	154	110
Endosperm	133	115

The uptake of P-32 by the roots of young Rideau Wheat plants and its subsequent translocation to the shoots is shown in Table XXV.

Table XXV. Uptake of P-32 by young Rideau wheat plants

	Activity, cpm/10µg ash wt			
Form of P-32 Solution	Roots(±200)		Shoots(±50)	
	C,C*	5K,C	C,C	5K,C
Water (Non-Sterile)	3081	2256	431	462
Culture Solution	2210	804	222	159
Water(Sterile)	811	607	441	439

* Treatment

P-32 appeared to accumulate more readily in the roots of the control series than in those of the sound-exposed series. However, transport to the shoots did not appear to be affected by the sound-exposure. Uptake by the roots and transport to the shoots was inhibited in both series when the P-32 was added to culture solution instead of to distilled water. The effect was greater in the sound-exposed series than in the control series. Uptake by sterile plants from sterile water was only 25% that from non-sterile water in both series, but rate of transport to the shoots was unaffected. Growth of sterile plants was inhibited when compared with that of non-sterile plants (Fig. 25).

xi) Effect of audible sound on Rideau wheat contaminants

The rates of growth of colonies of Mucor rouxianus are shown in Fig. 26. No differences were observed between colonies grown in control chambers and colonies grown in chambers exposed to 5K sound. Sporangia appeared on all plates approximately 48 hr after innoculation.

The number of colonies of Bacillus sp., Pseudomonos fluorescens and Fusarium solani appearing two days after innoculation are shown in Table XXVI. There was no significant differences in the number of colonies on plates grown in 5K-exposed chambers compared with those grown in control chambers. However, colonies in the 5K-exposed chamber were only 1.5 mm in diameter compared with the 5.0 mm diameter of colonies grown in the control chamber (Fig. 27).

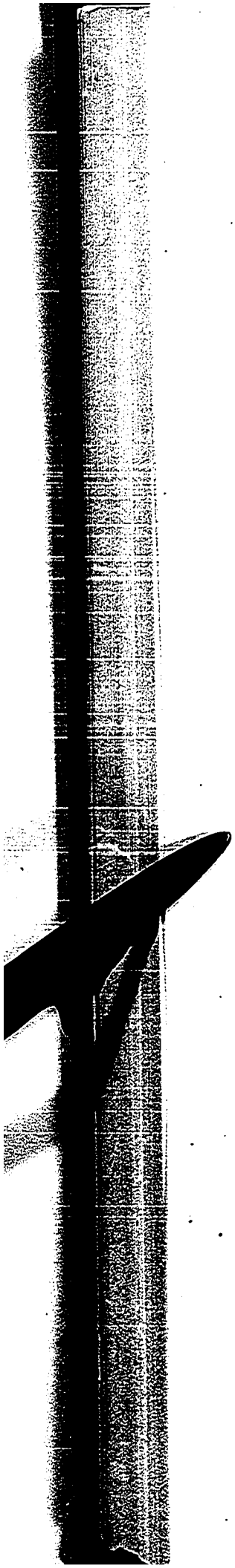


Fig. 25. Relative size of sterile (A) and non-sterile (B) ten-day old Rideau wheat seedlings

e (B)

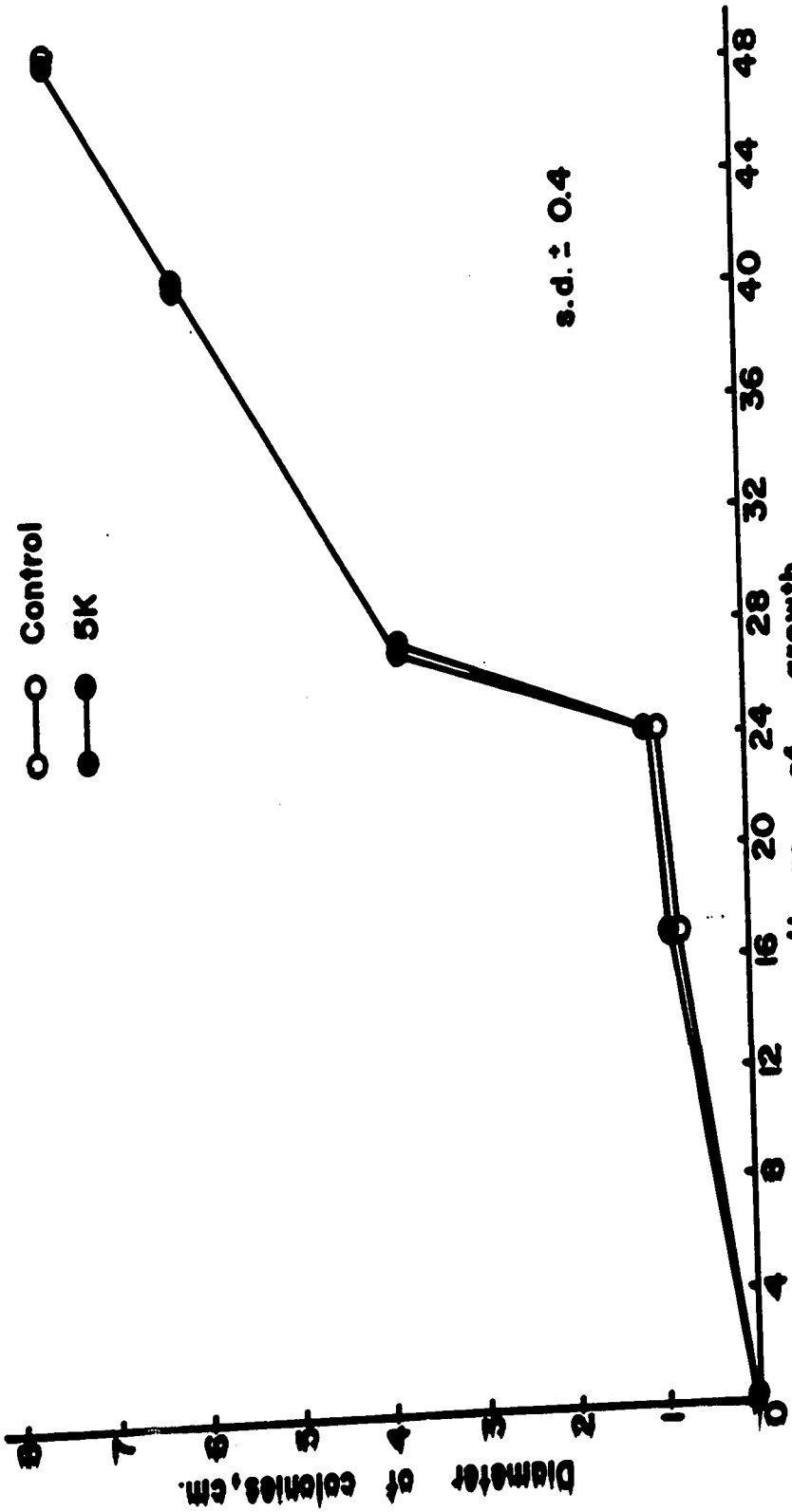


Fig.26.Rate of growth of colonies of Mucor rouxianus

Fig. 27, A-B. Effect of Audible Sound on Colonies of *Pseudomonas fluorescens*. (A) C. (B) 5K.

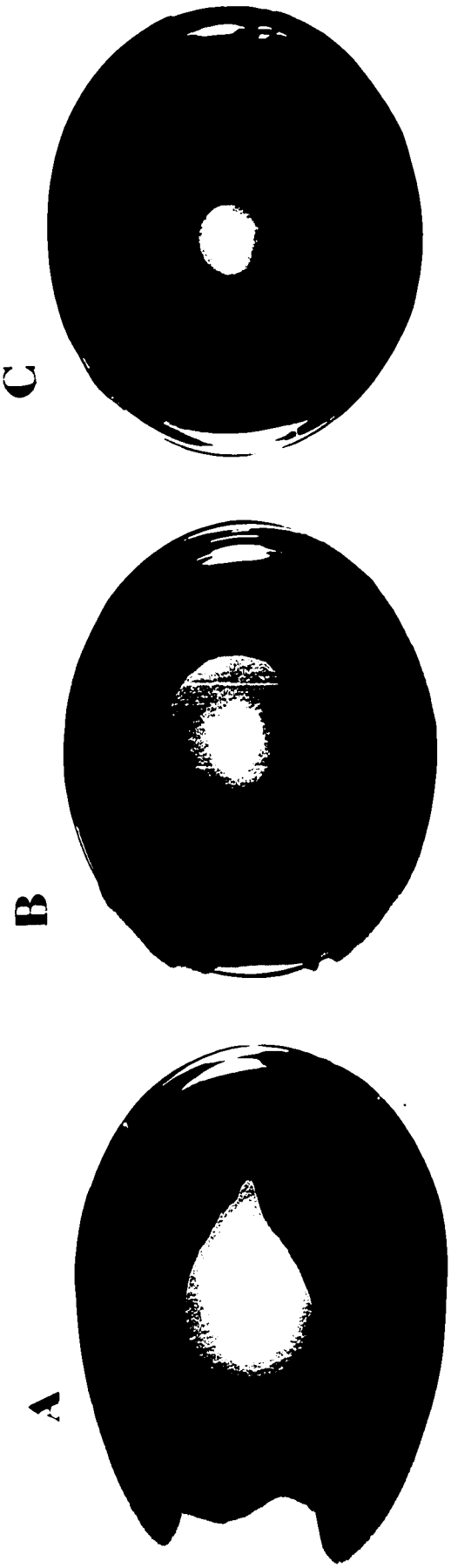
B
V

Table XXVI. Numbers of colonies of mico-organisms per petri dish apparent two days after innoculation.

Organism	Sound Exposure	
	Control	5K
Bacillus sp.	20 ± 3	20 ± 3
Pseudomonos fluorescens	150 ± 32	185 ± 25
Fusarium Solani	159 ± 8	155 ± 6

Examination of P. fluorescens under the microscope showed these bacteria to be fairly motile. Therefore further tests, designed to determine whether audible sound has an effect on bacterial motility, were carried out using an extremely motile strain of E. coli. Swarming of these bacteria was greatly inhibited in plates placed in growth chambers exposed to 5KHz sound at an intensity of 92 db (Fig. 28). The inhibiting effect was even more pronounced at an intensity of 105 db.

Fig. 28, A-C. Effect of audible sound on swarming, of *E. coli*. (A) C. (B) 5K (92 db). (C) 5K (105 db)


C
B
A

B) Field Studies

i) Wheat

a) First Generation

Results of growth studies of sound-exposed and control imbibed Rideau wheat grown to maturity at the Central Experimental Farm are shown in Table XXVII. Sound treatment during the chilling period had no effect on the number of heads, dry weight or yield of grain, thus indicating that the effects of sound exposure were lost when plants were grown to maturity in the field; i.e., under non-controlled conditions. In addition, head emergence of almost the whole field, regardless of pre-treatment, was noted on the same day.

Results of growth studies from non-imbibed grains are shown in Table XXVIII. Differences between treatments were insignificant.

A comparison of Tables XXVII and XXVIII illustrates the necessity of water for vernalization to be effective. Plants grown from non-imbibed grains exhibited a 46-64% increase in number of tillers and a 64-81% increase in dry weight when compared in plants grown from fully imbibed grains. However, by the end of the summer these plants had not produced any grain and were only approximately 60 cm in height, compared with 100 cm, the height of the grain-producing plants.

Table XXVII. Rideau wheat field trial, 1968 (imbibed grain)

Treatment During Vernalization	Number of Heads per Plant	Dry Weight per Plant, g	Yield of Grain per Plant, g
C	8.6	18.1	8.4
5K	8.2	17.7	8.9
12K	8.7	17.8	8.9

Table XXVIII. Rideau wheat field trial, 1968 (non-imbibed grain)

Treatment During Vernalization	Number of Tillers per Plant	Dry Weight per Plant, g	Yield of Grain per Plant, g
0	14.1	32.8	0
5K	13.4	29.0	0
12K	12.7	30.9	0

Table XXIX. Second generation plants; i.e., grains sound-treated, grown at farm then harvested grains vernalized and grown in growth chamber (t-test at 5% level)

Height, cm	F 5K,C	F C,C
	61.4	58.7
Number of tillers	F 5K,C	F C,C
	4.9	4.8
Number of roots	F C,C	F 5K,C
	32.3	29.9
Fresh wt. shoots, g	F 5K,C	F C,C
	9.83	9.09
Dry wt. shoots, g	F C,C	F 5K,C
	1.55	1.52
Fresh wt. roots, g	F C,C	F 5K,C
	2.23	2.10
Dry wt. roots, g	F C,C	F 5K,C
	0.25	0.22

b) Second Generation

The growth of plants from grains harvested from the original imbibed control and 5K-exposed grains and grown without sound exposure is shown in Table XXIX. When plants were sampled after eight weeks growth, there were no significant differences in any growth parameters. Thus it appears that the potential for an enhanced growth rate does not carry over into the second generation.

ii) Alfalfa and Grasses

a) 1968 Sowing

The fresh weights of Chinook orchard grass, frontier reed canary grass and vernal alfalfa, sound-treated in Ottawa and grown for two months in the field in British Columbia, are shown in Table XXX.

Table XXX. British Columbia field trial (1968)

Species	Treatment	Total Fresh Weight of Plants g
Chinook Orchard Grass	C,C	311
	5K,C	762
	12K,C	218
Frontier Reed Canary Grass	C,C	1228
	5K,C	2208
	12K,C	2457
Vernal Alfalfa	C,C	2612
	5K,C	3701
	12K,C	3452

The total weight of Chinook orchard grass grown from seeds exposed to 5KHz sound was 2.5 times that of the controls. That of plants grown from seeds exposed to 12KHz sound was only 70% that of the controls.

The total fresh weights of frontier reed canary grass grown from seeds exposed to either frequency were double those of controls, while fresh weights of vernal alfalfa were approximately 1.4 times those of controls.

Fresh weights of the second year's growth of frontier reed canary grass and vernal alfalfa are shown in Tables XXXI and XXXII. The weight of the first cut of frontier reed canary grass, grown in the fertilized plots from seeds exposed to either 5KHz or 12KHz sound frequency, was 20% larger than that of control plants. This increment was not apparent in the second cut. However, the weight of plants grown from 5KHz-exposed seeds in the unfertilized plots was double that of the controls in the first cut and 1.5 times that of the controls in the second cut. Weights of plants grown from 12KHz-exposed seeds were 1.5 times that of controls in the first cut and 1.1 times that of the controls in the second cut.

It appears, therefore, that stimulation of plant growth by exposure to 5KHz sound is apparent in the second years growth only in unfertilized plants. The total fresh weight of clippings from the 5K-unfertilized plot is comparable with that of the clippings from the control unfertilized

Table XXXI. Fresh weight (g / row) of frontier reed canary grass sown in 1968 and cropped in 1969

Plot	Cut	Sound Treatment C	5K	12K
Fertilized	1	930	1129	1151
	2	366	360	244
	Total	1296	1489	1395
Unfertilized	1	527	1043	771
	2	181	255	204
	Total	708	1298	975

Table XXXII. Fresh weight (g / row) of vernal alfalfa sown in 1968 and cropped in 1969

Plot	Cut	Sound Treatment C	5K	12K
Fertilized	1	1826	1675	1755
	2	716	693	649
	3	582	508	569
	Total	3124	2876	2973
Unfertilized	1	817	884	782
	2	771	757	680
	3	391	522	400
	Total	1979	2163	1862

plots is only half the weight of clippings from the control fertilized plot.

Differences between fresh weights of the control and sound-treated series of vernal alfalfa were very small in both the fertilized and unfertilized plots. Fresh weights of plants grown in the fertilized plots were approximately 1.5 times those of plants grown in the unfertilized plots.

b) 1969 Sowing

The fresh weights of reed canary grass are shown in Table XXXIII. Weights of plants grown at the Central Experimental Farm, Ottawa, Ontario, from seeds originally treated with 5KHz sound at an intensity of 92 db were significantly larger than those of plants grown from non-exposed seeds. Other treatments had no significant effects on the weights. None of the treatments had any significant effects on the fresh weight of plants grown at Cardston, Alberta. Again, this is probably related to the difference in fertilizer regimes. At the Experimental Farm the plot was fertilized (8-16-16) at a rate of 400 lb/acre only once before seed planting while at Cardston fertilizer (10-30-10) at a rate of 250 lb/acre was applied three times in the course of the growing season.

The effects of infra-red, cold- and sound-exposure of seeds on the growth of vernal alfalfa are shown in Table XXXIV. None of the treatments had any significant effect on the fresh weight of the plants.

Table XXXIII. Fresh weight (g / plot) of reed canary grass (1969)

Location of Plot	Fresh Weight (g)			
Ottawa, Ontario	92,C	NC,C*	105,C	C,C
	4037	3447	3357	3221
Cardston, Alberta	C,C	NC,C	105,C	92,C
	4639	4259	4210	3974

* NC Non-chilled control

Table XXXIV. Fresh weight (g / plot) of vernal alfalfa grown in Ottawa, Ontario, 1969 (Scheffé's test at the 5% level)

Infra-red	Treatment of Seed C,C	NC,C	92,C	105,C
+	4037	3810	3084	3765
-	3583	3583	3720	3493

The weights of the June 4, 1970 clipping of the 1969 - sown alfalfa, reed canary grass and Kentucky blue grass are shown in Table XXXV. There were no significant differences between any of the treatments.

Table XXXV. Dry matter yield (g / plot) of second year cropping (June 1970) of plants sown in 1969.

Species	Treatment of Seeds C,C	NC,C	92,C	105,C
Reed Canary Grass	1969	1721	1675	1753
Kentucky Blue Grass	1171	1188	1139	1227
Vernal Alfalfa	1719	1725	1692	1659
Vernal Alfalfa IR	1650	1783	1698	1735

III Environmental Effects on Plant Growth with Specific Reference to Electromagnetic Fields

Weights of the heads of Marquis wheat plants grown from grains exposed to magnetic fields are shown in Tables XXXVI and XXXVII.

The weights of heads of plants grown from grains exposed to a field of 14,000 Gauss for either 18 or 0 hours were significantly larger than those from grains exposed for 47, 24 or 7 hr. An analysis of variance showed the overall effect of embryo position during exposure, to be insignificant regardless of exposure period. However Fig. 29 illustrates the significant interaction between exposure period and embryo position. Effect of embryo position is insignificant from zero to 18 hr exposure time. However after a 24 or 47 hr exposure period, weights from plants from grains treated with the embryo parallel to the field of force were 25-70% larger than those from grains treated with the embryo perpendicular to the fields of force.

The weights of the heads of plants exposed to a field of 220 Gauss for 7 hr were increased by 30%, when compared with controls. Exposure at the higher field intensities resulted in a 15-30% decrease in the weight of the heads when compared with controls. The overall effect of embryo position was insignificant when averaged over the range of field intensities. Interactions between embryo position and field intensity were insignificant.

Table XXXVI. Effect of magnetic field of 14,000 Gauss on weight of heads of Marquis wheat (Scheffé's Test at 5%)

1) Effect of Exposure Period.

Exposure, hr.	18	0	47	24	7
Dry wt, g	1.09	0.98	0.78	0.67	0.61

2) Effect of Embryo Position in Relation to N⟶S field of force.

Embryo Position	⟶	←	↓	↑
Dry wt, g	0.99	0.80	0.76	0.74

Table XXXVII. Effect of exposure to magnetic field for even hours on weight of heads of Marquis wheat (Scheffé's test at 5% level)

1) Effect of Field Strength

Field Strength, Gauss	220	0	3500	1700	7000	14000
Dry Weight, g.	1.40	1.01	0.82	0.79	0.70	0.60

2) Effect of Embryo Position in Relation to N ⟶ S Field of Force

Embryo Position	↑	↓	⟶	⟵
Dry wt. g.	0.86	0.83	0.81	0.74

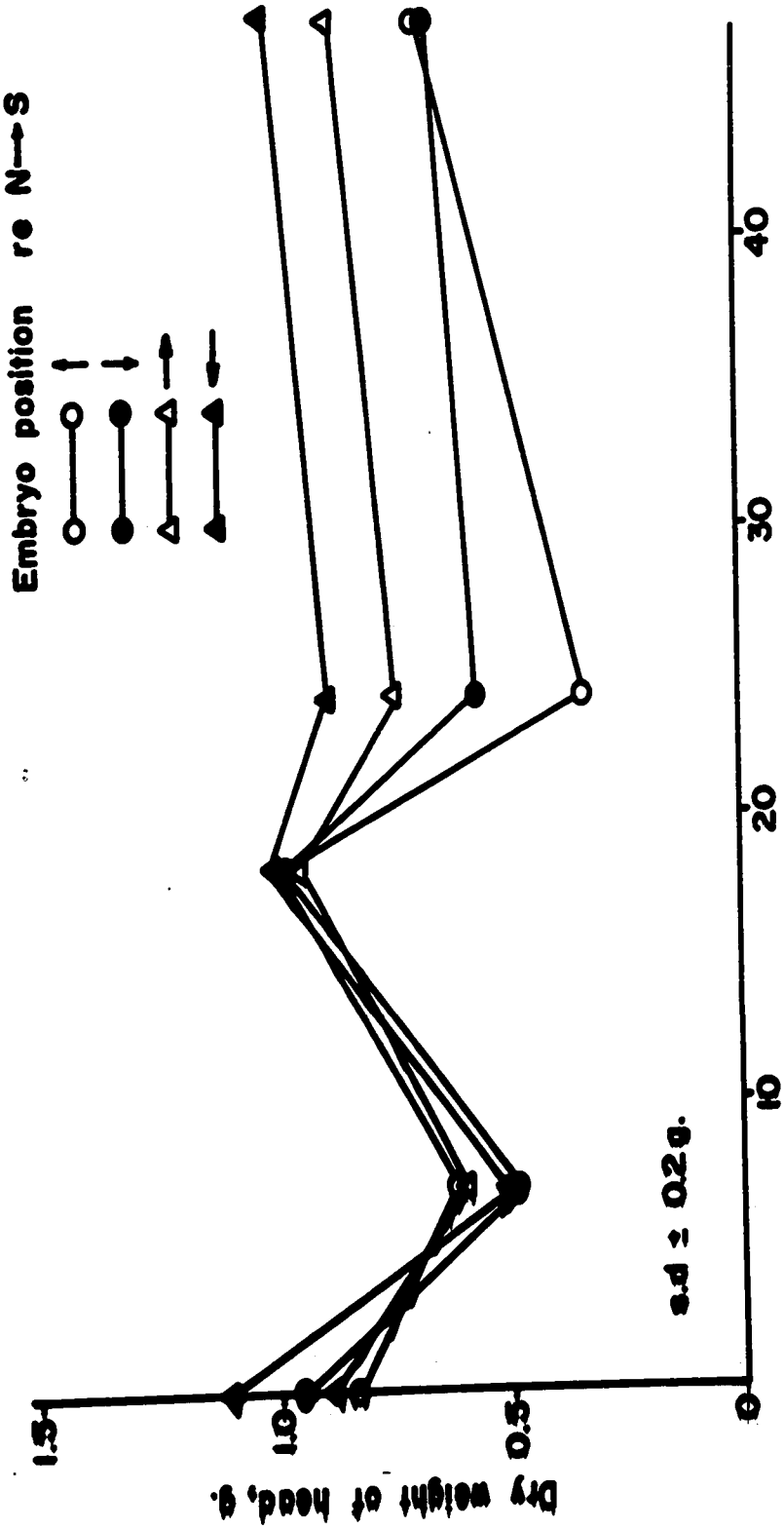


Fig.29. Effect of length of exposure period to a magnetic field (14,000 Gauss) on the dry weight of heads of Marquis wheat.

The average number of grains per plant of Marquis wheat exposed to magnetic fields for 7 hr did not vary with embryo position, but was increased by 33% in a field of 220 Gauss and decreased by 33% in a field of 1400 Gauss (Table XXXVIII).

Table XXXVIII. Effect of exposure to magnetic field for 7 hr on average number of grains per Marquis wheat plant (Scheffé's test at 5% level)

1) Effect of Intensity

Intensity	220	0	14,000
Dry wt, g	24.6	18.2	13.6

2) Effect of Embryo Position in Relation to N⟶S Field

Embryo Position	↑	↓	→	←
Dry wt, g	18.9	18.8	18.6	18.0

DISCUSSION

I Controlled Environment VS Field Studies

Many studies of crop plants have been made in growth chambers and greenhouses where the plant environment can be controlled to some extent. In many chambers, the temperature, relative humidity, daylength, light intensity and light quality can be varied within defined limits, specific to the growth chamber. Hence detailed studies have been made concerning the relative effects of temperature, light intensity, wavelength, soil moisture stress and soil aeretion on the vegetative production (Friend 1961 , Friend et al 1962a, 1962b, 1965; Welbank et al 1968, Campbell and Ferguson 1969, Hofstra et al 1969) and grain yield (Friend et al 1963; Friend 1964, Canvin 1967; Campbell and Read 1968, Campbell and Ferguson 1969, Rawson 1970) of wheat and yield of forage crops (Smith 1970).

Conversely, during field trials the majority of growth limiting factors cannot be controlled. Those which can be manipulated to some extent often interact with non-controllable factors. For example, yields of wheat and alfalfa are related to plant density, but the intensity of the effect depends on the amount of available moisture present in the soil (Kilcher and Heinrichs 1969, Pelton 1969). Fertilization of the soil will often increase crop yield (Peterson 1965, Lawrence and Ashford 1969b, Ledingham 1970), but much of the applied fertilizer may become precipitated and hence be unavailable to the plants.

Weather and climate, and thus rainfall, temperature, light intensity, light quality and wind interact to modify plant growth and development. In addition, the plants modify their own environment and horizontal, as well as vertical, gradients of temperature, carbon dioxide and relative humidity are set up over vegetation stands (Penman and Long 1960, Huskin 1968). Although such gradients are described in connection with vegetative stands, they are usually neglected in growth chamber studies.

In the growth chamber used in the present study, temperature at any one point varied within 1.5^{0} and the difference between two points was generally less than 2^{0} (Fig.2) Differences between points were consistent though small. The cooling system of the chamber appeared to be efficient enough to cope with heat production and even over-compensated when the room temperature was high (Fig. 3). Hence it appears that the air circulation may be inadequate to maintain a constant temperature throughout the chamber. This could strongly affect plant growth since gradients of carbon dioxide might also build up. Unfortunately, the equipment necessary for such measurements was not available.

The effects of both temperature and carbon dioxide concentrations interact with those of light intensity to modify plant growth and development (Thomas 1965). Air circulation is also important to the diffusion of carbon dioxide because of resistance to diffusion caused by the presence of air and the leaf surfaces (Gaastra 1963, Monteith 1963).

In general, rates of photosynthesis and plant growth are accelerated by increments of carbon dioxide concentration (Lemon 1963), temperature and light intensity (Friend et al 1962a, 1962b). Friend et al (1963) Friend (1965b) also found that floral initiation of wheat was earlier with increases in temperature from 10 to 30°C and light intensity from 200 to 2500 ft-c, although the spikelet number was higher at the lower temperatures. At 25°C, the dry weight of Marquis wheat plants was five times higher at 100 ft-c than at 500 ft-c (Friend et al 1962b). This suggests that the range of 710 to 940 ft-c found in the growth chamber in the present study may have important effects on plant growth. At 1000 ft-c Friend et al (1962b) also found dry weight to be the same at 20°C and 25°C, but to be less than half at 30°C. At 500 ft-c, dry weight was less at 25°C than at 20°C and least at 30°C.

In the present study, plants were smaller at the sides of the chamber when compared with plants in the middle, although the temperature was higher on one side and lower on the other. Hence it appears that temperature was not one of the major factors in the growth pattern observed in the growth chamber study. Light intensity and possibly carbon dioxide concentration may be more important.

The present study suggests that extreme caution must be taken when growth chambers are used for plant growth studies. Plants should be rotated in an attempt to lessen gradient effects and chambers should not be overcrowded to allow ade-

quate circulation of air. Light intensity should be checked often since fluorescent bulbs become dimmer before they burn out and light intensity can decrease by 200 ft-c over a two month period. Experiments should also be repeated in alternate chambers to exclude the possibility of a chamber effect instead of an experimental effect.

II Germination and Growth of Plants

A) Early studies on the effects of ultra-sound and audible sound

Stimulation of germination and growth of barley, sunflower, pine, spruce and Siberian pea tree seedlings by treatment with ultra-sonic waves has been demonstrated by Obolensky (1953, 1957), Kozubov and Gamyushkina (1964) and Timonin (1966). This effect was accompanied by increased activity of some enzymes, including α-amylase and catalse and an increased respiration rate. Obolensky and Timonin both found the effects of frequency and exposure time to vary with species. Both found a threshold level, below which germination and growth were enhanced, but above which, they were inhibited. Thefrequency and amount of energy required to produce these effects appears to vary widely among species.

Very few studies concerning the effects of audible sound have been made. Singh and Gnanam (1960, 1962, 1965), Singh and Krishna (1960) report increases in growth and yield of paddy and tobacco

following exposure to music. The rate of photosynthesis (Singh 1969, Gnanam and Rajaraman 1961) and the rate of transpiration (Singh 1961) are also reported to increase following exposure to music. Unfortunately, most of the above data appear in the literature in the form of abstracts, so the actual figures are not available for inspection and the data therefore subject to question. However, Smith (Hicks 1963), an Illinois farmer, reported an increased yield, faster seedling emergence, more rapid plant growth, greater uniformity of plants and earlier silking of corn grown in outdoor plots subjected to continuous music throughout the growing season. He found a further increase in growth when he substituted a single note for the music. This increment was greater when a low note, 450Hz, was employed than when a high note, 1800Hz was employed (Smith 1967).

The main points of similarity between plant response to ultra-sound and plant response to audible sound lie in the importance of frequency and intensity of the sound and species of plant in the nature of the response (Rubtsova 1967).

B) Present Study

The effects of audible sound on rates of germination and growth appear to depend on temperature and species of seed as well as on the frequency and intensity of the sound.

Germination of Marquis and Rideau wheats was stimulated by exposure to audible sound at 2°C, but not at 25°C. At 25°C,

the audible sound may not have had time to assert its effect since the grains germinated rapidly, requiring only 18 hours for 98% germination of Rideau wheat and 26 hours for 98% germination of Marquis wheat. In the few cases (Figs. 18-20) where audible sound did appear to alter rates of germination at 25°C, the effect was temporary and was soon lost. For example, while the germination of Genesee wheat was slightly inhibited after 8 hours of exposure to 5Hz sound, the effect was no longer apparent 4 hours later when 90% of the grains had germinated. However, the respiration rates of Marquis and Rideau wheats appeared to be accelerated during germination at 25°C (Tables XII and XIII).

At 2°C, germination of Rideau wheat was accelerated by exposure to 5KHz, but not 12KHz, audible sound. The germination of Marquis wheat, however, was stimulated by exposure to either frequency.

The initial stages of germination involve the imbibition of water by the seed. During imbibition, the seed increases in volume as water molecules become adsorbed onto the surfaces of hydrophilic coloids (Ferry and Ward 1959). Proteins, mucilages, cellulose and pectic substances are the major seed components which have the capacity to imbibe water (Mayer et al 1963). The presence or absence of a semi-permeable membrane appears to have little effect on this process (Shull 1920). Hence, the three main factors controlling imbibition are the availability of water, the permeability of the seed

coat to water and the composition of the seed (Mayer and Poljakoff-Mayher 1963). Each species has a specific moisture content which must be attained before the seeds can germinate (Hunter et al 1952). Any treatment which facilitates imbibition may also enhance the rate of germination.

In the present study, it is apparent that audible sound did not affect the rate of imbibition of Rideau wheat grains either at 2°C or at 25°C. Indeed, neither the rate of water uptake nor the rate of P-32 uptake appeared to be significantly affected by audible sound (Figs. 15, 16; Table XXIV). However, Bushnel and Obolensky (1954) found imbibition, as well as germination, of barley grains to be accelerated following treatment with ultrasound. They concluded that the membranes and integuments of the grains were modified by the ultrasound, thus permitting a more rapid uptake of water and hence a more rapid rate of germination of the sonicated grains. While this may be true at ultrasonic frequencies our own work does not indicate that audible sound affected the membrane structure in relation to the rate of uptake of water.

Hard-coated seeds such as those of alfalfa and clover are reported to germinate more quickly following exposure to infra red radiation (Works and Erickson 1963, Nelson and Wolf 1964a, 1964b). In the present study however, infra red radiation appeared to have no effect on the germination rate of alfalfa seeds as all plots sprouted with equal vigour (Baenziger 1969) and yields were unaffected by this treatment (Table XXXIV).

During imbibition of grains, the cellular structure of the endosperm is broken down to produce a milky tissue which contains reserve carbohydrates, lipids and proteins (Mayer et al 1963, Peterson 1965). Enzyme systems are activated and nutrients from the endosperm are transported via the scutellar vascular system to the young embryo (McCall 1934). Marcus et al (1966) report that the protein-synthesizing ability of young embryos develops during the first thirty minutes of imbibition in young seed, but is delayed in aged seed. Mounfield (1936) also noted that protein breakdown in wheat grains was increasingly delayed with storage time. This may account for the longer time required for the germination of stored Marquis grains (Fig. 20).

Following enzyme activation, storage proteins in the endosperm are broken down into amino acids which are then transported to the embryo where they are synthesized into metabolically active proteins (Chibnall 1939, Folkes and Yemm 1958). Jones and Pearce (1967) found that the loss of many amino acids from the endosperm was identical with the gain in the embryo. This was not true for all amino acids and the result of germination was a net loss of glycine and glutamic acid and gain of proline, lysine and aspartic acid. However, changes in the ratio of amino acids (Aronoff 1962, Wang 1968) and proteins (Daussant and Abbott 1969) varies with species and stage of germination. Oaks (1965) suggested that degradation of storage protein in the endosperm may be regulated by amino acid requirements in the embryo.

The amino acid pattern in the present study is further complicated by vernalization of the grains. As vernalization proceeds, new proteinsare formed in the embryo (Teraoka 1967) and the patterns of amino acids change (Trione 1966, Trione et al 1967, Jones 1969). Trione et al (1967) found that, in general, the free amino acid content of wheat seedlings increased during the first two weeks of vernalization, decreased sharply during the third week and increased again during the fourth week.

In the present study, evidence of protein break-down in the endosperm, transfer of amino acids to the embryo and protein synthesis in the embryo are suggested by the larger amounts of free amino acids in the embryo, when compared with the endosperm (Tables XVI and XVIII). The changes in the proportion of amino acids in the embryo, compared with proportion in the endosperm suggest the interconversion of many amino acids during these processes. Our results support those of Steward and Beevers (1967) which similarily indicated that interconversion may occur in the endosperm as well as in the embryo. The higher amino acid content in both the embryo and endosperm of the control series, when compared with the sound-exposed series suggest that more protein may have entered the respiratory cycle or have been converted to some other nitrogenous compound in the sound exposed series. Steward et al (1956) found that protein synthesis and respiration are closely related and McConnell (1957) noted that protein is used as an obligatory

substrate during seed respiration. Respiration measurements in the present study do not indicate an increased rate of respiration in the sound-exposed grains at 2°C although respiration appears to be accelerated when grains are germinated at 25°C (Table XIII). However, Folkes and Yemm (1958) found that the relationship between the rate of respiration and protein metabolism is not a simple quantitative one and depends both on the amount of protein and its rate of formation. In addition, the rate of respiration fluctuates during germination (Mayer and Poljakoff-Mayher 1963, Yemm 1965). Therefore respiration measurements of germinating grains is questionable unless continuous measurements are made during germination. Also amino acids may enter other metabolic pathways than those leading to respiration and protein synthesis.

Steward et al (1956), using cultured carrot cells, showed that although the total amount of protein produced in the cultures depended on the rate of growth of the cells, the relative proportions of amino acids was almost unaffected. The same relationship can be seen in the present study when amino acid content is expressed on a percentage basis (Tables XVII and XIX). Differences in total amino acid concentration between the control and sound-exposed grains are minute. However differences of some of the free amino acids appear significant.

The percentage of free alanine in the embryo of the sound-exposed grains is almost double that of the control series, while that in the endosperm is almost half that of the control

series. This may partly be due to an accelerated transport from the endosperm to the embryo, since Jones and Pierce (1967) found that losses from the endosperm of germinating barley grains parallels gains in the embryo. Steward and Durzan (1965) found that alanine was present in large quantities in the soluble nitrogen component of rapidly growing cells. Hence the larger proportion in the embryo of sound-exposed grains might indicate a more rapid rate of development. Alanine may also affect morphogenetic development since Virtanen and Linkola (1946) found that pea plants fed with this amino acid showed an abnormally high degree of branching. Hence the presence of large amounts of this amino acid in the embryosof the sound-exposed series may be related to the large number of tillers produced by these plants.

The percentages of free glycine and aspartic acid are larger in the endosperm of the sound-exposed series, while that of asparagine is higher in the control series. Experiments performed by Oaks (1965), suggest that break-down of storage protein in the endosperm is controlled by the demands of the embryo. Also Steward and Beevers (1967) suggest that amino acid interconversions may occur in the endosperm. Thus large quantities of glycine in the endosperm may indicate a large demand by the embryo for this amino acid. In addition, the presence of smaller amounts of asparagine in the sound-exposed series compared to amounts of asparagine in the control series suggests that ammonia released during deamination is entering some alternate pathway in the sound-exposed series.

Since glycine and ammonia are used as precursors to RNA (Webster 1959, McKee 1962), RNA synthesis may have occurred at a more rapid rate in the sound-exposed series than in the control series. Oota and Osawa (1954a, 1954b) noted that intensive protein synthesis occurs only during the early stages of germination and appears to be related to RNA metabolism. Marcus and Feeley (1964) suggest that the soluble enzymes necessary for protein synthesis may be active before germination, but that imbibition is necessary for the activation or formation of messenger RNA. Webster (1959) found evidence to suggest that nucleic acid synthesis is necessary for, and coupled, with protein synthesis. In addition, although RNA, DNA and protein content all increase during cellular elongation of roots (Heyes 1959), the amount of RNA synthesis while cells are still in the apex determines both the rate of elongation and final size of the cell (Woodstock and Skoog 1962). The amount of DNA synthesis has no effect on rate of elongation (Woodstock and Skoog 1960). Smillie and Krotkov (1959) found that RNA content of pea leaves gave a good index of their metabolic activity.

In the present study, embryos of sound-exposed grains showed a significantly larger amount of RNA than embryos of control grains (Table XX). This indicates that the glycine demand by the embryos of the sound-exposed grains is indeed larger than that of the embryos of the control grains. In addition, glycine, together with succinyl CoA is utilized in the initial stages of chlorophyll synthesis (Devlin 1967).

Boulter and Barber (1963) suggest that loss of glycine from bean seeds may be correlated with an increase in chlorophyll. Hence the larger amounts of glycine in the endosperm of the sound-exposed grains may reflect a larger demand by the embryo of these grains to be used in both RNA, and protochlorophyll synthesis.

However, the total amino acid content in the embryo of the control grains is higher than that of the sound-exposed grains. Coupled with the higher growth rate of sound-exposed grains, this suggests a rapid turnover and utilization of these precursors of protein synthesis.

Since enzymes are part protein, changes in amino acid and protein metabolism could affect enzyme activity. Kozubov and Gamyusukina (1964) and Obolensky (1957) found enzymatic activity to be stimulated in seeds exposed to ultrasound. The activity of peroxidase was tested in the present study since this enzyme is widely distributed throughout the plant kingdom (McHargue 1920), is easy to detect (Buttery and Buzzell 1968), and peroxide content of corn grains has been reported to be affected by exposure to ultrasound (Elpiner and Bronskaya 1960). In addition, Ridge and Osborne (1970) found that both protein synthesis and RNA synthesis are related to peroxidase activity.

The activity of this enzyme did not appear to be affected by audible sound, but the testswere made on whole grains at only two stages of development. However, the activity of this

enzyme changes with plant part (Buttery and Buzzell 1968) and age Daussant and Abbott 1969). The tests, while not conclusive, do indicate that at the stages of growth used here, a change in peroxidase activity in the grain cannot be used as an indicator of the subsequently observed sonic-stimulated growth.

Since cell division and cell elongation are two separate processes which occur during germination (Haber and Luippold 1960) it was decided to count the mitoses in the root tips of recently sprouted Rideau wheat grains. It was found that cell division (Table XXI), as well as elongation (Fig. 21), was unaffected by audible sound. This was reflected in the DNA content of the embryos of vernalized grains (Table XX), since DNA content was the same in both the control and the sound-exposed series.

During the germination of grain, nutrients from the endosperm are transported to the embryo via the absorbing epithelium of the scutellum (McCall 1934). Since the permeability of cytoplasmic membranes is one major factor which controls the movement of substances from one cell to another, any process which alters the permeability could conceivably alter the metabolism and hence germination and growth patterns of the young embryo.

Howkins (1967) found the rate of dialysis across a semi-permeable cellophane membrane to be increased during exposure to 14KHz sound. Ste Marie (1970), similarily, found an increase in the rate of dialysis of glucose during exposure to ultrasonic frequencies of 25KHz and 45KHz. The effect was more pronounced at the higher frequency.

The rate of diffusion across a semi-permeable membrane depends on the difference in concentrations on the two sides of the membrane, the permeability of the membrane for the solute and the surface area of the membrane (Renkin 1956). Synthetic membrane permeability is dependent on the distance a molecule must travel through the membrane, the ratio of molecular diameter to hole diameter and the frequency with which the molecules hit the membrane (Leonard and Bluemle 1958). The latter in turn is dependent on the diffusion constant of the solute since the molecules nearest the membrane are transferred first, leaving a concentration gradient from the membrane to the centre of the solution.

Two factors which increase the rate of dialysis are temperature rise (Howkins 1967) and rapid stirring of the solutions (Leonard and Bluemle 1960). Since the dialysis measurements made by Howkins and Ste. Marie were made while the ultrasonic system was operating, these two factors could account, at least partially, for the increased rates of dialysis in the presence of ultrasound. The only permanent change, i.e., a change which was still apparent after the ultra-sound was turned off, reported by Howkins, was in an experiment where the sound amplitude was sufficient to cause cavitation. In this case, permeability was decreased, not increased, following exposures to ultrasound.

The present study, utilizing audible sound, gave no indication that the 5KHz frequency has any effect on the

permeability of synthetic membranes. Only permanent effects were studied because plant membranes and hence synthetic membranes were exposed to sound for a much longer time than that required for the diffusion of glucose through the membrane. This study was deemed adequate since audible sound had a lasting effect on plant growth as wheat plants grown from grains, exposed to sound only during the vernalization period, grew larger than plants grown from control grains. Hence the sound must have initiated some change inthe cells of the embryo that could be transferred to subsequent cell generations, although it could not be transmitted to subsequent plant generations (Table XXIX).

The transfer of ions across a biologically active membrane is much more complicated than that across an inert one since active transport, requiring metabolic energy, as well as diffusion takes place. Hence factors such as low temperature and low oxygen tension which inhibit metabolic activity of plants will also reduce ion accumulation by the plants.

Distribution of nutrients within a plant depends on their initial entry into the root, their transfer to the stele of the root, their upward rate of movement to the shoots and their downward movement back to the roots. Thus a nutrient entering a root may accumulate in the cell sap, be used in a metabolic process or be transported radially and secreted into the xylem (Russell et al 1953). The degree of circulation within a plant

depends on many factors including the nature of the molecule. For example, phosphorus is continually recirculated throughout the plant whereas calcium usually remains where it is deposited (Biddulph et al 1958). The nutrient status of the plant is also important, as there is an initial rapid entry of ions into a plant if the roots are transferred from one nutrient solution to another more concentrated one (Russell and Martin 1953). Conversely, when roots or tissues are transferred to more dilute solutions there is an initial rapid outflow of ions. Also, actively growing tissues such as meristems and young leaves successfully compete for nutrients with less active tissues and may accumulate large amounts of some nutrients by withdrawing them from older tissues (Steward and Sutcliffe 1959).

The initial entry of phosphate into a root may be partially due to diffusion and partially due to active transport since uptake is reduced in the presence of 2,4-dinitrophenol (Loughman and Russell 1957). Phosphates entering the root are rapidly incorporated into the organic form (Loughman and Russell 1957) and may be retained in the root if the nutrient status of the plant is low. The authors suggest that this retention of phosphates by the roots is metabolically controlled since metabolic inhibitors induce a more rapid upward movement to the shoots (Russell et al 1953).

In the present study, P-32 appeared to accumulate more readily in the roots of plants of the control series than in

those of the sound-exposed series (Table XXV). However, upward transport did not appear to be affected by sound-exposure since the radioactivity found in the shoots was the same for the two series. Therefore any changes in phosphorus metabolism or movement appear to be related to factors other than itsrate of movement into the stele.

Phosphate absorption can be affected by microbial contamination of the roots (Bowen and Rovira 1966, Bowen 1969). Barber (1968a) and Shone (1970) suggest that many phenomena attributed to absorption by plant roots can be explained by the presence or absence of micro-organisms. For example, Epstein (1966) reports that curves showing the effect of concentration on the rate of ion absorption by plant roots have two peaks of maximum uptake. The presence of two peaks may be due to different rates of transport across the plasmalemma and across the tonoplast (Laties 1967) or to the ability of micro-organisms located within and around the root (Barber 1968b) to absorb ions at a lower nutrient concentration than that required by plant roots (Barber 1968a). Rapid incorporation of the majority of phosphates into nucleic acids in roots of plants absorbing phosphorus from dilute solutions (Loughman and Russell 1957) may also be due to absorption by micro-organisms since the percentage incorporated is much higher in non-sterile plants than in sterile plants (Barber 1966). In addition, since the relative amounts of $H_2PO_4^-$ and $HPO_4^=$ in solution depend on pH, and since the activity of micro-organisms cultured

from plant roots also depends on pH (Barber and Loughman 1967), the different sites for absorption of these two ions (Hagen and Hopkins 1955, Hagen et al 1957) may, in reality, be the effect of increased absorption by micro-organisms at higher levels of pH (Barber and Loughman 1967).

Although chloramphenicol inhibits bacterial growth and division by preventing the incorporation of amino acids into proteins, the cells are not killed and continue to incorporate amino acids into soluble RNA (Wilson 1966). However, moderate bacterial contamination (10^6 to 10^8 cells per g tissue) does not significantly affect measurements of P-32 incorporation into RNA by soybean plants (Sobota et al 1968). In the present study P-32 concentration in the roots was reduced by 74% in both the control and sound-exposed series when the plants were grown under sterile conditions although translocation to the shoots appeared to be unaffected. This suggests that much of the P-32 which appeared to be in the root fraction of the non-sterile plants was actually in the microflora associated with the roots, and that differences in absorption between the control and sound-exposed series were probably direct effects on the roots themselves and not indirect effects caused by inhibition of the micro-organisms by the audible sound.

Rovira and Bowen (1966) also found P-32 uptake to be higher in non-sterile wheat roots than in sterile ones. In addition they found that the roots of sterile plants had a higher fresh weight than roots of non-sterile plants. In the present study, however, root and shoot growth of sterile plants was greatly

reduced when compared with that of non-sterile plants (Fig. 25). This may have partly been due to the addition of the chloramphenicol to the culture solution since antibiotics may be harmful to plants when applied in amounts necessary for the control of micro-organisms (Leben and Keitt 1954). Antibiotics have been shown to inhibit ion absorption (MacDonald et al 1966), cause root damage (Purdy 1967) and inhibit amino acid incorporation into chloroplast proteins (Margulies 1962, 1964, Margulies 1970). However, the concentration of chloramphenical used in the present study was 20 µg/ml, the concentration suggested by Jones and Varner (1967b) to effectively inhibit bacterial activity without affecting plant metabolism.

The results of the present study contradict those of Barber and Loughman (1967) and Barber (1968a) who found that sterile barley plants both accumulated more phosphorus in the roots and translocated more to the shoots than non-sterile plants. Bowen and Rovira (1966) found that both the roots and shoots of non-sterile plants accumulated more phosphorus than those of sterile ones. They and Subba-Rao (1961) concluded that changes in root absorption in the presence of micro-organisms are caused by alteration of root metabolism of infected roots, as well as by nutrient uptake by the organisms themselves.

Uptake of a particular ion may be stimulated, inhibited or unaffected by the presence of other ions in the external solution (Lazaroff and Pitman 1966, Legget 1968). For example, Tanada (1955) found that phosphate absorption of mung bean roots was

increased when either calcium or magnesium was added to the culture solution, but was decreased when sodium chloride was added. In the present study, Rideau wheat plants absorbed less P-32 from culture solution than from distilled water. Absorption appeared to be inhibited much more in plants grown from sound-exposed grains than in control plants. This suggests that a portion of the active transport mechanism (Bowling et al 1966) may have been altered when the grains were exposed to audible sound. Since phosphorus is a nutrient that is constantly recirculated within the plant (Steward and Sutcliffe 1959), an alteration of the movement of this element could conceivably affect plant development.

Evidence that audible sound may affect mineral transport was shown in the British Columbia field trial (Table XXXI). During the second year of growth, yield of unfertilized plots of reed canarygrass grown from sound-exposed seed was comparable to that of control fertilized plots, whereas yield of control unfertilized plots was greatly reduced. Gregory (1953) reported that by the time a developing cereal plant has grown to 25% of its final size, measured, by dry weight, it has accumulated 90% of its total phosphorus. Williams (1938) suggests that accumulation of nitrogen and phosphorus in fertile tillers at the expense of non-fertile ones is probably the cause of the death of non-fertile tillers.

In the present study, wheat plants which produced heads (field trial) showed no difference in tiller number between the control and sound-exposed series, whereas plants which

reached only the initial stages of head production (laboratory studies) showed an increase in tiller number in the sound-exposed series. In the field trial, the developing heads may have produced a strong drain on non-fertile tillers, thus exhausting their phosphorus supply and promoting an early death. In the laboratory studies, drain from older tillers may have been less severe, hence the slower rate of movement of phosphorus noted in the sound-exposed series may have helped to prolong the life of older tillers, thus inducing a larger number of tillers per plant.

However, very few dead tillers were found on any of the plants grown for the laboratory studies. This suggest that the larger tiller number in the sound-exposed series was due to a stimulation of tiller growth rather than to the retention of older tillers.

The axillary buds, which give rise to the tillers of the grass family are produced very early in the development of the young plant (Hamilton 1948). In wheat plants, these buds are usually present in the axils of all leaves, including the coleoptile and the prophylls (Friend 1965c). Environmental conditions, such as temperature, light intensity (Aspinall and Paleg 1964, Friend 1965c), soil fertility and plant density (Peterson 1965), determine whether these buds will develop into tillers or will remain dormant. Usually, no new tillers are produced after the head emerges (Ashby and Hellmers 1959, Friend 1965c), but those already initiated may continue to grow (Troughton 1955). Since wheat plants have a terminal inflo-

rescence, leaf number per tiller is restricted to the number of leaves differentiated on the apical meristem at the time of floral induction (Friend et al 1963). Some species can be induced to continue tillering after the head emerges if the plants receive an adequate nutrient supply (Joffe and Small 1963).

During early growth of young wheat plants, photosynthetic assimilates are translocated throughout the plant. The main shoot supplies the carbohydrates necessary for early stages of tiller development (Evans et al 1964), although this inhibits growth of the main shoot (Tincker and Jones 1931). During ear development, tillers may become autotrophic (Lupton 1966) or may still depend on the main shoot for assimilates for their developing ears (Rawson and Hofstra 1969).

Since the rate of tillering is dependent on the rate of assimilation (Friend 1965c), studies were undertaken to determine whether photosynthetic rates of plants grown from sound-exposed grains were higher than those of plants grown from control grains. However, neither the CO_2 production in the dark nor the net CO_2 uptake in the light were different in the two series (Table XVI), thus indicating that neither respiration nor net photosynthesis were affected by exposure to the sound. The results of the present study contradict those of Singh (1959) and Gnanam and Rajaraman (1961) who found photosynthesis of water plants and blue-green algae to be stimulated by exposure to music. Also Kozubov et al (1964) found respira-

tion rates of germinating pine and spruce seeds to be accelerated following exposure to ultrasound.

In the present study, the rates of respiration of control and sound-exposed grains were measured in two ways, namely by means of the manometric technique and by means of an infrared carbon dioxide analyzer. The Warburg apparatus has the advantage that both oxygen uptake and carbon dioxide production can be measured. However, the absolute values of the volumes of gas exchange measured by this method are subject to question.

Comparative measurements using this apparatus require that the total volume of materials be the same for each flask and be the same as the volumes used in the calculation of the flask constant (Kurtz and Kellor 1966). As there is a high degree of variability in the size of grains, constant volume would be possible only if the volume of each test lot of grains were measured and an appropriate volume of buffer was added to each flask. However, this would require the addition of extra buffer to flasks with smaller grains and less buffer to flasks with larger grains. Thus smaller grains would become more submerged than larger grains and their rates of respiration might be altered. Therefore, although grains were selected for equal size, as well as was possible by eye, no correction was made to allow for the 10% variation in size. Hence some degree of error was introduced into the calculated results.

Also, since manometric measurements are based on changes

in volume, there is no way to distinguish between the exchange of oxygen and carbon dioxide and the evolution of other gases. Several authors have shown that ethylene, not only affects many physiological stages of plant growth, but is also produced in large quantities during various periods of plant development (Burg and Burg 1967, Pratt and Goeschl 1969). Two peaks of ethylene production occur during the germination of seeds and the growth of young seedlings (Meheriuk and Spencer 1964 and Spencer et al 1965). Since our experiments covered these two stages of early development, ethylene production may have affected our measurements. In addition, Spencer and Olsen (1965) found that ethylene production was correlated with carbon dioxide production throughout the germinating period. Thus fast-growing tissues with a high respiration rate are also producing large volumes of ethylene.

In addition, microflora present on the surface of seeds produce enough carbon dioxide to significantly affect respiration measurements (Denny 1948). Although the grains in the present study were treated with chloramphenicol, the microflora could still be present in large numbers since this antibiotic inhibits, but does not kill, bacteria (Wilson 1966).

Wilson et al (1969) also questioned the use of the Warburg apparatus for photosynthesis measurements. Their criticism lay in the necessity to detach the leaves of large plants in order to make the measurements, and in the inability to measure the exact amount of carbon dioxide available to each leaf

because of gradients set up in the absence of a rapidly moving air stream. They concluded that the Warburg method was satisfactory only for comparative studies of photosynthetic rates. It is proposed that in the present study, the volumes of oxygen uptake and carbon dioxide production be considered only as a relative measurement between control and sound-exposed grains and that the actual numerical values be considered only a rough approximation of the rates of respiration. Measurements made by means of the infra-red carbon dioxide analyzer are considered to be more reliable. However, they are not exactly comparable to measurements made by the manometric technique because the two sets of data were accumulated during different stages of embryo development.

III Effect of Audible Sound on Bacterial Motility

Although P-32 uptake by the bacterial fraction did not appear to be affected by exposure to audible sound, it was decided to isolate some of the micro-organisms present on the seed coat of Rideau wheat grains and subject them to sound while they were growing. It was interesting to note that colonies of one motile bacterium grew more slowly in the presence of sound than in control chambers. Further tests on a strain of E. coli selected for its extreme motility showed that these bacteria migrated on soft agar at a much slower rate when left in high intensity audible sound than when left in low intensity sound (Fig.28). This suggests that the sound

may have had some effect on the bacterial flagella. Flagella are extremely sensitive and can be removed from cells by boiling, extremes of pH or violent shaking (Oginsky and Umbreit 1959, Salle 1961). The sound waves may have impaired the flagella of some of the cells, so the colonies were able to grow only when the, presumably few, non-sensitive cells swam out.

IV Practical Applications of Present Study

Since sonic equipment is expensive, yield increases must be significant to warrant the sonic treatment of seeds on a large scale.

Although wheat plants could be induced to produce a larger number of tillers in sonic studies in the laboratory, tiller numbers in the field were identical with these of control plants. This was very disappointing since each tiller has the potential to produce a head of grain. In a country such as Canada, where large quantities of wheat are produced annually, there is no need to increase crop yield. However a pre-sowing treatment that could induce an increased yield would be extremely beneficial to overpopulated areas of the world. Growth increments induced by ultrasound (Albu and Auslander 1967) or by audible sound (Table XXIX) do not appear to carry over to the next generation. Crop maturity has been reported to be hastened following exposure to ultrasound (Johnson and Obolensky 1954) but morphogenetic development appeared to be unaltered in the present study (Table XI).

Although yield increases were not found in the present study, audible sound may still prove to be a useful tool since the present study scanned only the effects of four frequencies and three intensities.

Yield of wheat can also be increased by pre-sowing treatment of grains in a magnetic field. However this phenomenon must be carefully studied before it is applied commercially, since long exposure periods and high gauss levels decrease, rather than increase, yield.

It appears that it may be possible to increase the yield of forage crops, such as alfalfa, by treatment with audible sound. This would be extremely beneficial to ranchers, since a twofold increase in yield would allow a doubling of the number of cows raised per unit area.

Thus it appears that such studies have the potential for practical applicability, but that much more research must be done before such methods can be used on a large scale.

V Possible Mechanisms Involved in Plant Response to Audible Sound and Magnetic Fields

A) Audible Sound

a) Transfer of energy from Sound Waves to Cellular Constituents

In the present study, growth of Marquis wheat was accelerated by both 300Hz and 5KHz frequencies, whereas Rideau wheat responded to only the 5KHz sound. Siekman (1969) found that

the absorption coefficient of Merion bluegrass, another member of the Graminae, increased with frequency rise over the range of 120 to 4KHz. At 4KHz the absorption coefficient was 0.99 thus indicating that the sound was almost completely absorbed by the bluegrass at this frequency. Unfortunately, his measurements did not extend to higher frequencies. Nevertheless, his results suggest that the maximum effect noted in our studies at 5KHz may be related to maximum absorption near this frequency. Maximum absorption probably does not account for the effect of 300Hz sound on Marquis wheat since the absorption coefficient of the bluegrass was only 0.26 at 250Hz. Smith (1967) also found plant growth to be stimulated by a low frequency (450 Hz). Since both germination and later stages of growth of Rideau wheat appear to be accelerated by exposure to audible sound (Fig. 3, Table VI) whereas intermediate stages of growth appear to be unaffected (Table IV), it seems likely that at least two different systems are affected by audible sound.

The total amount of energy received by treated seeds or grains seems to be important in both audible sound and ultrasonic studies. In the present study, growth of Rideau wheat was stimulated by exposure to 5KHz sound when the intensity was 92 db. However, when the intensity was increased to 105 db, the plants were no longer stimulated. Jonas (1955) found that the germination rate of onion seeds was accelerated by exposure to ultrasound, when the power input was 10-15 watt/cm^2 but was no longer affected when the power input was increased

to 20 watt/cm^2. Timonin (1966) found a similar effect when he increased the exposure period of white spruce trees to ultrasound. Exposure for one minute had no effect; exposure for two minutes doubled the percentage germination; exposure for four minutes stimulated germination slightly, but not as much as exposure for two minutes; exposure for six minutes was lethal.

However, the effects of audible sound are not comparable with those of ultrasound because of the differences in energy. The 92 db level used in the present study is roughly equivalent to 7.5×10^{-6} watt/cm^2 whereas the intensity used in most ultra-sonic studies approximates 10-50 watt/cm^2. Hence the amount of energy added by the sound frequencies in the present study is very minute being only a fraction that required to break a chemical bond and too low to cause cavitation of water.

However, the amount of energy is sufficient to produce a resonance effect in any cellular organelle or molecular structure which has a natural period of vibration corresponding to that of the added sound. In such a case, the effects of individual successive vibrations of the sound waves would be cumulative and might induce larger vibrations. This might, in some manner, affect cellular biochemical or biophysical processess which are connected with germination and growth. If this were the case, it would seem probable that harmonic frequencies would produce similar effects. However, 1250Hz was found to have no significant effect on the growth of

Marquis and Rideau wheats, although growth was stimulated by exposure to 5KHz, the fourth harmonic of 1250Hz, in both cases. On the other hand, a resonance effect could explain the lack of response to random white noise, since a larger vibration induced by sound of one wave length could be negated by sound of another wavelength.

Goodwin (1969) has suggested the following hypothesis to explain cell dynamics in terms of thermodynamic oscillations. He suggests that living cells are resonating systems made up of coupled oscillators which shift spontaneously between various resonant modes. The talandic temperature, which increases as the size of the oscillations increase, indicates how energy will be distributed among feedback control systems. The synthesis of mRNA and proteins is an example of strongly coupled oscillators while the interaction between oscillators through pools of metabolites are examples of weak coupling. Interactions may occur when two oscillators with different frequencies and phases lock together in the same frequency and phase or when one oscillator is beating at a frequency that is subharmonic to that of the other oscillator. Goodwin suggests that resonance produced in this way could be important in controlling the phase and time relations of cellular biochemical activities.

Since the talandic temperature is a measure of energy and since, according to Goodwins theory, oscillations increase as the talandic temperature increases, energy added

to the system at the same frequency as the coupled oscillators could increase the oscillations and hence certain biochemical activities. In the present study, the audible sound frequencies which stimulated plant growth may have been the right wavelength to increase the resonance of one or more sets of coupled biochemical oscillators, whereas the other frequencies were not. The two frequencies affecting Marquis wheat, namely 300Hz and 5KHz may have stimulated plant growth by increasing the oscillations of two different systems.

b) Effects of Sound on Water

Another hypothesis involves the effects of sound on water. Since there is no thermal expansion of water at $4^{o}C$, a phase shift of the sound wave may occur at lower temperatures. If the rate of germination is not linear with regards to temperature and pressure, grains may germinate faster in one part of the sound wave than in another. If this is true, then temperature and pressure may re-inforce one another at $2^{o}C$ but not at $25^{o}C$. This then could account for increased germination rates of grains subjected to sound at $2^{o}C$ but not at $25^{o}C$.

Also, one of the effects of high intensity ultrasound is to fracture water molecules into H, OH and HO_2 radicals

(Ackerman 1962, Hamill 1969). These radicals are very active, chemically, and may produce biological effects. Although the energy used in the present study is insufficient to cause cavitation, the energy could be absorbed by only a few molecules, instead of being evenly distributed throughout each cell. Hence local sites of high chemical activity might be set up. This in turn could alter the biological activity of the cell. Lisenkov (1966) found the germination of larch and maple seeds to be accelerated by treatment with sound-treated water, as well as by treatment with sound and ultrasound.

3) Magnetic Fields

The growth rates of bacteria (Gerencser et al 1962), cells in tissue culture (Mulay and Mulay 1961, Butler and Dean 1964, Drosophila (Levengood 1967) and mice (Barnothy 1963) have been reported to be inhibited by exposure to magnetic fields. In contrast, growth of barley (Montgomery and Smith 1962, Mericle et al 1964) and wheat (Pittman 1963) is reported to be stimulated. The present study (Tables XXXVI-XXXVIII) indicates that growth of Marquis wheat can be either accelerated, inhibited or unaffected by pre-germination exposure to magnetic fields. The type and degree of response depends on the length of the exposure period, the field strength and embryo position in relation to the field and/or interactions between these three parameters.

Akoyunoglou (1964) has found the activity of the enzyme carboxydismutase to be increased in the presence of a magnetic field, whereas Maling et al (1965) and Rabinovitch et al (1967) found the activities of ribonuclease, peroxidase and aldolase to be unaffected. Respiration is stimulated in yeast cells (Pereira et al 1967) but inhibited in mouse embryo kidney cells (Reno and Nutini 1963). Gross (1964) postulated that inhibition of metabolic processes in the presence of a magnetic field may be due to a distortion of bond angles on paramagnetic molecules. Butler and Dean(1964) have suggested that since most chemical substances are diamagnetic, but may become paramagnetic in the ionic form, the presence of a permanent magnetic field could interfere with ionic exchanges and thus alter cellular metabolism. However, the effects of magnetic fields on plant growth appear to be permanent effects, since the result of exposure of many kinds of seeds to magnetic fields is revealed in their later growth.

Montgomery and Smith (1963) postulated that permanent effects might be induced by the action of magnetic fields on cobalt, manganese and iron. These elements play an important regulatory role in meristematic growth of young seedlings. Since the availability of these elements is often limiting and since they are magnetically sensitive, their rate of transport in young embryos might be accelerated and the growth rate of the plant increased when seeds are placed in magnetic fields. At high intensities, or long exposure periods, these elements might accumulate in toxic levels and thus inhibit plant growth.

Such a theory could therefore explain the importance of field strength, and exposure period in determining the effects of magnetic fields on plant growth. High intensities and long exposure periods might also affect the configuration of protein molecules and thus affect enzyme activity.

VI Statistical VS Biological Significance

The level of significance chosen for any statistical analysis is very arbitrary and open to criticism. If α, the probability of rejecting a true hypothesis, is too small, then β, the probability of accepting a false hypothesis becomes very high. Thus a level of significance which balances these two types of error must be chosen.

The choice of an α in botanical studies is very difficult since there is a high degree of variability within plant populations (Heinrichs et al 1969). Variability in the present study was further increased by growth chamber effects in the laboratory studies (Fig. 5-14) Owing to the controversial nature of the audible sound and magnetic field studies, it was deemed preferable to risk choosing an α that was too low and risk a high β rather than choose an α that was too high. Since the hypothesis was always one of equal means, some of the data considered statistically insignificant may in fact be biologically significant.

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