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Some Effects of Simulated Acid Rain on Cool Season Turfgrasses

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SOME EFFECTS OF SIMULATED ACID RAIN ON

COOL SEASON TURFGRASSES

(TITLE)

BY

Robert C. Morrow

THESIS

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ABSTRACT

Six varieties of cool season turfgrasses were exposed to simulated acid rainfall with treatments consisting of a sulfuric acid solution, a nitric acid solution, and a 50-50 mixture of both. Each solution was used to make "acid rain" of pHs 3.0, 2.5, 2.0, and 1.5.

Height measurements showed decreases in growth throughout the experiment for all treatments except the nitric and 50-50 acid treatments at pHs of 2.0 and 1.5, which maintain fairly constant growth. Analysis of nitrate, phosphorous, and potassium levels in the soil indicated heavy leaching of the nitrates and potassium from most soil samples, which probably account for the reduced growth observed. There appeared to be an increase in leaching of potassium from samples recieving the more acidic treatments. Grasses with little decrease in growth showed greater foliar injury than did the stunted plants. Greater foliar injury was also observed at the beginning of the experiment when all the plants were fairly uniform in height. Soil pH showed little change except for the pH 1.5 sulfuric acid treatments, which caused some increase in acidity. There was no correlation between the soil pH and turfgrass height or foliar injury.

A separate, related experiment was conducted to investigate a new chlorophyll extraction procedure reported in the literature for obtaining chlorophyll concentrations

expressed as mg chlorophyll per gram dry weight.

Chlorophyll extracts from the injured plants showed a reduction in chlorophyll A, chlorophyll B, and total chlorophyll. Injured plants also showed a decrease in chlorophyll A to B ratios. In addition, higher percentages of chlorophyll were extracted from uninjured tissue than from injured tissue.

Length of storage studies indicated that chlorophyll extracts were stable for at least ten days when stored in the dark.

ACKNOWLEDGMENTS

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I would also like to acknowledge my parents, who can finally relax, now that this thesis is completed.

Special thanks are due my wife, Janet, for her help during this study, and for putting up with the long hours spent in the Life Science building and the library during this study.

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INTRODUCTION

Acid precipitation is generally defined as precipitation with a pH of less than 5.6, which is considered the lower limit of natural rain or snow. The presence of carbonic acid formed from atmospheric CO_2 and H_2O accounts for this slight acidity (Anonymous, 1979).

The major increase in the acidity of precipitation observed over the past two decades is believed to be the result of sulfur and nitrogen oxides emitted by various anthropogenic sources. These oxides undergo oxidation and hydration in the atmosphere to become nitric and sulfuric acids (Gunnerson et al., 1979).

Some of the environmental effects attributed to acid precipitation thus far are acidification of lakes, rivers, and ground waters (resulting in damage to fish and other aquatic organsims), acidification and release of metals from soils, deterioration of man made materials such as buildings, statuary, metal structures, and paint, possible contamination of drinking water from metals released from soil and pipelines, and damage to vegetation (Anonymous, 1979). The most commonly observed plant injuries include leaf deformations (wrinkling and curling) and necrosis of leaf tissue (Hindawi et al., 1980; Ferenbaugh, 1976; Wood et al., 1974), reductions in chlorophyll content and integrity (Hindawi et al., 1980; Ferenbaugh, 1976), reductions in carbohydrate concentrations (Ferenbaugh, 1976), foliar leaching of nutrients (Hindawi et al., 1980; Fairfax and Lepp, 1975; Wood and Bormann, 1975; Eaton et al., 1973), reductions in yield and biomass (Harcourt and Farrar, 1980; Hindawi et al., 1980; Lee et al., 1980; Mohamed, 1978; Ferenbaugh, 1976; Wood and Bormann, 1974), and alterations in host-parasite interactions (Shriner and Cowling, 1980; Shriner, 1977).

This study was concerned with general morphological and physiological changes in cool season turfgrasses exposed to simulated acid rain. Studies on a variety of crop grasses seem to indicate that these grasses (and monocots in general) are much more resistant to acid rain than are dicots, but lesion development has been observed in many of them (Lee et al., 1980; Mohamed, 1978).

Six varieties of cool season turfgrasses (Table 2) were utilized in this experiment because of their great economic and aesthetic importance in the midwestern United States, being used in lawns, parks, cemeteries, athletic fields, and other similar areas which require durable, attractive ground covers (Beard, 1973).

LITERATURE REVIEW

For the past twenty years a sharp increase in the acidity of rain and snow has been recorded for the Eastern United States, as well as for other parts of the world. This increase has been generally attributed to the oxidation and hydration of nitrogen and sulfur oxides emitted from anthropogenic sources such as coal burning power plants and vehicle exhausts.

The oxides of nitrogen present in the atmosphere are N_2O, NO, and NO_2. NO is the most common form, being formed by combustion at high temperatures (Haagen-Smit, 1976). In the atmosphere NO reacts with O_2 to form NO_2:

$2NO + O_2 \text{ --- } 2NO_2$ (Schuck et al., 1958)

At high concentrations of NO this reaction occurs in a matter of seconds, but at low concentrations it is much slower. However, ozone will oxidize NO rapidly, even at low concentrations:

$NO + O_3 \text{ --- } NO_2 + O_2$ (Air Pollution Found., 1960)

In the presence of ozone, NO_2 is readily oxidized to NO_3^-:

$NO_2 + O_3 \text{ --- } NO_3^- + O_2$ (Cox & Penkett, 1971)

Then to N_2O_5:

$NO_2 + NO_3^- \text{ --- } N_2O_5$ (Hellner & Keller, 1972)

Which can then be hydrated to nitric acid:

$N_2O_5 + H_2O \text{ --- } 2HNO_3$ (Adel, 1951)

The same results are attained in fog droplets, where hydration and catalytic oxidation lead to complete conversion of NO_2 to nitric acid:

$$4NO_2 + 2H_2O + O_2 \text{ --- } 4HNO_3$$ (Johnston & Yost, 1949)

The most common oxide of sulfur present in the atmosphere is SO_2, which is released by the combustion of sulfur containing materials. The processes by which SO_2 is oxidized to sulfuric acid in the atmosphere are not well understood. One proposal is that the SO_2 released during combustion is oxidized to SO_3, which then reacts with water vapor to form a sulfuric acid mist (Kellogg et al., 1972):

$$SO_2 + O + m \text{ --- } SO_3 + m$$

were m is a molecule of O_2 or N_2 which carries off the excess energy, preventing the prompt reversal of this reaction. The SO_3 formed in this reaction reacts with water vapor almost immediatly to form H_2SO_4:

$$SO_3 + H_2O \text{ --- } H_2SO_4$$

which then combines with water to form droplets of H_2SO_4 solution. The amount of SO_2 oxidized to SO_3 depends upon a number of factors, including the duration and intensity of light, the amount of moisture present, and the amount of catalytic, sorptive, and alkaline materials present. A more common process is probably when SO_2 is dissolved in fog or cloud droplets, thus becoming sulfurous acid, which can then be rapidly oxidized to H_2SO_4 by dissolved oxygen.

Deleterious effects caused by the dry deposition of sulfur oxides on plants have been fairly extensively studied

(Guderian, 1977), and include foliar damages such as necrosis and chlorosis, reduced growth, decreased yields, changes in enzyme levels, and alterations in the composition of plant communities. Oxides of nitrogen exhibit basically the same effects, but usually must be present in higher concentrations to do so, and there is some indication that the combined effects of NO_2 and SO_2 are more harmful to vegetation than are the effects of either pollutant separately (Ashenden & Mansfield, 1978). Injuries caused by acidic precipitation formed by the hydration of these pollutants have not been thoroughly studied, but the growing concern over this problem has stimulated much new research, both on the government and university levels (Acid Rain, 1980, Anonymous, 1980).

The most commonly observed injuries affecting plants exposed to acid precipitation seem to be leaf deformations (i.e. leaf curling and wrinkling) and necrosis of leaf tissues (Hindawi et al., 1980, Ferenbaugh, 1976, Wood & Bormann, 1974). Experiments show that lesion development in Phaseolus vulgaris, Helianthus annuus (Evans et al, 1977), and Pteridium aquilinum (Evens & Curry, 1979) was very similar when these plants were exposed to simulated acid rain at pH's of less than 2.3. Initial injury was characterized by collapse of adaxial epidermal cells, later followed by the collapse of adjacent palisade parenchyma cells. When injury was near vascular tissue supportive cells also collapsed. The next stage of lesion development was characterized by cell wall distortions and subsequent collapse of spongy parenchyma cells adjacent to collapsed epidermal and palisade parenchyma cells. The final stage of lesion development was the collapse of all tissues except xylem and phloem.

Lesion development in Glycine max (Evans & Curry, 1979) was characterized by a collapse of epidermal cells followed by distortions of palisade parenchyma cells. Extensive hyperplasia was seen in these cells, with occasional enlarged cells present. Hyperplasia and hypertrophy occured prior to cell collapse.

Responses of 2 of 6 clones of Populus sp. (Evens et al., 1978) exposed to simulated acid rain were markedly different from the other 4 clones (2 of which showed typical lesion development and 2 of which showed limited hypertrophy and hyperplasia in tissues surrounding lesions) by the production of gall like outgrowths. Cells of the epidermis and surrounding tissues of these galls were collapsed. Elevations in both the adaxial and abaxial leaf surfaces due to hypertrophy and hyperplasia in palisade and spongy parenchyma cells characterized later stages of gall formation.

Exposure of Tradescantia sp. (Evans & Curry, 1979) to simulated acid rain resulted initially in the collapse of outer epidermal cells in trilaminar sites and the upper mesophyll. Lesion development showed a progressive increase in cell deterioration from the exterior of the leaf to the interior. About 50% of all lesions exhibited hypertrophy with affected cells obtaining volumes 3 times their normal size. No hyperplasia was present, and hypertrophy occured only in mesophyll cells.

Foliage of Quercus palustris (Evans & Curry, 1979) showed a different sequence of lesion development than did the previously mentioned plants. Epidermal cell collapse occured simultaneously with the collapse of palisade parenchyma cells, followed by mesophyll tissue abnormalities. Hyperplasia and hypertrophic responses in spongy mesophyll gave rise to galls. The final stage of injury, normally characterized by severe necrosis of all tissue layers did not occur in this species.

Foliar damage has also been noted in Phaseolus vulgaris, Acer saccharum (Wood & Bormann, 1975), Betula alleghaniensis (Wood & Bormann, 1974), Tortula ruralis (a moss) (Sheridan & Rosenstreter, 1973), and in a variety crop plants (Table 1).

Most lesion development observed in these studies occured at pH's of less than 3.0. Enlargement of these lesions was probably accelerated by the collection of acidic rainfall in the depressions of newly formed lesions (Evans & Curry, 1979). The majority of lesion development occured near vascular tissue, stomates, and trichomes (Evans et al., 1979), possibly because vascular tissues and cells at the base of trichomes create natural basins in the leaf surface

Table 1. Some crop plants which are susceptible to foliar damage when exposed to acidic precipitation (From Lee et al., 1980 and Mohamed, 1978).

Plants showing extensive foliar damage at pH 3.0	Plants showing moderate foliar damage at pH 3.0	
Radish	Beet	Corn
Mustard greens	Carrot	Okra
Swiss chard	Spinach	Watermelon
Cauliflower	Bibb lettuce	Muskmelon
Tomato	Head lettuce	Pepper
Cucumber	Tobacco	
Green pepper	Cabbage	
Carrot	Broccoli	
Dry beans	Ryegrass	
Snap beans	Orchid grass	
	Fescue	
	Potato	
	Green pea	
	Peanut	
	Soybeans	
	Alfalfa	
	Red clover	
	Strawberry	
	Timothy grass	
	Bluegrass	

were rain water could pool (Evans & Curry, 1979). A study done on Phaseolus vulgaris (Evans et al., 1977) showed lesions originating in epidermal cells adjacent to trichomes 75% of the time, near the stomata 20% of the time, and in all other areas 5% of the time.

Exposure to simulated acid rain has also been shown to destroy chlorophyll in mosses and to alter the chlorophyll A to B ratio by increased hydrolysis of chlorophyll A. Reductions in chlorophyll content and chloroplast integrity in P. vulgaris was also reported, though the chlorophyll A to B ratio remained unchanged (Hindawi et al., 1980, Ferenbaugh, 1976). In addition, increases in photosynthesis and respiration along with reductions in starch and sugar concentrations have been demonstrated in this plant (Ferenbaugh, 1976). An increase in photosynthetic rates was also noted in conifers (Gordon, 1972), while photosynthetic depression was seen in Tortula ruralis (Sheridan & Rosenstreter, 1973).

Reductions in root and foliar biomass caused by exposure to simulated acid rain have been recently reported in P. vulgaris (Ferenbaugh, 1976) and Raphanus sativus (Harcourt & Farrar, 1980). Reductions in leaf biomass in P. vulgaris has also been demonstrated by Hindawi (1980), along with reductions in reproductive organs (i.e. seeds and pods). Reductions in leaf, and total plant biomass was reported in yellow birch (Wood & Bormann, 1974), and there is some concern that a continued decrease in plant productivity because of acid rain could have adverse effects on future food supplies (Pearson, 1974). Studies on crop plants (Lee et al., 1980, Mohamed, 1978) have indicated reductions in fresh weight of crops such as radishs, carrots, beets, mustard greens, broccoli, lettuce, tomatoes, peppers, cabbages, peas, spinach, okra, muskmelon, watermelon, cucumbers, and snap beans. Tomatoes in particular showed a significant decrease in fruit weight and number along with a reduction in marketable fruits (Mohamed, 1978). Many of the leaf crops were damaged enough to affect marketability (Lee et al., 1980). Other observations (Lee & Neely, 1980) seem to indicate that dicot yield is more adversly affected by acid rain than is the yield of monocots, and that among dicots, yields of root crops are most likely to be damaged, followed by leaf, cole, and tuber crops, while legume, forage, and fruit crops may be stimulated by acid rain, and grain crops seem to be unaffected. There also seems to be no correlations between foliar injury and yield effects. Acid precipitation may also be responsible for decreases in forest productivity noticed in southern Sweden (Johnsson & Sundberg, 1972) and the deciduous forests of the Eastern United States (Whittaker et al., 1974).

Another response to acidified precipitation is foliar leaching of nutrients. Studies done on <u>Nicotiana tabacum</u> (Fairfax & Lepp, 1975) showed increases in foliar calcium loss and decreases in foliar potassium loss. The rate of magnesium loss was not affected. The loss of calcium is a passive ion exchange process depending upon hydrogen ion concentrations in rainfall. The increased concentration of this ion in acid rain therefore causes an increase in calcium loss (Mecklenburg et al., 1963). The decrease in foliar leaching of potassium was not readily understood. Experiments on P. vulgaris (Hindawi et al., 1980) showed significant losses in foliar nitrogen, phosphorus, magnesium, and calcium, while potassium was not affected. Other studies have demonstrated acid rain leaching of calcium, magnesium, and sulphur from the canopy of a sugar maple stand (Eaton et al., 1973), increases in the leaching of potassium, magnesium, and calcium from pinto beans and sugar maple (Wood & Bormann, 1975), a ten-fold increase in calcium concentrations in litter beneath an Acer pseudoplatanus stand (Fairfax & Lepp, 1975), and increases in calcium and magnesium concentrations in litter beneath sugar maple and red alder stands (Lee & Weber, 1980).

Another question now being asked is how acid precipitation and host parasite interactions are related. It has been suggested that alterations of these interactions may increase susceptibility of plants, alter their capacity to tolerate disease or injury, or alter virulence of the pathogen (Gunnerson & Willard, 1979). A study done on 5 host parasite systems (Shriner, 1978) of kidney beans showed a 66% inhibition in the reproduction of root knot nematode, a 29% decrease in the percentage of leaf area affected by *Uromyces phaseoli*, and a stimulatory or inhibitory effect in the development of halo blight depending upon the stage of disease cycle at which treatments were applied. Treatment before innoculation increased disease severity by 42%, suspension of bacteria in acid rain resulted in no infection, and application of acid rain after infection inhibited disease development by 22%. Shriner later reported (Shriner & Cowling, 1980) that simulated acid rain seemed to stimulate infection by facultative parasites due to lesion development, while obligate parasites tended to be inhibited, possibly because of imbalanced host metabolism. In growth studies on radish and onions exposed to simulated acid rain (Lee et al., 1981) it was observed that treated plants recieved less damage from slugs and twelve spotted beetles than did control plants.

There have been no reports of beneficial effects on vegetation exposed to acid precipitation as yet (Jacobson, 1980), but benefical responses may be found with further experimentation. One possibility is that the sulfur and nitrogen in acid precipitation may act as fertilizer in soils deficient in these materials (Jacobson et al., 1980, Tveite & Abrahamsen, 1978). Another possibility may be the inhibition of plant pathogens by exposure to acid rain. A variety of bacteria and fungi are known to be repressed under acid conditions (Shriner, 1978), but little work has been done in this area.

Other research which has been done on effects of simulated acid rain have demonstrated reduced fruit set, retardation of pollen tube elongation, and pollen germination in tomatoes (Kratky et al., 1974), abnormal needle development in some species of pine (Gordon, 1972), deleterious effects on developmental morphology of various trees (Gordon, 1972) (Wood & Bormann, 1974), inhibited decomposition of leaf litter from the forest floor (Abrahansen et al., 1976), reductions in nodule formation on legumes (Shriner, 1977), increased susceptibility to drought, biotic pathogens, and other stress factors, adverse effects on symbiotic associations, accelerated leaching of organic and inorganic materials from the soil, and synergistic interactions with other environmental stress factors such as gaseous sulfur, ozone, flouride, drought, etc. (Gunnerson and Willard, 1979).

Several good review articles (Linzon, 1981; Anonymous, 1979; Gunnerson & Willard, 1979; Likens & Borman, 1974; Likens et al., 1972) are now available. In addition to the literature discussed above, there are transcripts of various acid rain conferences (Hutchinson & Havas, 1980; Toribura et al., 1980; Applied Science Assoc. Inc., 1978; Guderian, 1977; Stern, 1977; Naegele, 1973; Thomas & Hendricks, 1956). Also available are the proceedings of a congressional hearing (Acid Rain, 1980), which gives a good overview of the economic, social, and political significance of acidic precipitation.

METHODS AND MATERIALS

Six varieties of turfgrass were used to determine their susceptibility to damage when exposed to simulated acid rain. The varieties used and their characteristics are given in Table 2.

Soil for this study was obtained from Burgner Acres, a nature area owned by Eastern Illinois University. This soil was dried at room temperature for approximately two weeks and then crushed to pass through a 2mm sieve. Soil texture was determined by using the Bouycouous hydrometer method as described in Brower and Zar (1977), organic matter content was determined by the wet combustion, potassium dichromate-sulfuric acid method (Bull. No. 102 Southern Cooperative Series) utilizing a Baush & Lomb Spectronic 20 for absorbance measurements. N,P, and K levels were tested as described below. The properties of this soil are summarized in Table 3.

At the conclusion of this study soil samples from each treatment (including portions of the original sample) were tested for pH, nitrates, soluble phosphorous, and potassium. Testing for pH was done by mixing a 1-2½ ratio of soil with Milli-Q deionized water and allowing it to come to equilibrium for 12 hours at room temperature. The pH was then read on an Orion 407A Ionalyzer. Soluble phosphorus was determined by the colorimetric method described by Bray and Kurtz (1945) utilizing a Baush & Lomb Spectronic 20. Potassium was determined using a potassium electrode and an Orion 407A

Table 2. Nomenclature and characteristics of the cool season turfgrass varieties used in this study.

Scientific name	Variety	Common name	Characteristics
<u>Festuca</u> <u>dubra</u> L.	Pennlawn	Red Fescue	Medium dark green. Fine texture. High shoot density. Fairly rapid shoot growth rate.
<u>Festuca</u> <u>ovina</u> L.	Biljart (C-26)	Hard Fescue	Deep green turf. Very fine texture. High shoot density. Slow vertical shoot growth rate. Growth habit medium low.
<u>Lolium</u> <u>perenne</u> L.	Pennfine	Perennial Ryegrass	Bright, medium dark green. Medium fine texture. Medium shoot density. Leafy diminutive growth habit.
<u>Lolium</u> <u>perenne</u> L.	Manhattan	Perennial Ryegrass	Bright, medium dark green. Medium fine texture. Medium high shoot density. Diminutive growth habit. Profuse tillering. Slow vertical shoot growth rate.
<u>Poa</u> <u>pratensis</u> L.	Baron	Kentucky Bluegrass	Dark green color. Medium coarse texture. High shoot density. Low growth habit. Medium slow vertical shoot growth rate.

Table 2. Con't.

Scientific name	Variety	Common name	Characteristics
Poa pratensis L.	Victa	Kentucky Bluegrass	Dark green with medium texture and density.

Table 3. Physical and chemical properties of the soil used in this experiment.

Soil Texture	% Organic Matter (± 2 SD)	pH (± 2 SD)	Chemical components (ppm ± 2 SD)
Sand = 24 %	6.79 ± .21 %*	5.63 ± .15*	NO_3^- = 111.0 ± 2.0*
Silt = 62 %			$PO_4^{\equiv}$ = 34.2 ± 0.8
Clay = 14 %			K^+ = 103.0 ± 8.5

Soil type - Silt loam

* at a 95% confidence level

Ionalyzer. Nitrates were also determined with an Orion 407A Ionalyzer, using a nitrate electrode. Procedures for both of these tests are given in the Orion 93 series method manual (Orion, 1978b).

The sieved soil was placed in 7 ounce white Solo styrofoam cups (with perforated bottoms for drainage of gravitational water) and then saturated with Milli-Q deionized water. Planting was accomplished by sprinkling a constant volume of seeds over the soil, covering the seeds with approximately 1/8 inch of soil, and then rewetting the soil.

A total of 234 cups were planted, 39 for each variety. All treatments utilized three replicates and each cup was color coded for ease of identification. The cups were randomly placed on a 72" X 84" table which was marked off into 288 quadrants, and were illuminated by four 90" long double bulb fluorescent fixtures with F96T12 Slyvania cool white bulbs. The fixtures where 25cm apart and light intensities varied from 790 ft. candles between the fixtures to 1000 ft. candles directly beneath the fixtures. The photoperiod used was 16 hours of light (6 AM-10 PM) and 8 hours of dark (10 PM-6 AM).

The experiment was carried out in a 34' X 18' room where the environment could not be strictly controlled. Both the temperature and relative humidity were monitered on a recording hydrometer and the weekly averages for each listed in table 4.

Table 4. Mean weekly temperature ranges and relative humidity levels. Readings were obtained with a recording hydrometer.

Date		Temp. range (^{o}F)	% Relative humdity
3/8/82	Day	73.4 - 78.0	33.8
	Night	71.8 - 77.0	
3/15/82	Day	73.0 - 78.6	40.8
	Night	71.3 - 75.2	
3/22/82	Day	73.2 - 77.0	31.0
	Night	71.0 - 75.1	
3/29/82	Day	70.8 - 79.6	34.8
	Night	71.4 - 75.0	
4/5/82	Day	69.0 - 75.5	30.0
	Night	68.6 - 73.3	
4/12/82	Day	73.3 - 79.8	34.5
	Night	72.6 - 78.1	
4/19/82	Day	73.2 - 79.3	25.0
	Night	73.0 - 78.0	
4/26/82	Day	73.0 - 80.8	29.1
	Night	73.1 - 77.4	
5/3/82	Day	73.0 - 80.0	36.1
	Night	73.0 - 78.6	
5/10/82	Day	76.8 - 83.8	40.8
	Night	77.0 - 81.7	
5/17/82	Day	78.2 - 84.2	49.1
	Night	77.9 - 82.1	
5/24/82	Day	75.4 - 82.8	56.5
	Night	75.2 - 80.0	

Two different acid stock solutions were used to make the simulated acid rain used in this experiment. These acids were concentrated reagent grade sulfuric acid and nitric acid. Acid solutions of pH 3.0, 2.5, 2.0, and 1.5 were made from these acids by dilution with Milli-Q deionized water. Also, these acid solutions were mixed to give $\frac{1}{2}H_2SO_4$-$\frac{1}{2}HNO_3$ solutions of the same pH to test for possible synergistic effects. The pHs used were chosen because much of the literature indicated that most evident damage to plants occured at pHs of less than 3.0. In addition, a preliminary experiment which was destroyed by aphids showed no visible damage at pHs of 4.0, 3.5, and 3.0.

A total of 13 different treatments were used in this experiment, including a control of untreated Milli-Q deionized water. The other 12 treatments were as shown below:

	H_2SO_4	HNO_3	$\frac{1}{2}H_2SO_4$ - $\frac{1}{2}HNO_3$
(pH)	3.0 2.5 2.0 1.5	3.0 2.5 2.0 1.5	3.0 2.5 2.0 1.5

Plants were watered using a spray apparatus to simulate rainfall (Fig. 1). Four hundred milliliters of acid solution were placed in the flask and a hand pumped pressure sprayer was used to provide 18 psi (gauge pressure) of pressure for spraying the solution. An aerator nozzle was used to break the solution into a spray in the shape of a solid cone. Seven cups at a time were placed in a bucket for watering. Six of these had plants in them and the other was empty for

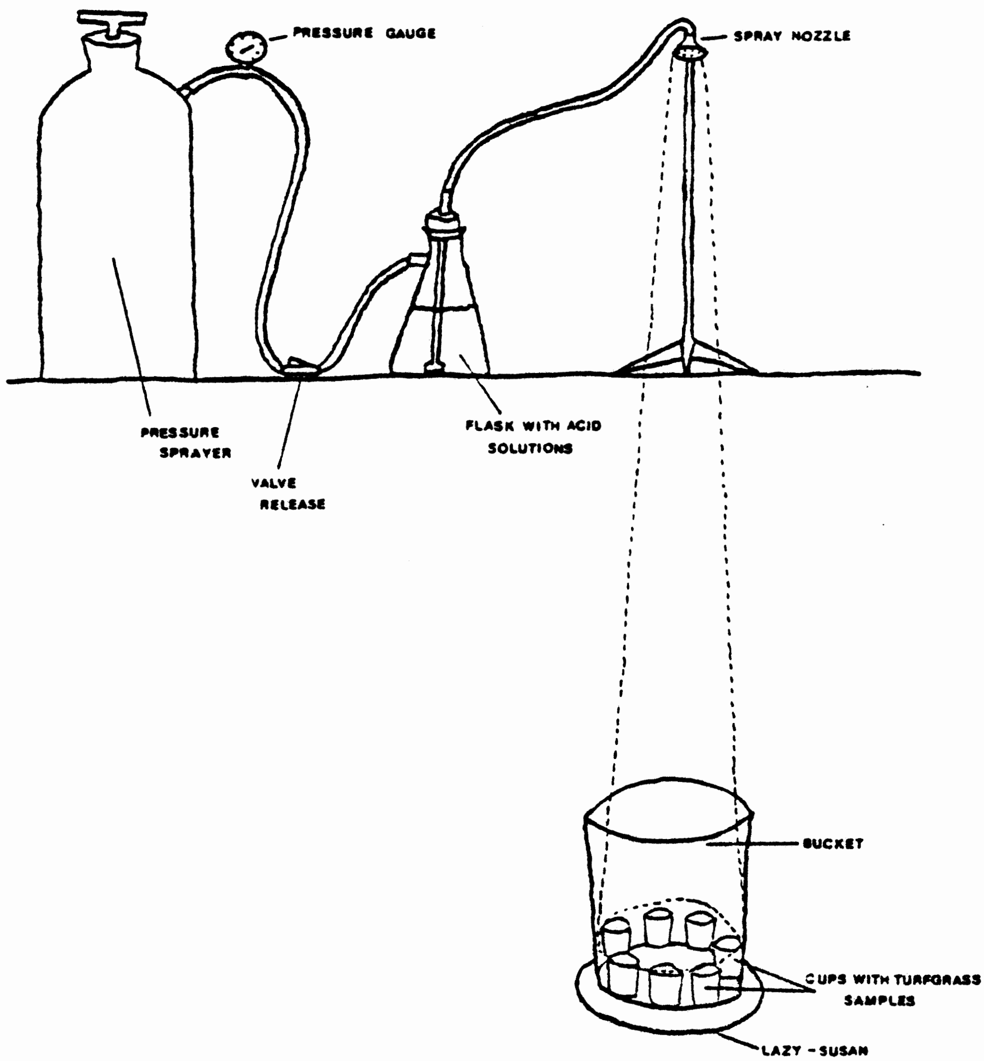


Fig. 1. The spray apparatus used to create the simulated acid rainfall used in this experiment.

the purpose of monitering the volume of water the cups received in each watering (Table 5). The bucket was placed on a lazy-susan and rotated during the waterings to provide more uniformity. Several trial runs of this watering system using empty cups were made to determine the deviations in amount of water recieved by each cup during a watering (Table 6). Every other watering was done using this spray apparatus. Between sprayings the soil in each cup was watered with 25ml of untreated Milli-Q deionized water to prevent wilting of the grass plants. After each spraying the plants were placed back on the table in a random fashion.

After 10 waterings (about 20 days), 5 with acid solutions and 5 with untreated Milli-Q water, the turfgrass plants were harvested by cutting the grass at a height of one inch above the soil. Clippings were disposed of and the newly cut grass exposed to another series of simulated acid rain treatments. Before each harvesting two parameters were measured to monitor plant injury, the average height of the grass in each cup (to the nearest ½cm) and the amount of visible foliar damage present. The amount of visual damage was recorded by using the following scale:

1 = little or no damage visible
2 = moderate damage visible
3 = heavy damage visible

Only three ratings were used to try to reduce bias in recording the amount of damage present. Photographs were pro-

Table 5. Mean volumes of water (in milliliters) of water recieved by each cup of turfgrass during this study.

Watering #	Volume (mls) ± 2 sd
1	22.5 ± 2.8*
2	21.3 ± 2.8
3	22.5 ± 2.5
4	21.3 ± 2.0
5	22.5 ± 3.3
6	24.0 ± 2.2
7	23.7 ± 2.9
8	22.6 ± 2.4
9	22.3 ± 2.7
10	22.2 ± 2.6
11	22.4 ± 3.2
12	22.8 ± 1.9
13	23.5 ± 2.3

* at a 95% confidence level

Table 6. Mean volumes expressed as milliliters of water per cup for trial runs of the watering system used in this study.

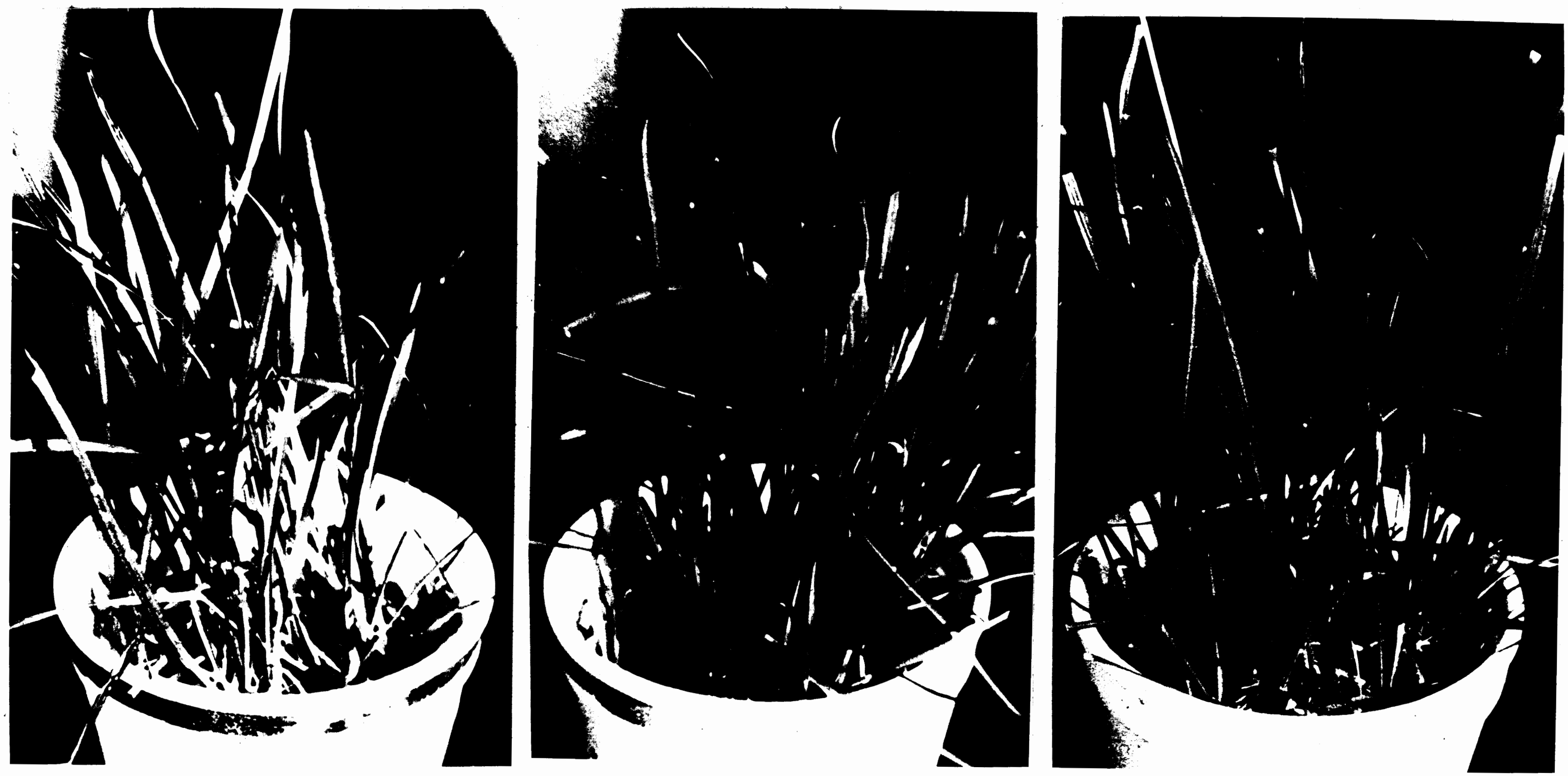
Trial #	Volume (mls) ± 2 sd
1	23.7 ± 2.3*
2	22.5 ± 2.0
3	21.8 ± 3.2
4	23.1 ± 3.8
5	21.3 ± 1.9
6	23.3 ± 1.9
7	20.5 ± 2.4
8	22.0 ± 3.3
9	22.5 ± 2.9
10	24.2 ± 1.9
11	22.8 ± 0.5
12	19.4 ± 1.5
13	24.0 ± 2.8

* at a 95% confidence level

vided for ease in visualizing the amount of damage present for each rating (Figs. 2 & 3).

Since no height measurements were taken in the first run, a fourth run was made (utilizing fresh soil from the original sample) duplicating the conditions of the first run. This run was then substituted for the first run, which was dropped from the study.

Commonly used methods of determing injury to plants have included estimations of the % leaf surface area which is necrotic and chlorotic, and measurements of chlorophyll loss (Knudson et al., 1977). Estimating the % leaf surface area which is damaged has inherent observer bias and is not very adaptable to turfgrasses were there are very large numbers of leaves. Most of the procedures used for determining chlorophyll loss are based on chlorophyll extraction methods using fresh weight and are therefore inaccurate because injured tissue becomes dessicated, causing a reduction in fresh weight that corresponds to a reduction in chlorophyll. As an alternative, researchers at the University of Wisconsin (Knudson et al., 1977) developed a simple procedure for determing the chlorophyll concentrations in plants as milligrams of chlorophyll per gram dry weight. This method was demonstrated to be of practical value in evaluating ozone injury to bean plants, and appeared to be applicable for evaluating injury to other plants, and injuries produced by other pollutants. Since

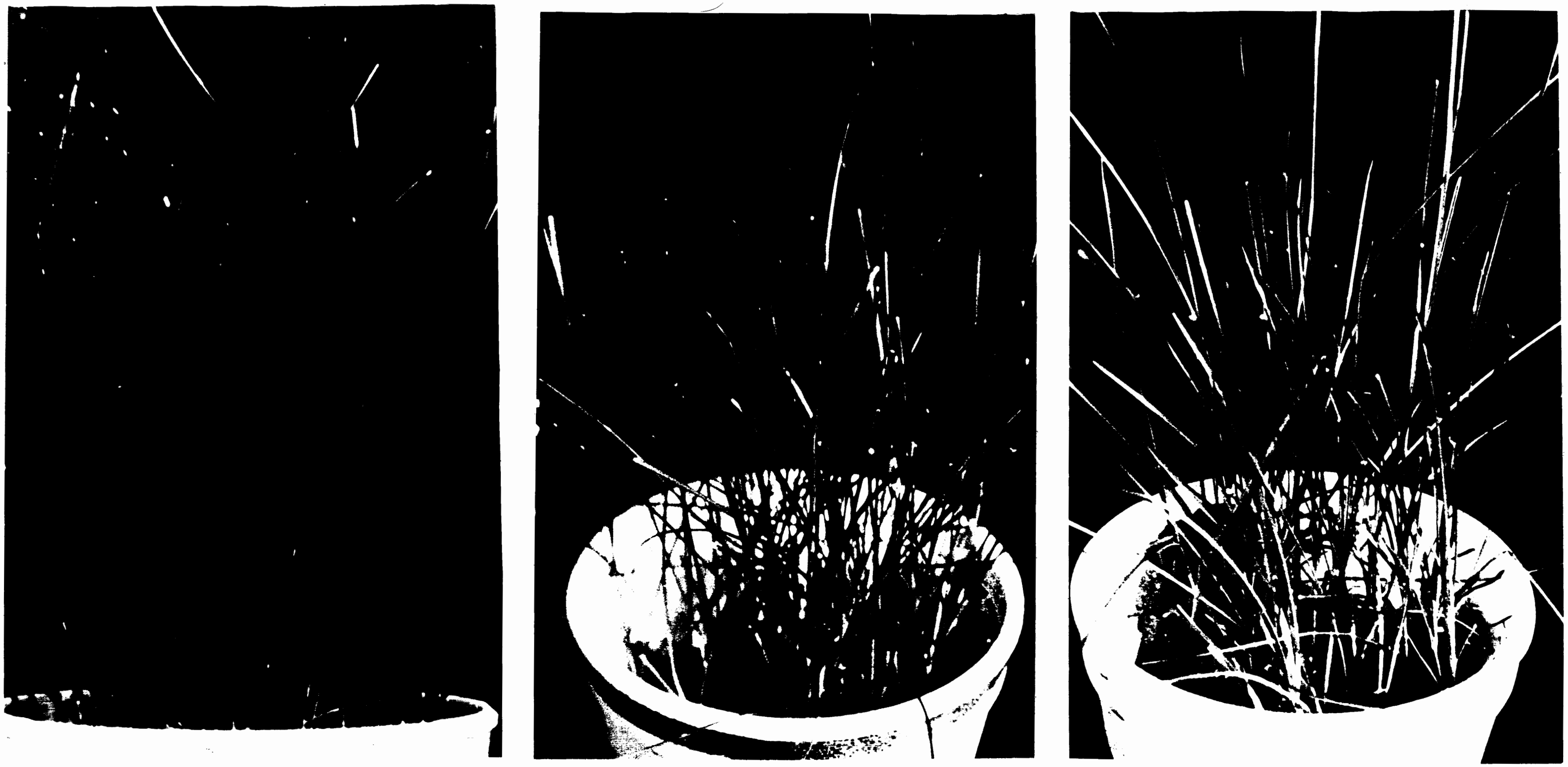


DAMAGE RATING – 3
HEAVY DAMAGE

DAMAGE RATING – 2
MODERATE DAMAGE

DAMAGE RATING – 1
LITTLE OR NO DAMAGE

Fig. 2. Photographs used to determine the three visual damage ratings used in this study. These pictures show typical damage seen in the broadleaf turfgrasses used in this experiment (Baron, Victa, Pennfine, and Manhattan).



DAMAGE RATING – 3
HEAVY DAMAGE

DAMAGE RATING – 2
MODERATE DAMAGE

DAMAGE RATING – 1
LITTLE OR NO DAMAGE

Fig. 3. Photographs used to determine the three visual damage ratings used in this study. These pictures show typical damage seen in the narrow leaf turfgrasses used in this experiment (Biljart and Pennlawn).

this method had not been previously tested for use with turfgrasses it was not used in the main experiment, but instead set up as a seperate small experiment which consisted of two parts. Planting was done using the same methods and materials used for the main experiment. The first part of the experiment consisted of six cups per turfgrass variety. All the cups were watered with untreated tapwater. Chlorophyll determinations for the grass in these six cups were done using a modification of the steeping method described by Knudson et al.(1977). A weighed amount of grass clippings from each plot were placed in test tubes which were filled with 95% ethanol and set in a refrigerator for two days. The ethanol was then poured off and the clippings rinsed with more ethanol and the tube refilled. This process was repeated a total of three times and then all the ethanol collected was brought up to volume. Some preliminary work with this method showed that the turfgrasses were not as easily extracted as bean leaves, taking from 10 days to 2 weeks to become totally "bleached", therefore 6 days was determined as a reasonable compromise between the amount of chlorophyll extracted and the length of the extraction process. Cutting the grass blades in half seemed to facilitate the extraction process, as did keeping the number of blades per sample between 15 and 25. Grass clippings from 3 of the tubes were dried in an oven for 3 days at 55°C and then reweighed. Aliquots of the chlorophyll extracted were then placed in a Beckman Acta IV spectrophotometer to determined the absorbance for each sample. The quantity of chlorophyll A and B were determined by using the following equations:

$$\text{mg. chl. A/g dry wt.}^{*} = 13.7(A_{665}) - 5.76(A_{649}) \times \frac{\text{Volume}}{1000 \times \text{dry wt.}^{*}}$$

$$\text{mg. chl. B/g dry wt.} = 25.8(A_{649}) - 7.60(A_{665}) \times \frac{\text{Volume}}{1000 \times \text{dry wt.}}$$

total mg. chl./g dry wt. = mg. chl. A + mg. chl. B

* to obtain the mg. chl./g fresh wt. the fresh wt. of the sample was substituted for the dry wt.

Clippings from the other 3 tubes were ground in 80% acetone, filtered through Watman's # 1 filter paper using a Buchner funnel, and then brought up to volume. Chlorophyll concentrations form these solutions were then determined to obtain an estimate of how much chlorophyll was extracted by the 95% ethanol.

In the second part of this experiment two different treatments were used. Three cups were treated with simulated acid rain ($\frac{1}{2}H_2SO_4$-$\frac{1}{2}HNO_3$) of pH 1.5 to induce damage, and three other cups were treated with untreated Milli-Q deionized water for use as controls. Chlorophyll extractions from these plants were made as previously described for the first part.

After chlorophyll determinations were made the remaining portions of the extracts were stored under various conditions for one week and rechecked to determine their stability. Of each three replicates, one was stored in a refrigerater, one in the dark at room temperature, and one in the light at room temperature.

RESULTS AND DISCUSSION

The rainfall in the North Eastern United States now has an average pH of approximately 3.9, with occasional values ranging as low as pH 2.1 (Likens & Bormann, 1974). These values are likely to become even more acidic if SO_2 and NO_3^- levels continue to increase in the atmosphere.

Visible damage to the turfgrasses used in this experiment occured in the form of white, necrotic lesions located along the margins and midveins of the leaves, and in some cases extended across the entire width of the blade. The size of the lesions varied from 0.5mm x 0.5mm to 1.0mm x 3.0mm. The results of the visual damage ratings are given in Figures 4-9.

There were definite relationships between height, solution pH, and the degree of damage. Because grass height decreased in each subsequent run for cups recieving the higher pH treatments (3.0, 2.5) (Figs. 10-15) it seemed probable that a decline in nutrient levels within the soil had occured.

Cups which had been watered with H_2SO_4 solutions showed little change in potassium levels, but those watered with HNO_3 and $\frac{1}{2}H_2SO_4$-$\frac{1}{2}HNO_3$ solutions showed fairly large decreases (Figs. 16-21). This may be due to the increased growth associated with these treatments, increased leaching due to the acid solutions, or a combination of both. Phos-

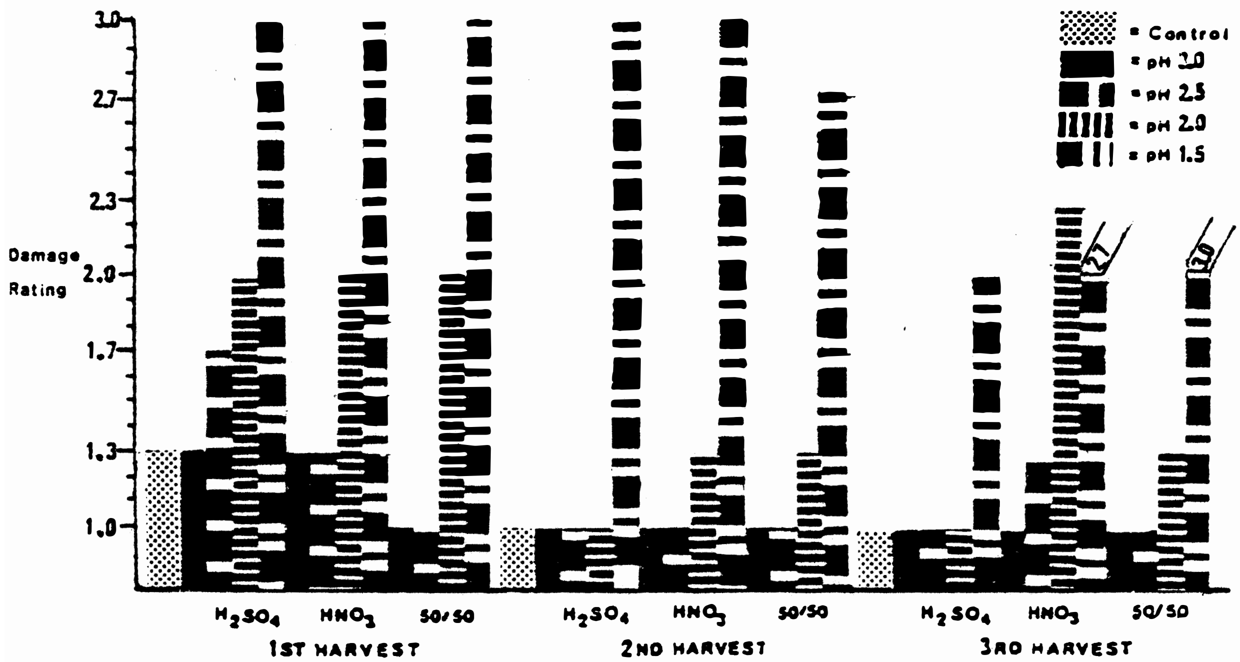


Fig. 4. Visual damage ratings for the Kentucky Bluegrass variety, Baron. Ratings from all 3 runs are given.

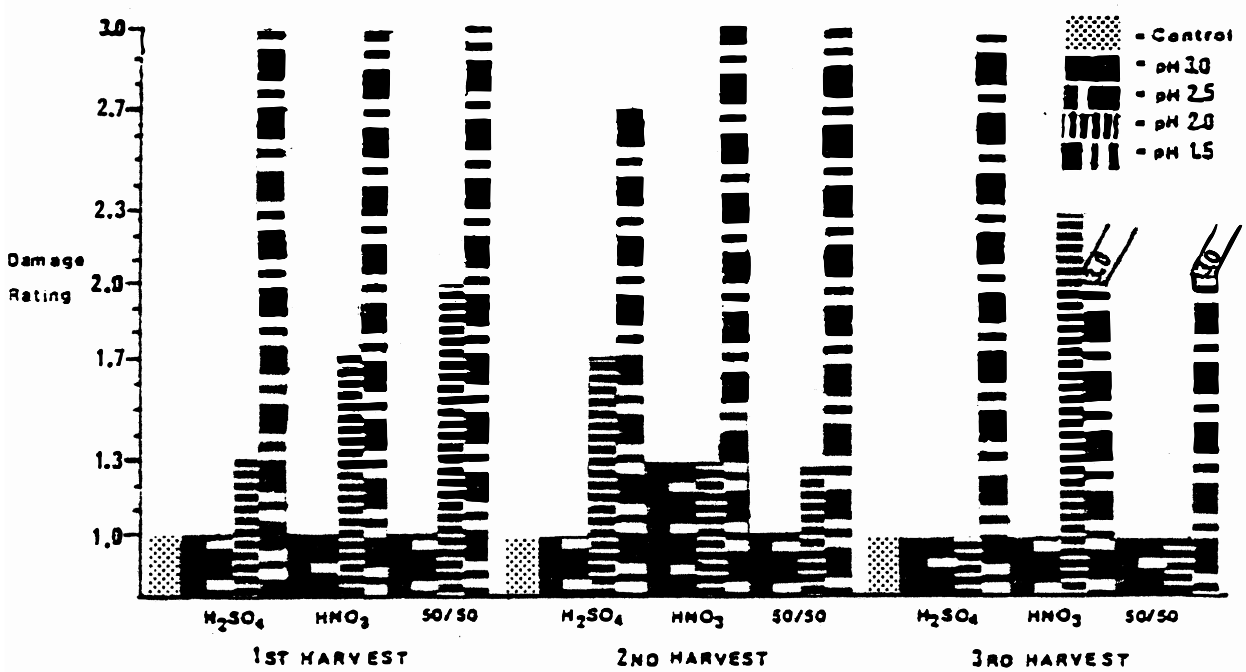


Fig. 5. Visual damage ratings for the Kentucky Bluegrass variety, Victa. Ratings from all 3 runs are given.

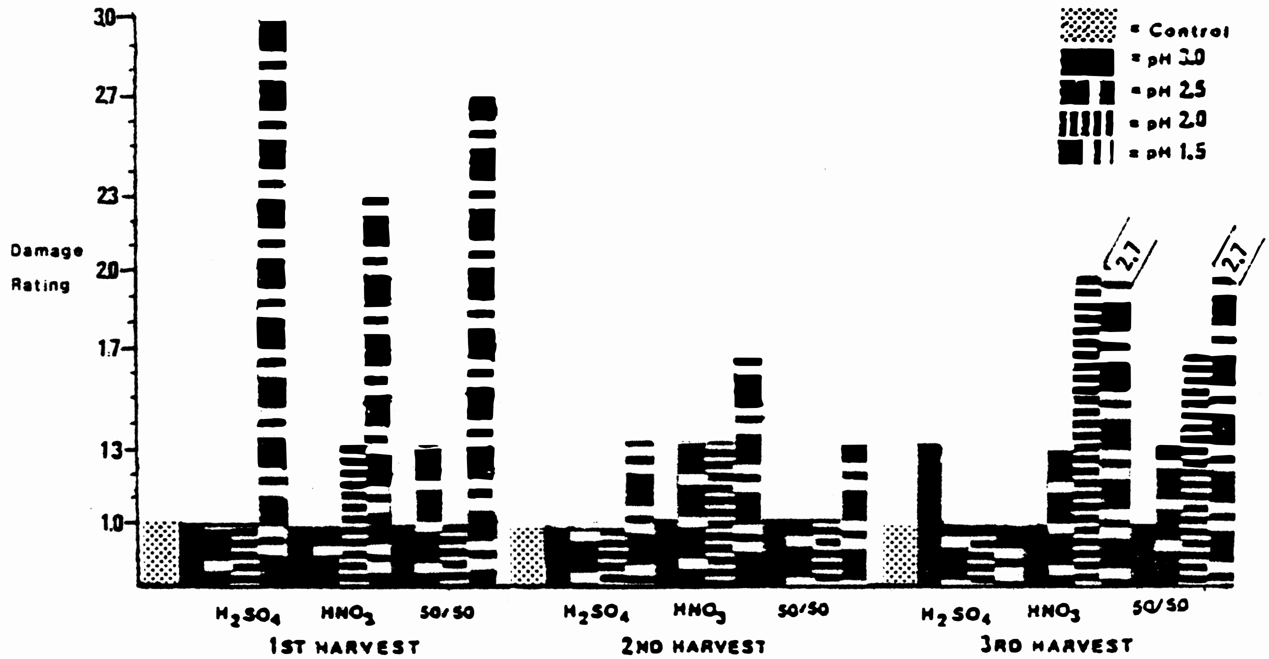


Fig. 6. Visual damage ratings for the Perennial Ryegrass variety, Manhattan. Ratings from all 3 runs are given.

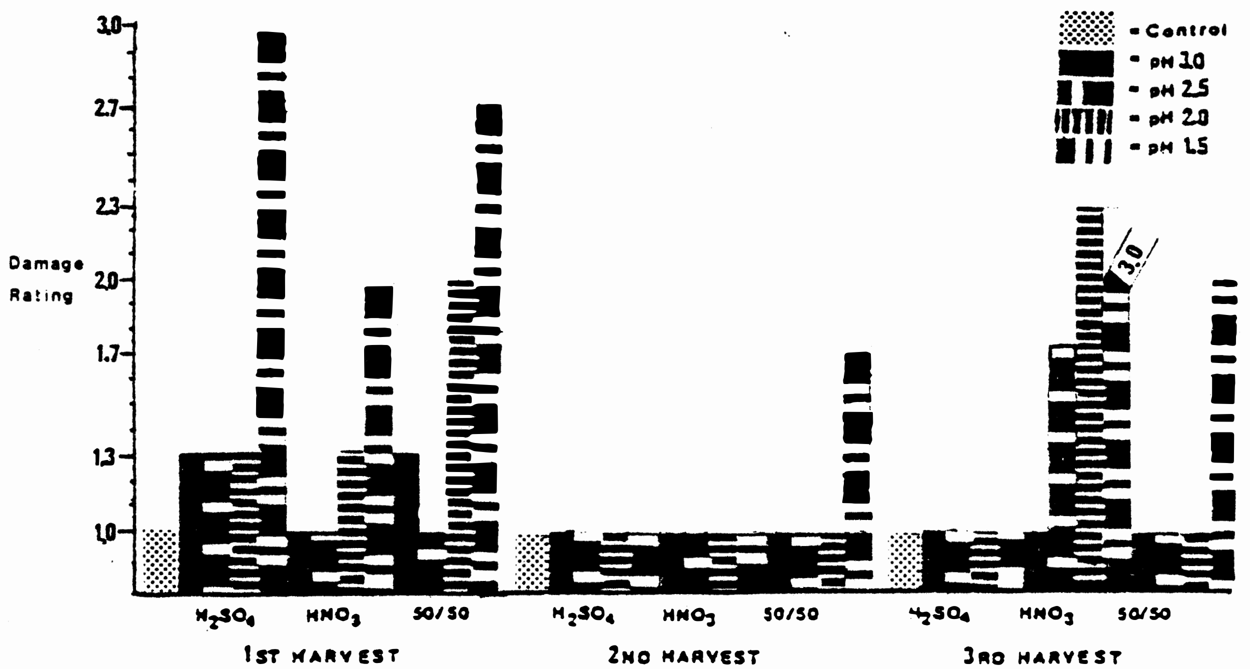


Fig. 7. Visual damage ratings for the Perennial Ryegrass variety, Pennfine. Ratings from all 3 runs are given.

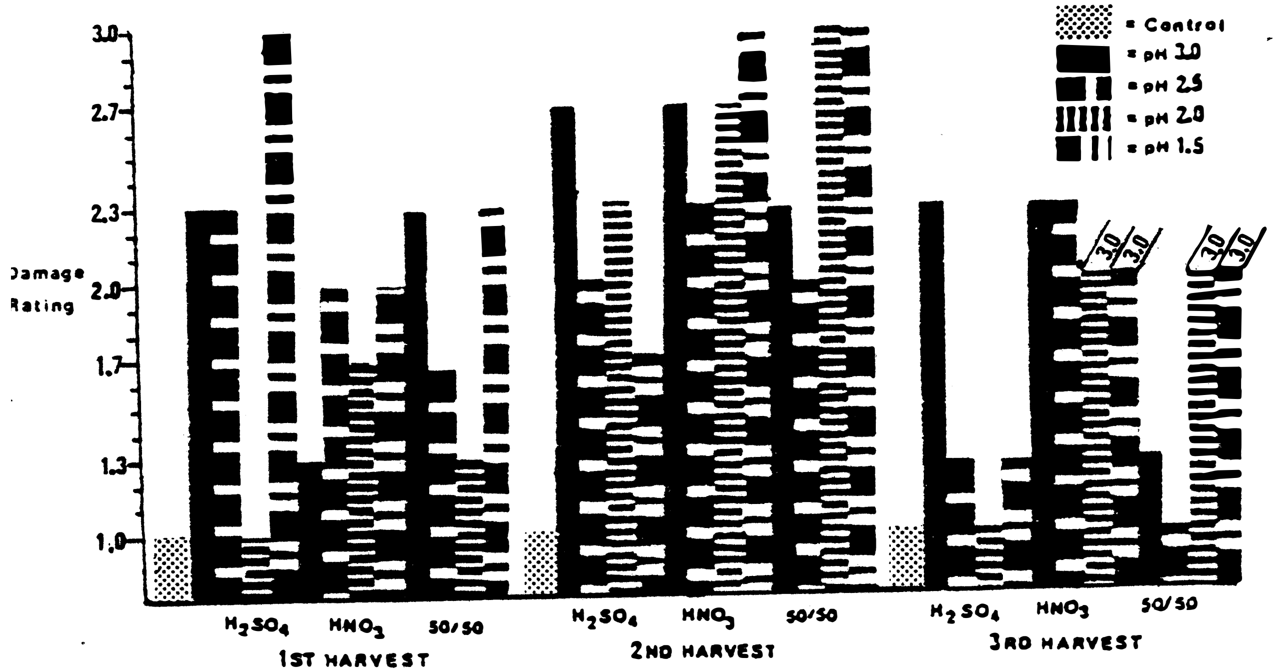


Fig. 8. Visual damage ratings for the Fine Fescue variety, Biljart. Ratings from all 3 runs are given.

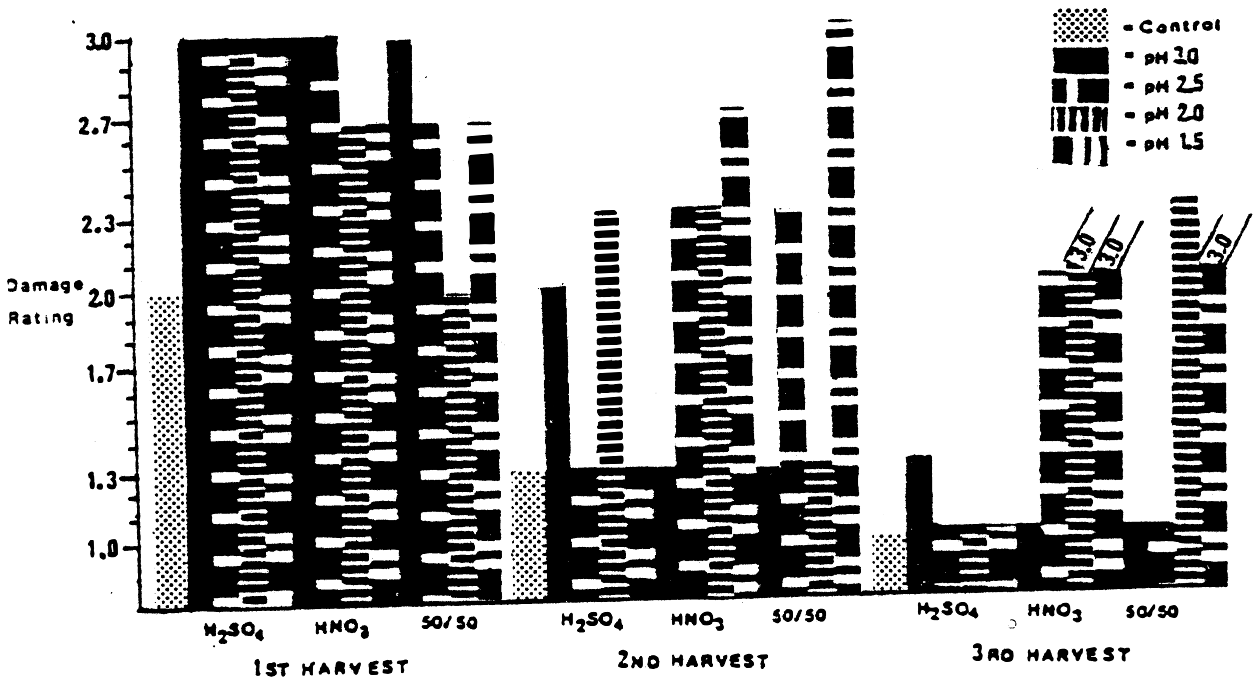


Fig. 9. Visual damage ratings for the Fine Fescue variety, Pennlawn. Ratings from all 3 runs are given.

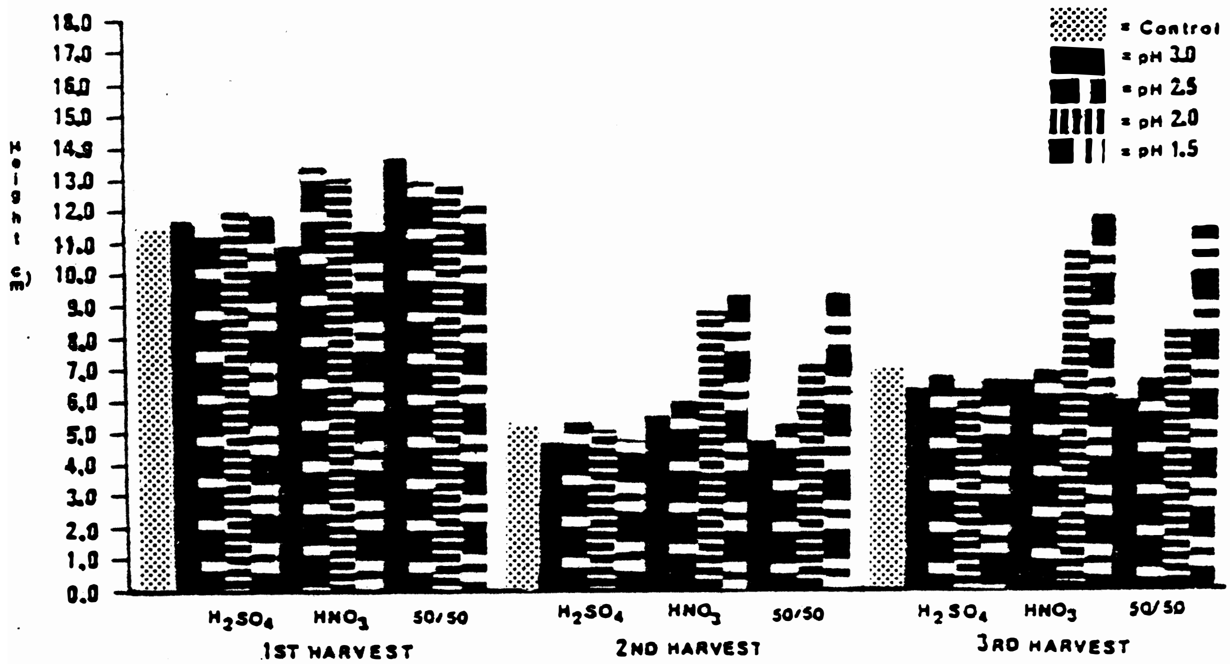


Fig. 10. height data for the Kentucky Bluegrass variety, Baron. Data from all 3 runs is given.

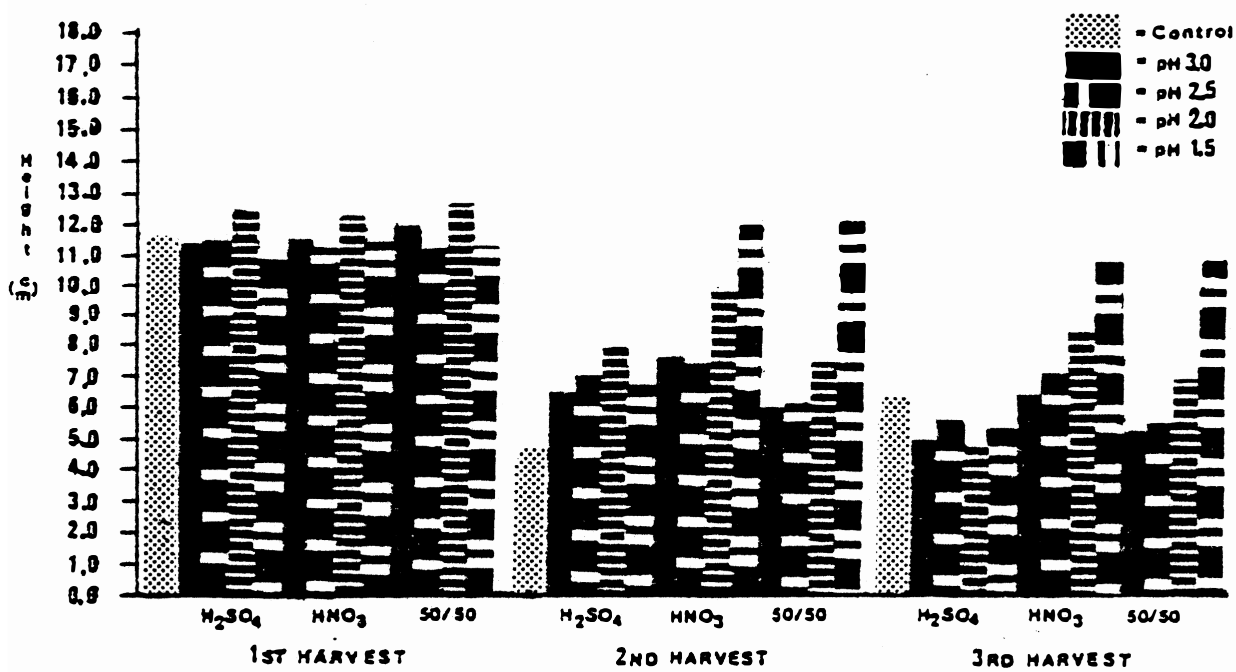


Fig. 11. Height data for the Kentucky Bluegrass variety, Victa. Data from all 3 runs is given.

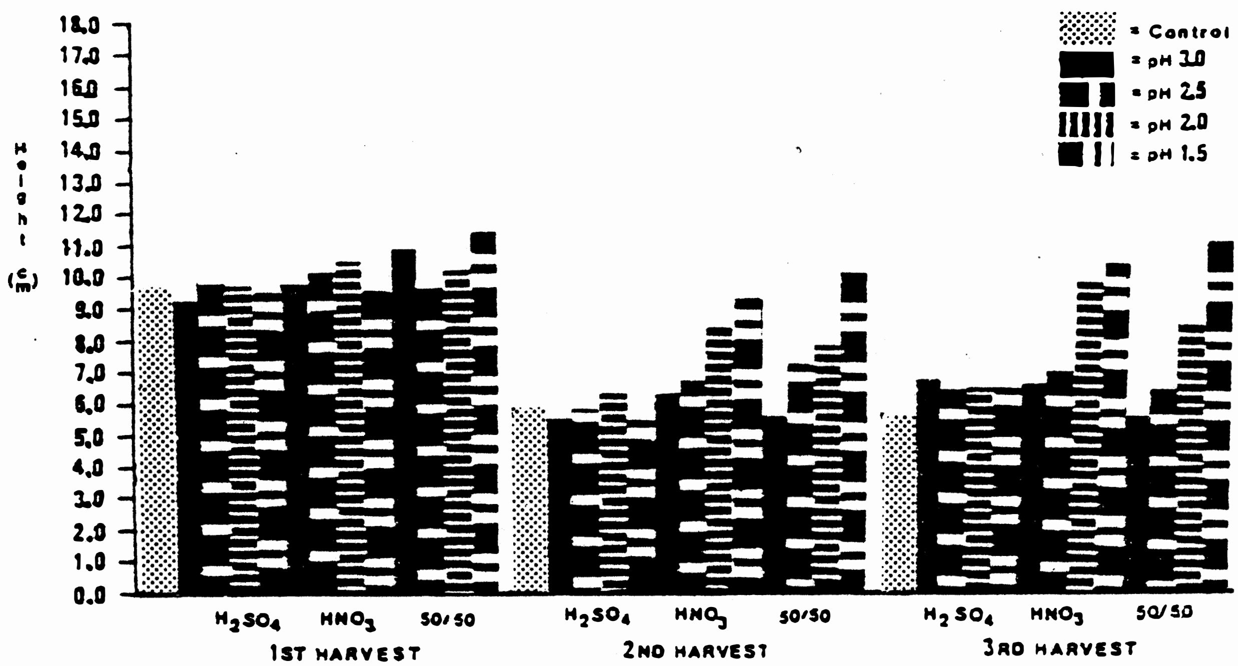


Fig. 12. Height data for the Perennial Ryegrass variety, Manhattan. Data from all 3 runs is given.

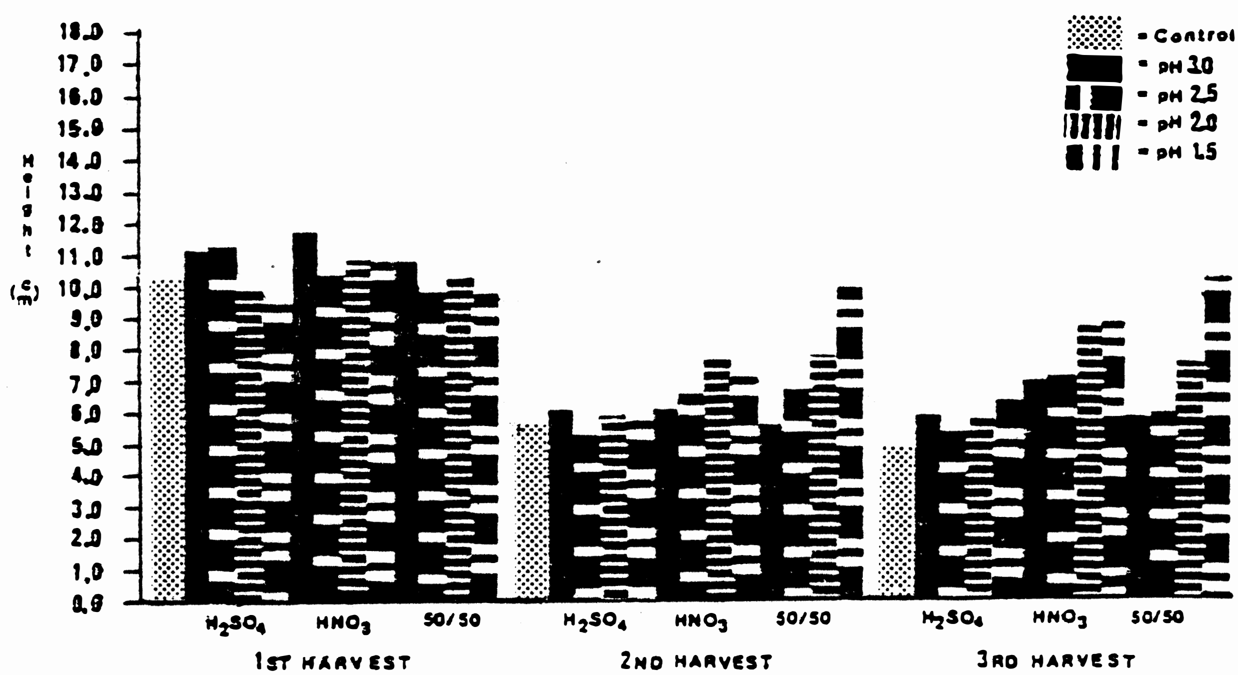


Fig. 13. Height data for the Perennial Ryegrass variety, Pennfine. Data from all 3 runs is given.

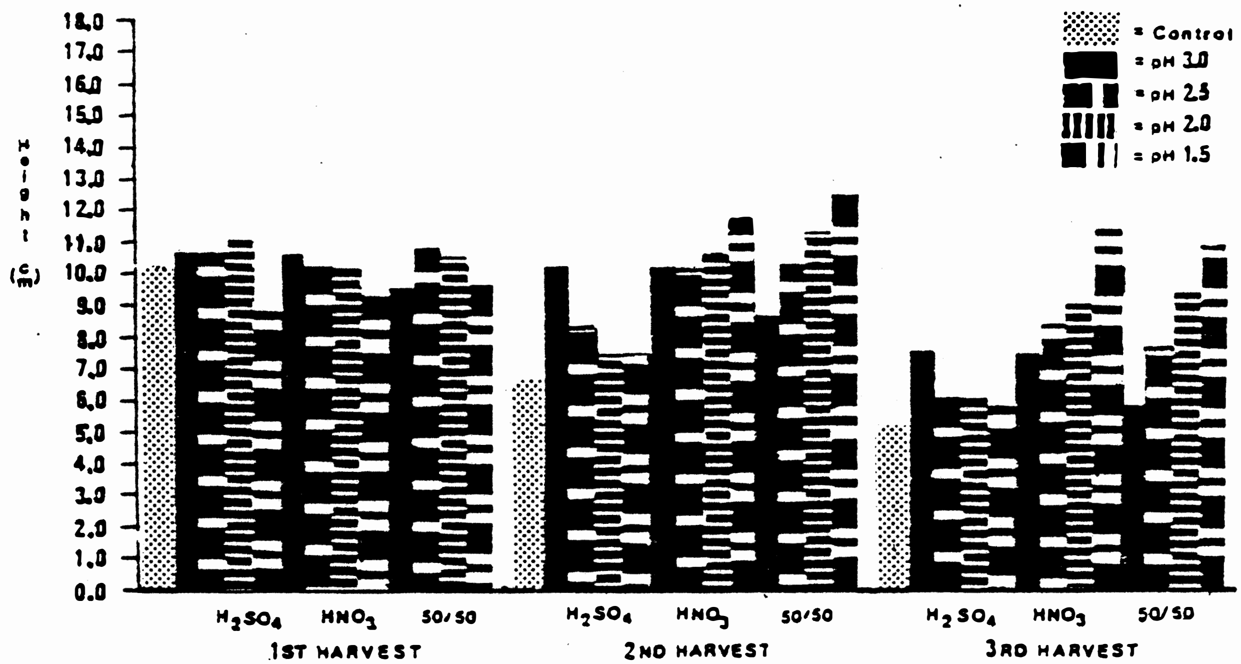


Fig. 14. Height data for the Fine Fescue variety, Biljart. Data from all 3 runs is given.

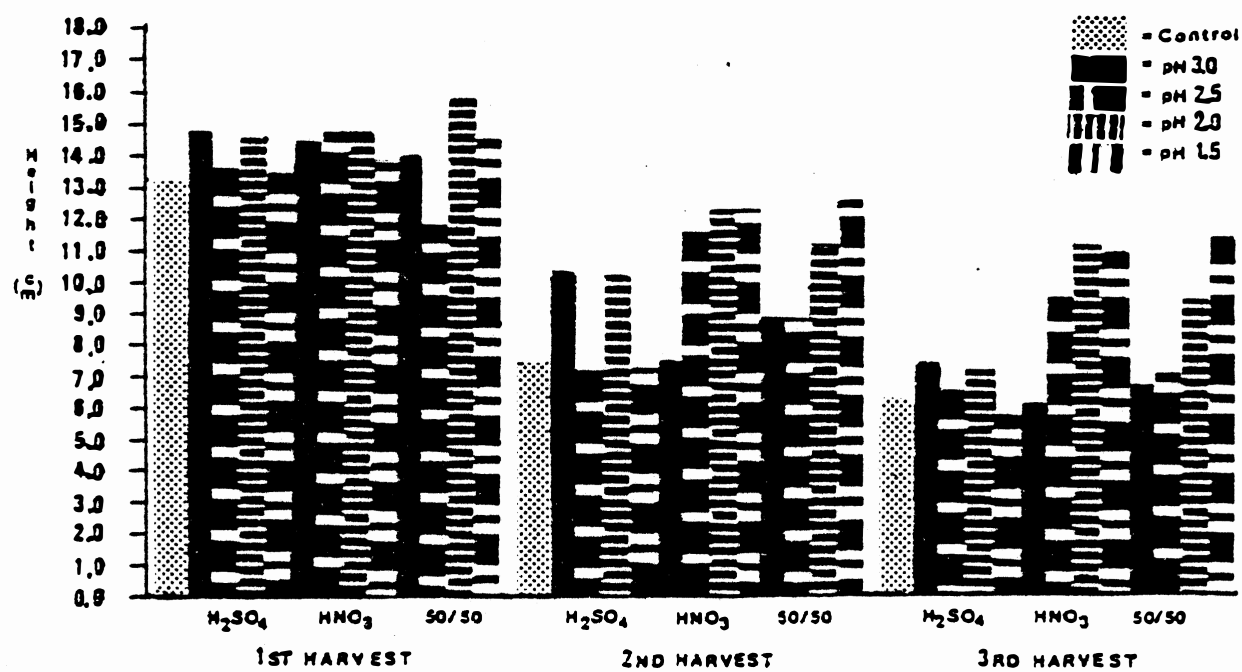


Fig. 15. Height data for the Fine Fescue variety, Pennlawn. Data from all 3 runs is given.

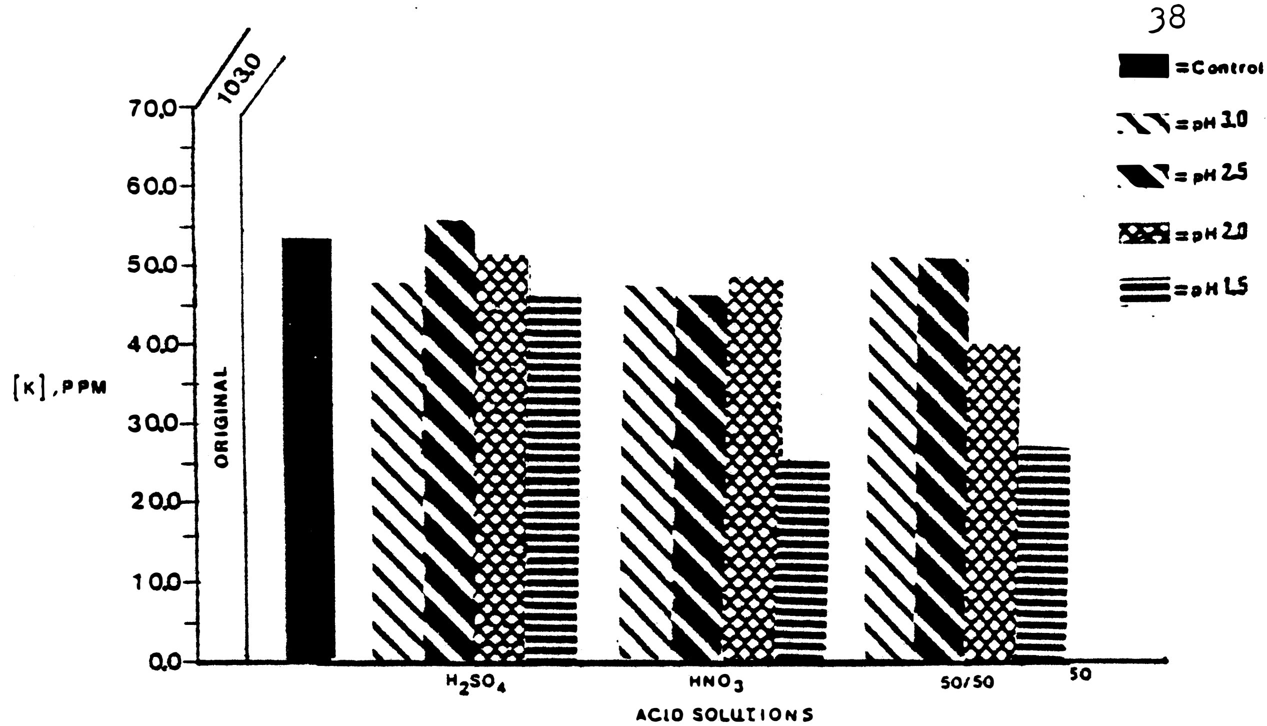


Fig. 16. Potassium levels for the soil in which the Kentucky Bluegrass variety, Baron, was grown.

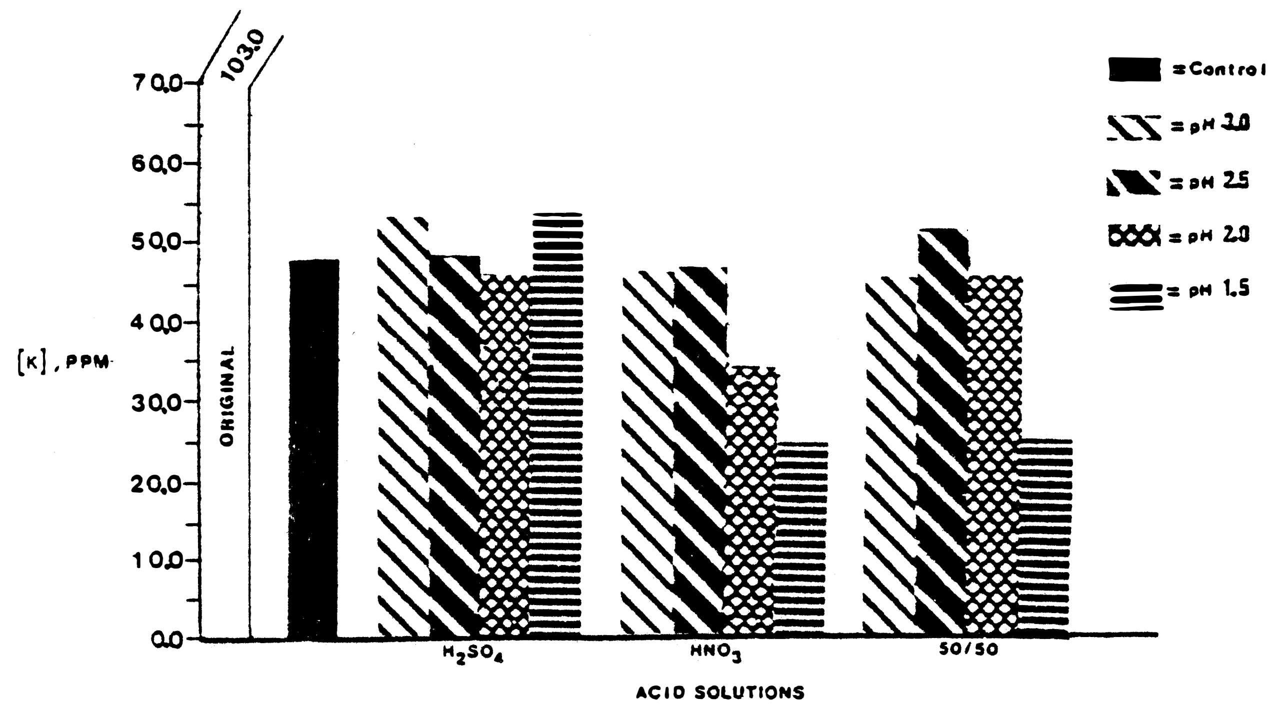


Fig. 17. Potassium levels for the soil in which the Kentucky Bluegrass variety, Victa, was grown.

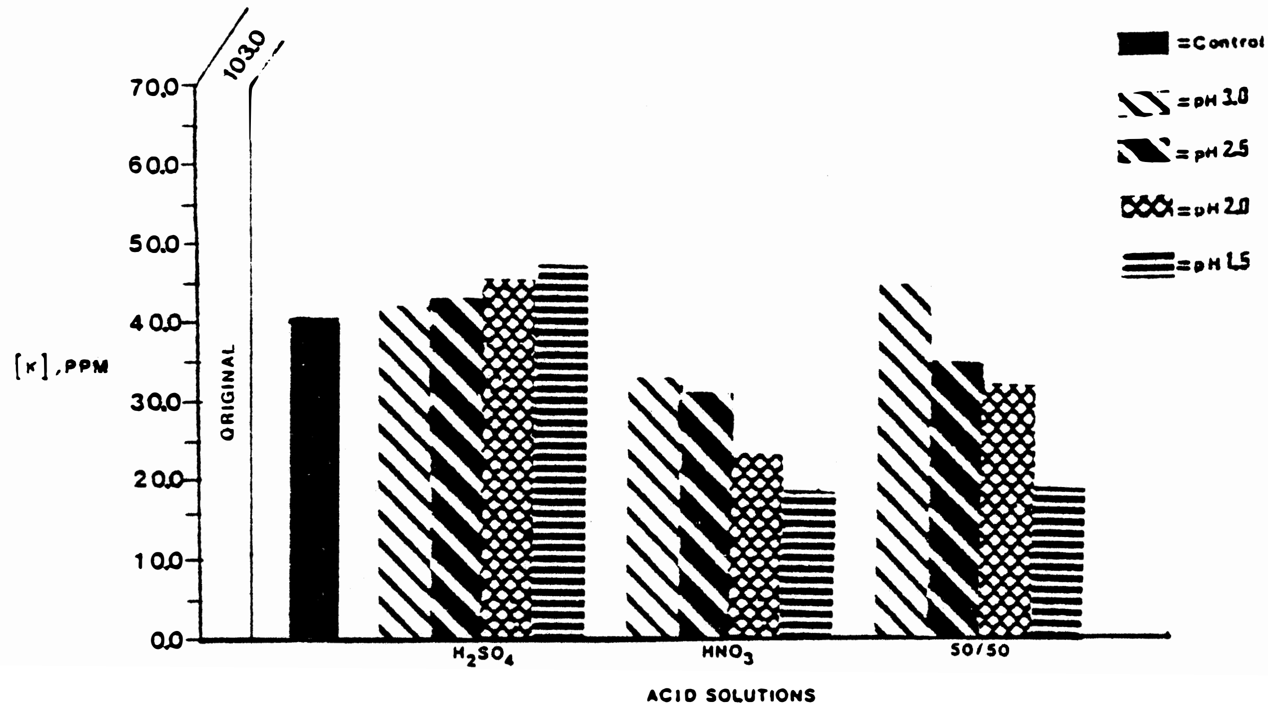


Fig. 18. Potassium levels for the soil in which the Perennial Ryegrass variety, Manhattan, was grown.

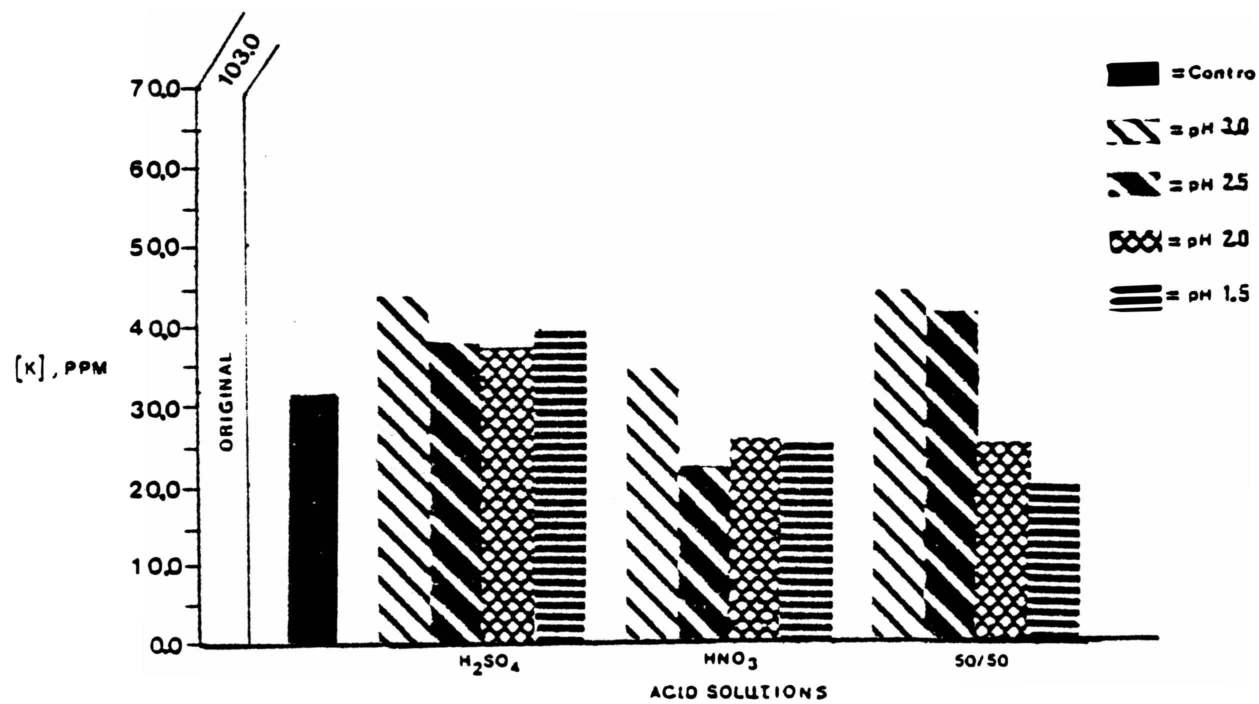


Fig. 19. Potassium levels for the soil in which the Perennial Ryegrass variety, Pennfine, was grown.

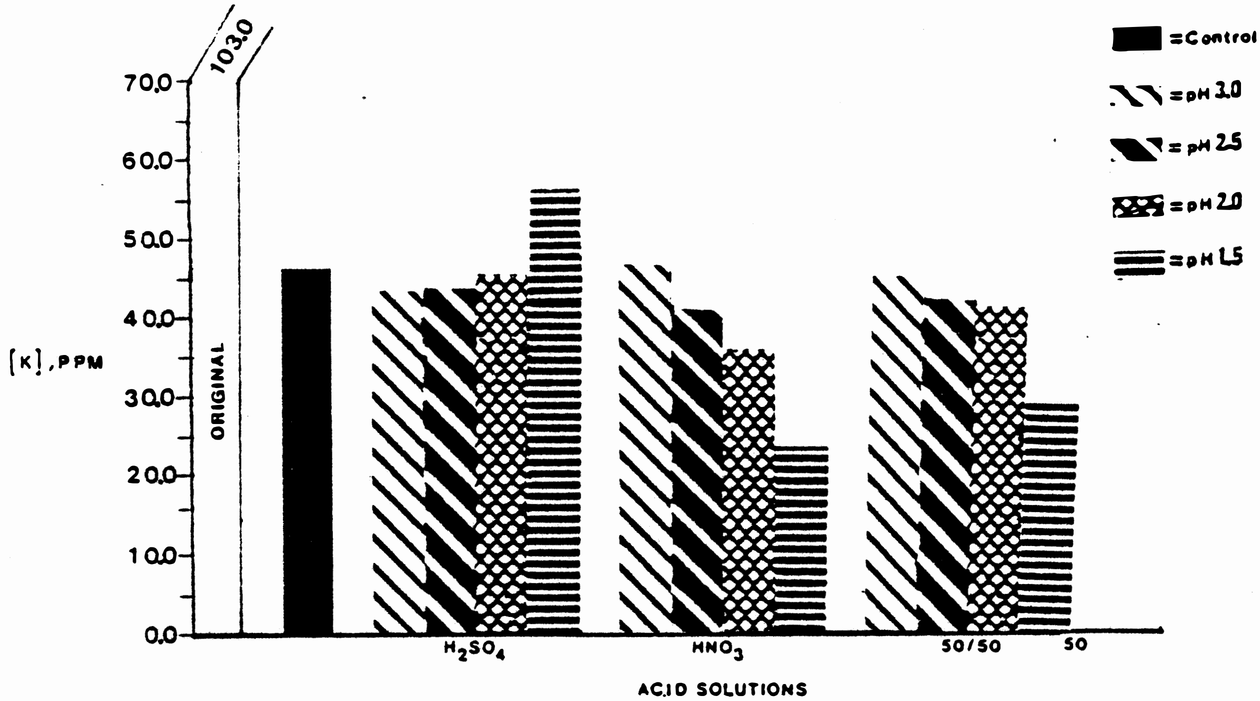


Fig. 20. Potassium levels for the soil in which the Fine Fescue variety, Biljart, was grown.

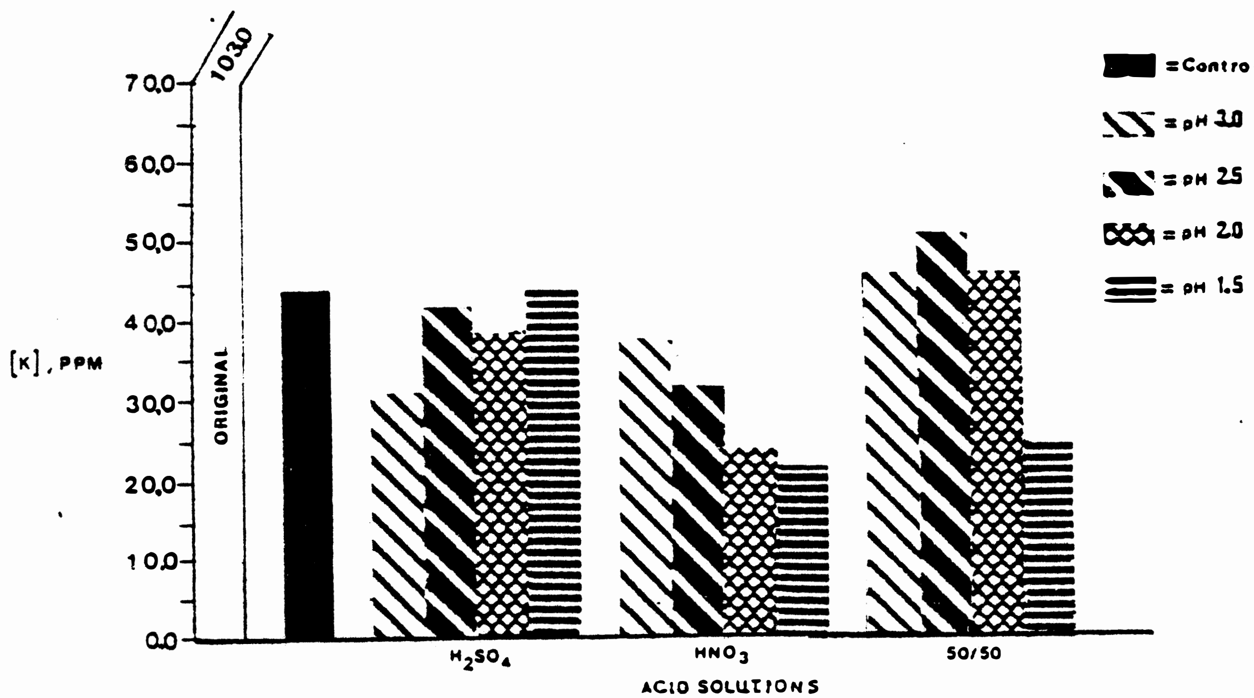


Fig. 21. Potassium levels for the soil in which the Fine Fescue variety, Pennlawn, was grown.

phorus levels showed little variation for any of the treatments and remained comparable to the levels found in the original soil samples (Figs. 22-27). This was not surprising since phosphorous is usually quite immobile in the soil (Beard, 1973). Nitrate tests showed significant nitrate levels in only the pH 1.5 HNO_3 solution, although these levels were below those found in the original soil samples (Figs. 28-33). All other treatments and the controls showed very little nitrate remaining in the soil, which was probably due to leaching. Since all treatments except the pH 1.5 HNO_3 treatment showed approximately the same amount of NO_3 as the controls, it seems likely that the acid solutions in this experiment did not increase the amount of nitrogen lost from the soil.

Soil from various treatments showed little change in pH for all but the very acidic treatments (i.e. pH 1.5). The H_2SO_4 solution appeared to acidify the soil the most (Figs. 34-39), but the pH changes noted probably had little effect on the height differences observed in this study since there was no correlation between height and soil pH. However, the literature indicates that acidification of soil may cause the loss of base ions, the reduction of cation exchange capacity, mobilization of aluminum ions, and changes in biological activity (Bache, 1980).

Height data (Figs. 10-15) showed that the samples recieving H_2SO_4 treatments had decreased growth for each sub-

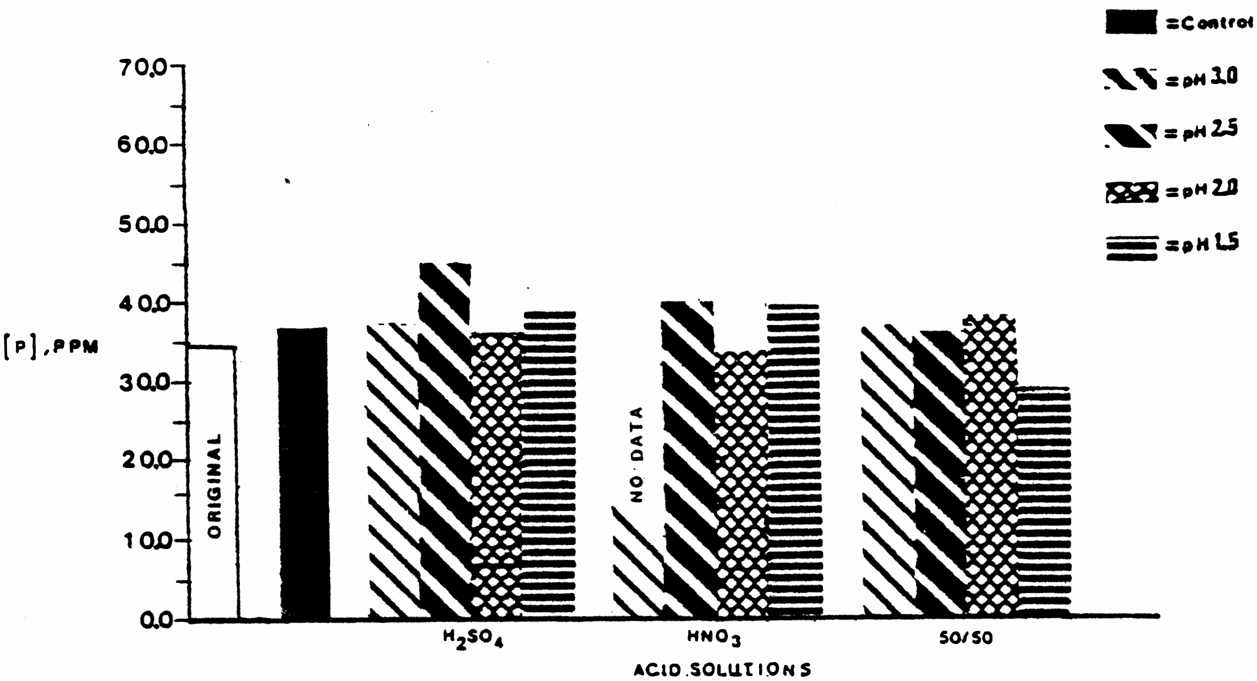


Fig. 22. Phosphorous levels for the soil in which the Kentucky Bluegrass variety, Baron, was grown.

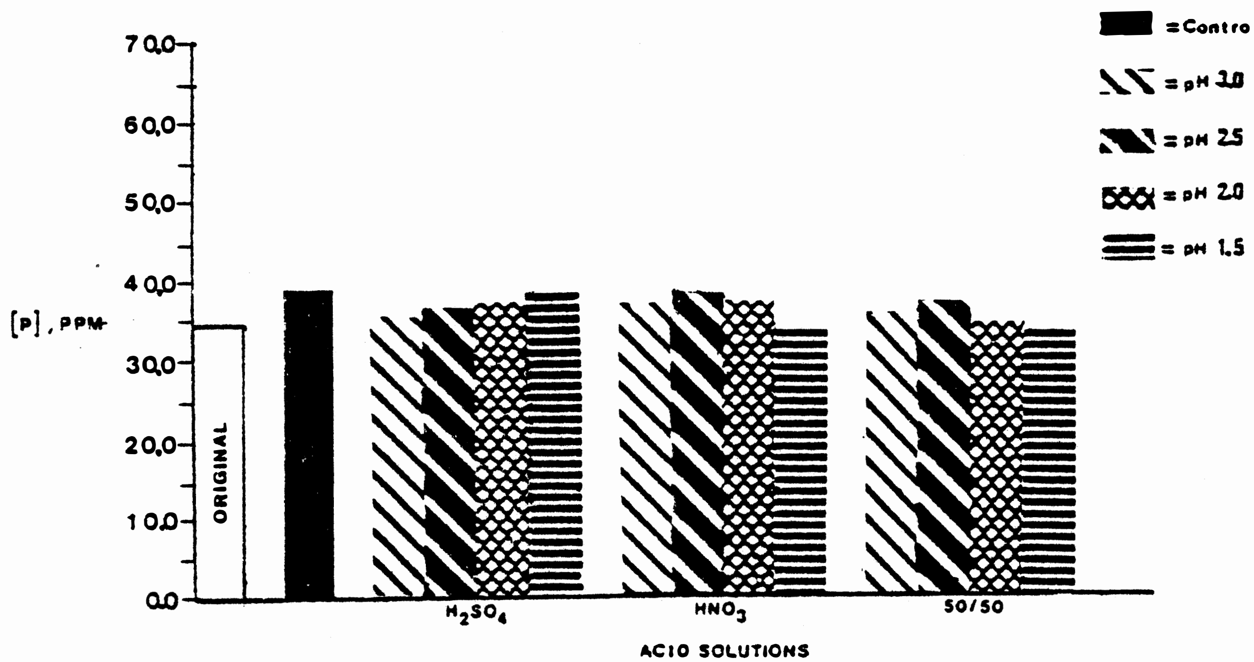


Fig. 23. Phosphorous levels for the soil in which the Kentucky Bluegrass variety, Victa, was grown.

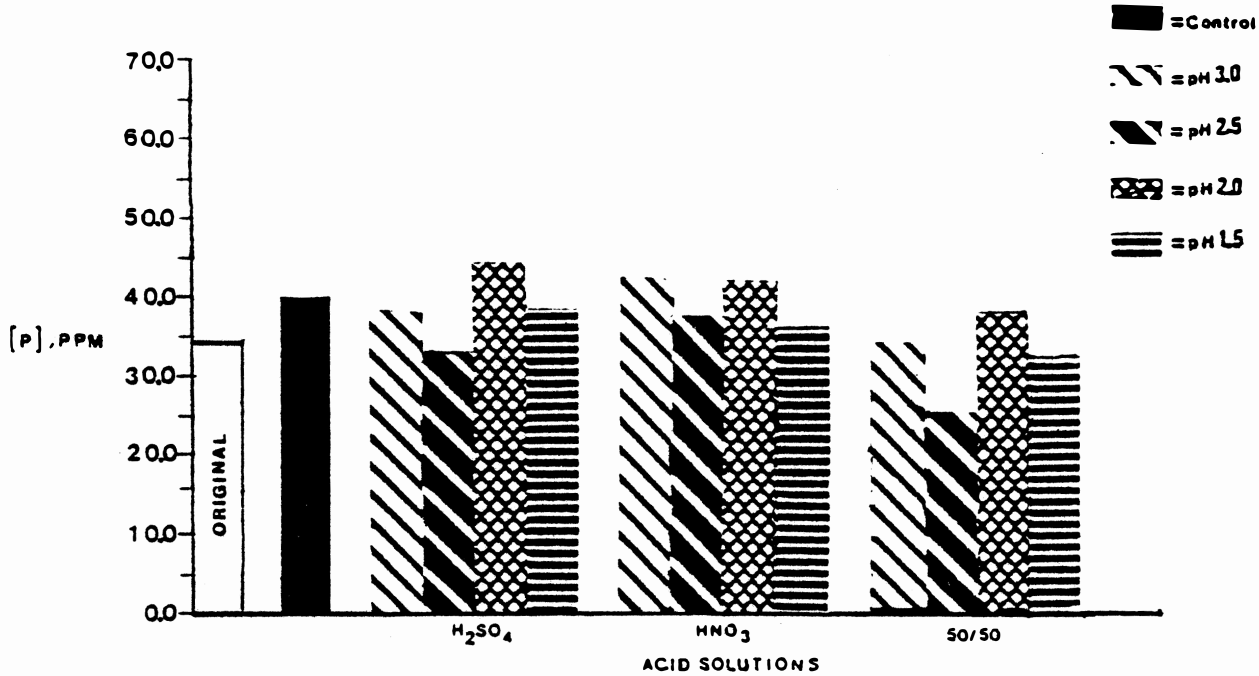


Fig. 24. Phosphorous levels for the soil in which the Perennial Ryegrass variety, Manhattan, was grown.

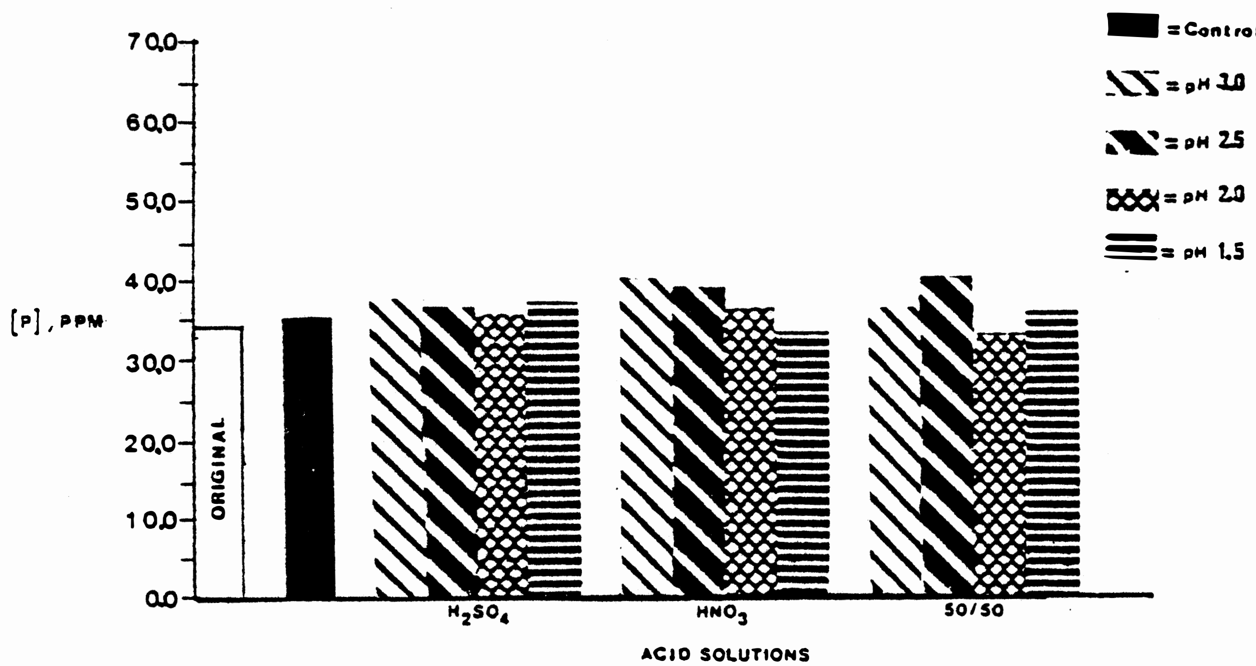


Fig. 25. Phosphorous levels for the soil in which the Perennial Ryegrass variety, Pennfine, was grown.

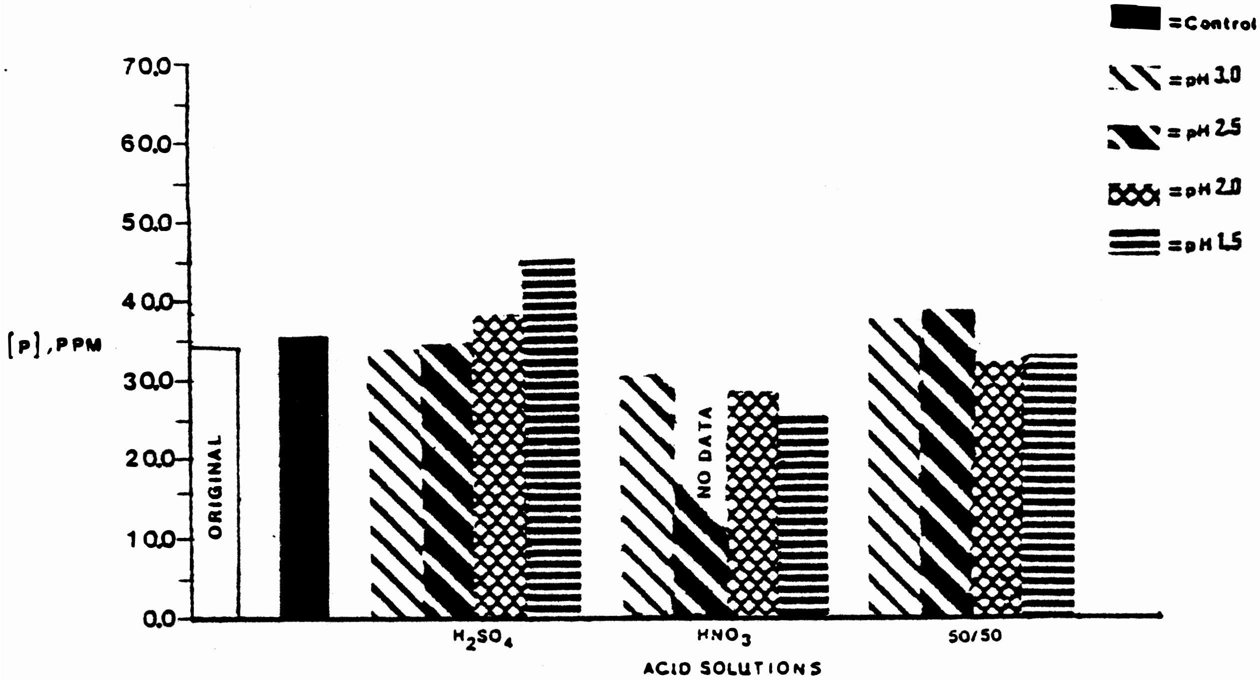


Fig. 26. Phosphorous levels for the soil in which the Fine Fescue variety, Biljart, was grown.

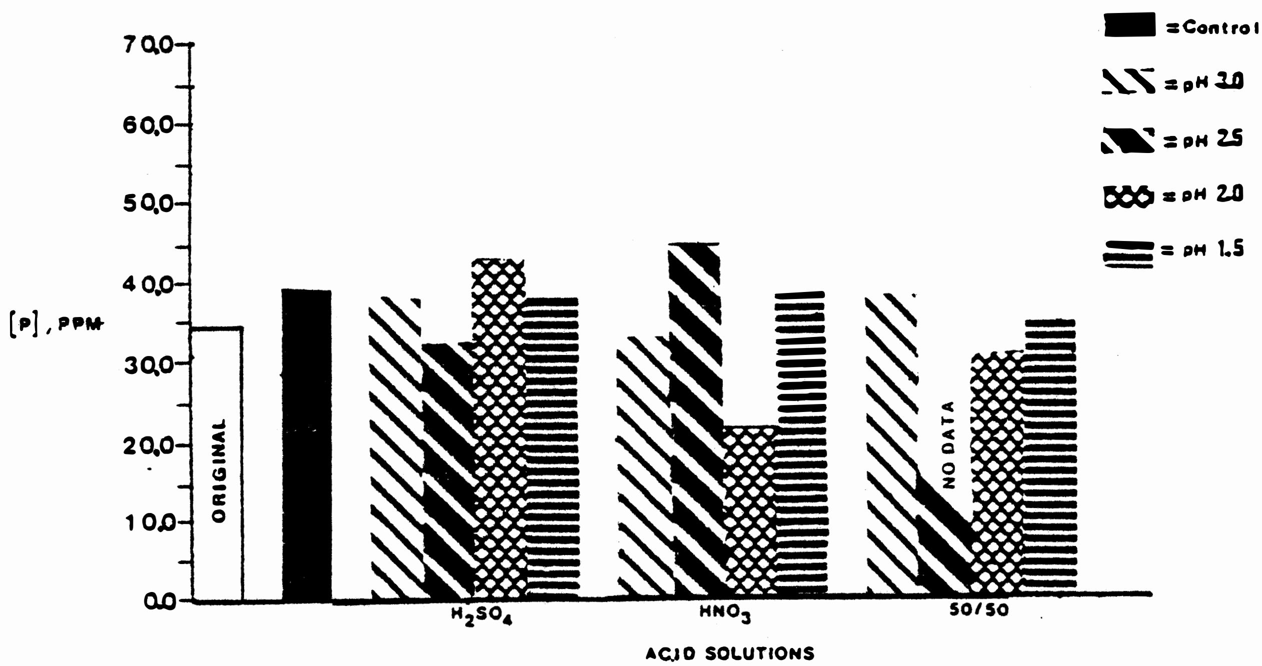


Fig. 27. Phosphorous levels for the soil in which the Fine Fescue variety, Pennlawn, was grown.

Fig. 28. Nitrate levels for the soil in which the Kentucky Bluegrass variety, Baron, was grown.

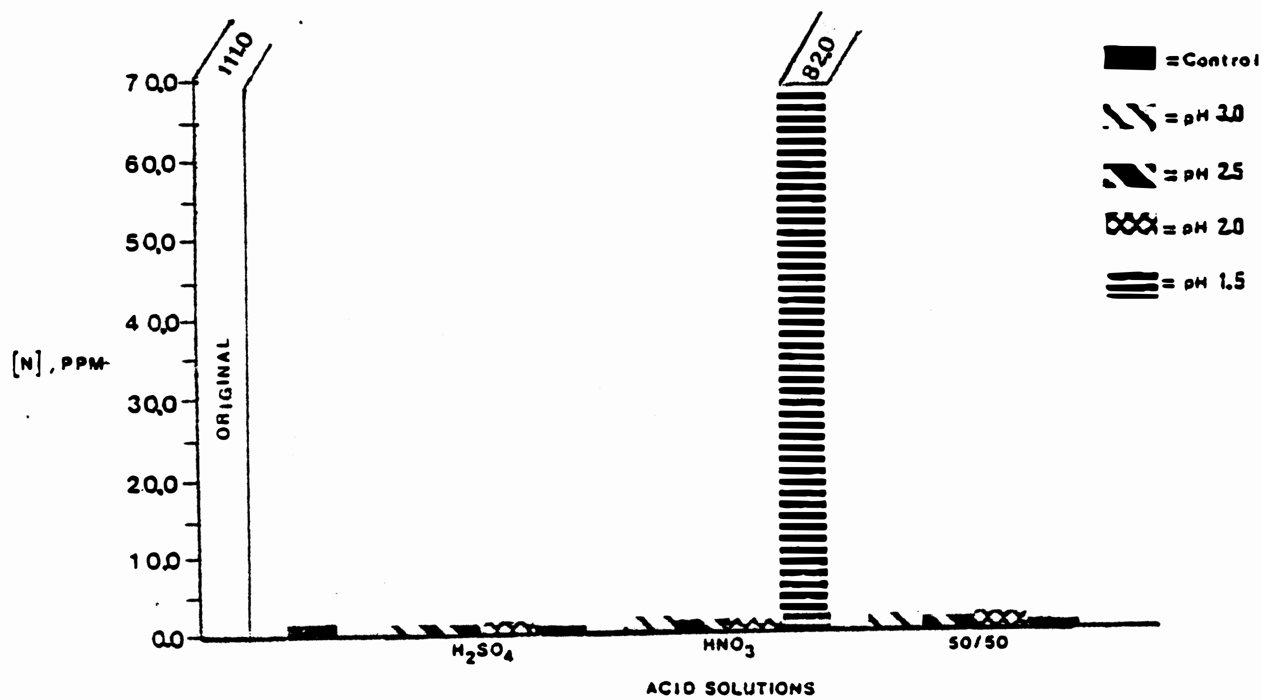


Fig. 29. Nitrate levels for the soil in which the Kentucky Bluegrass variety, Victa, was grown.

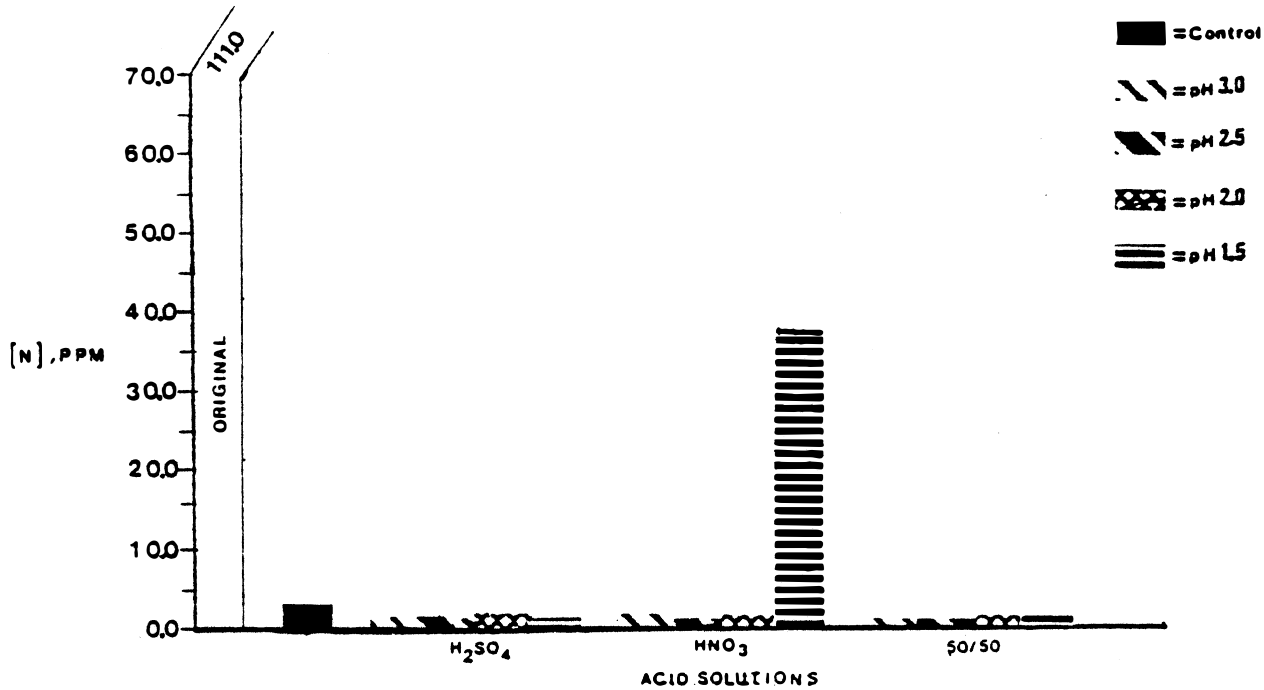


Fig. 30. Nitrate levels for the soil in which the Perennial Ryegrass variety, Pennfine, was grown.

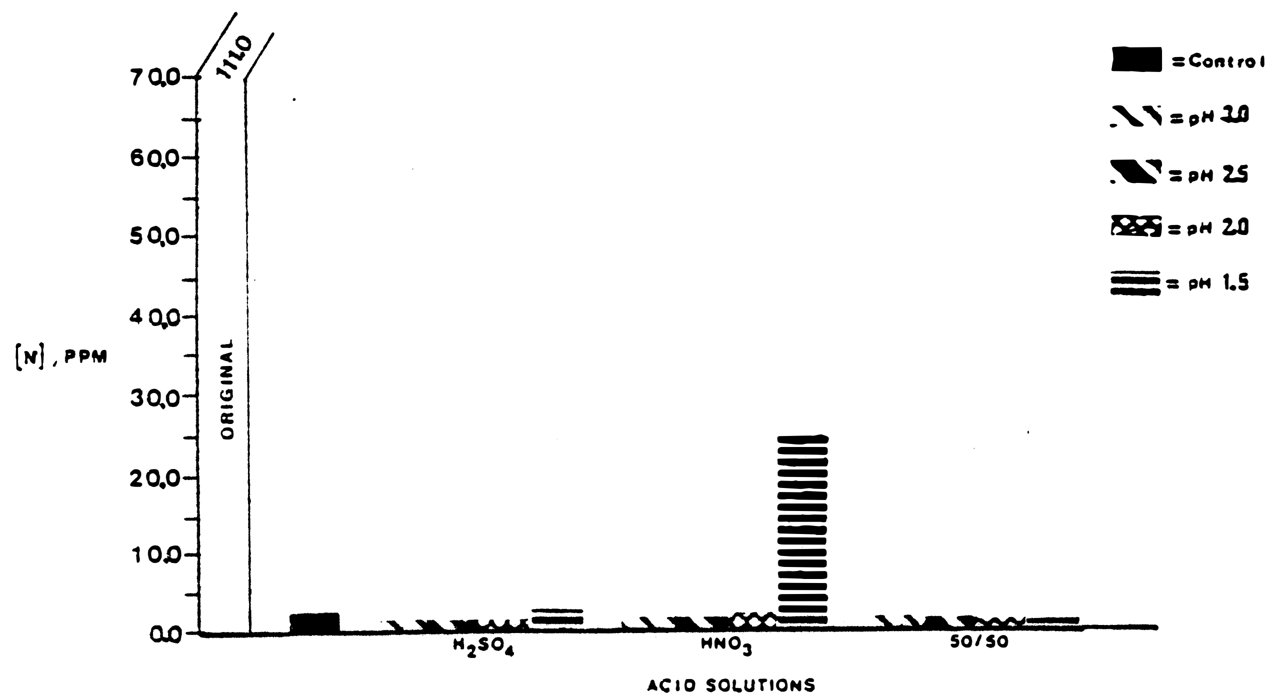


Fig. 31. Nitrate levels for the soil in which the Perennial Ryegrass variety, Manhattan, was grown.

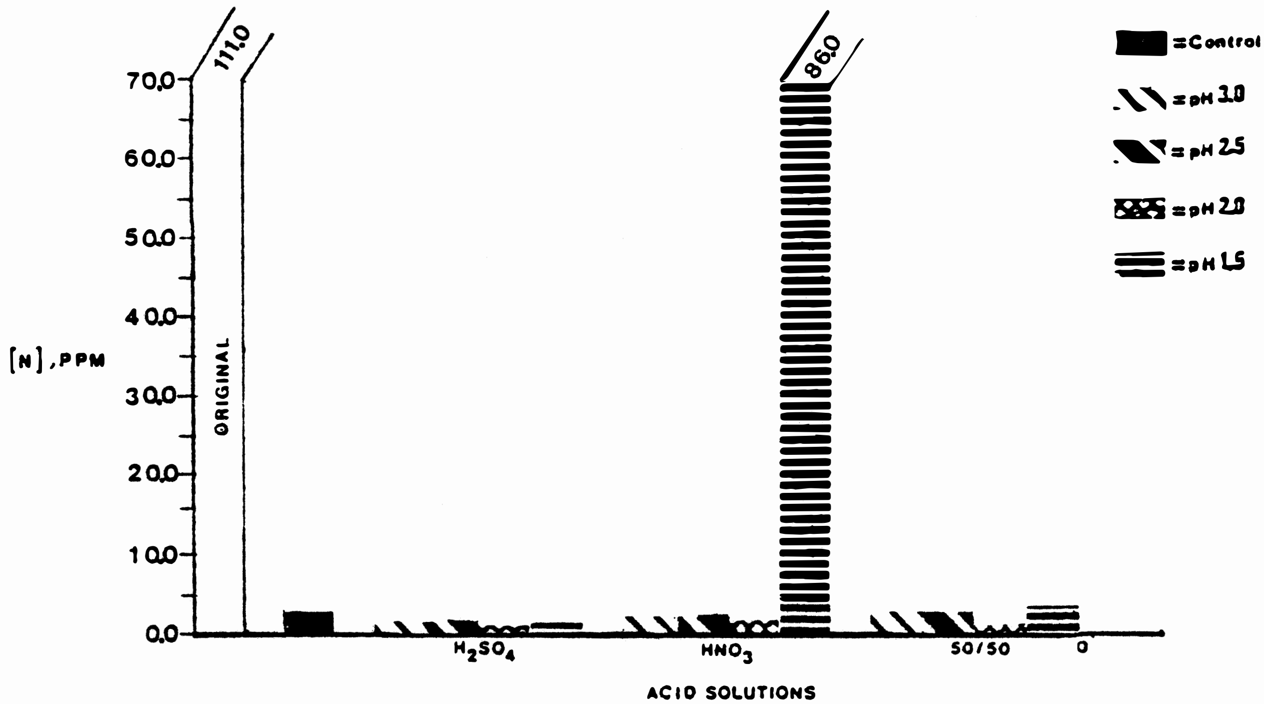


Fig. 32. Nitrate levels for the soil in which the Fine Fescue variety, Biljart, was grown.

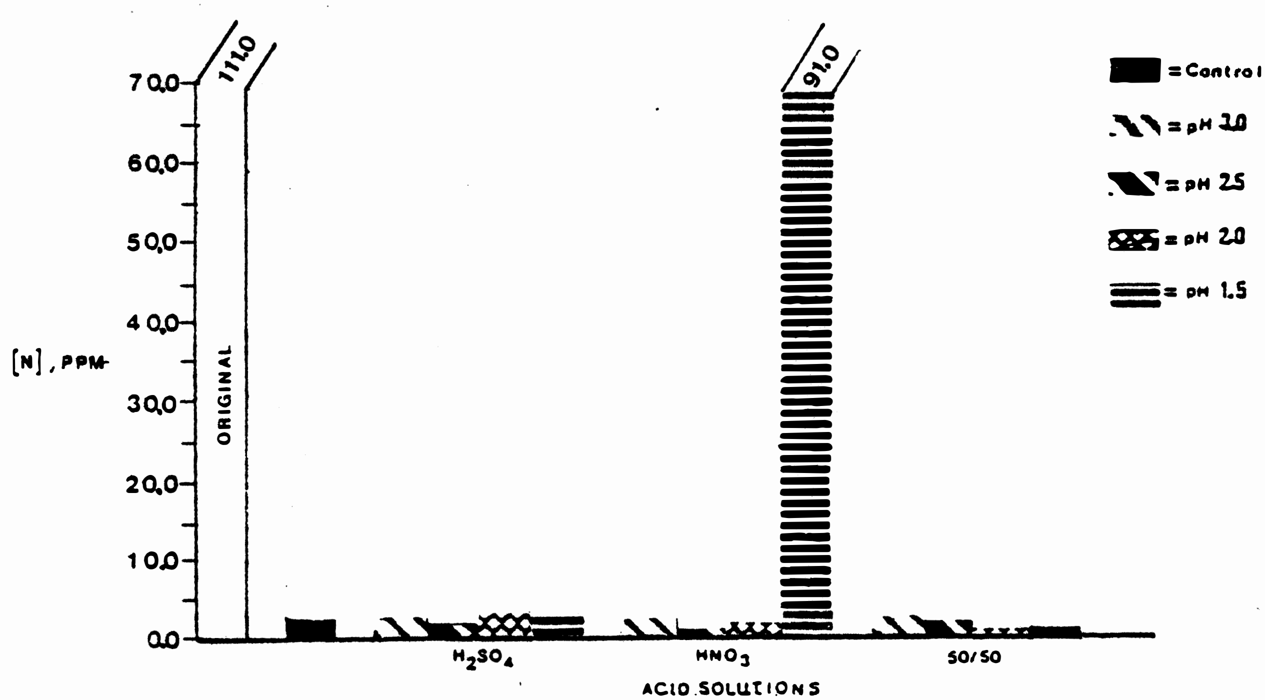


Fig. 33. Nitrate levels for the soil in which the

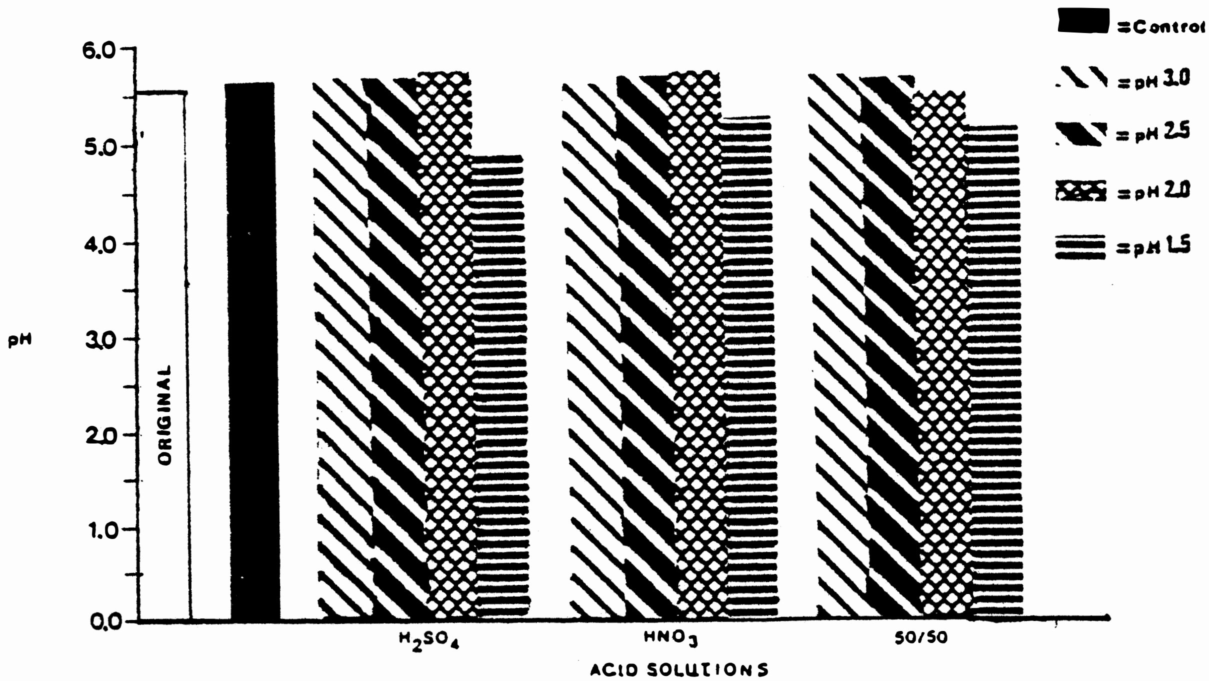


Fig. 34. pH levels for the soil in which the Kentucky Bluegrass variety, Baron, was grown.

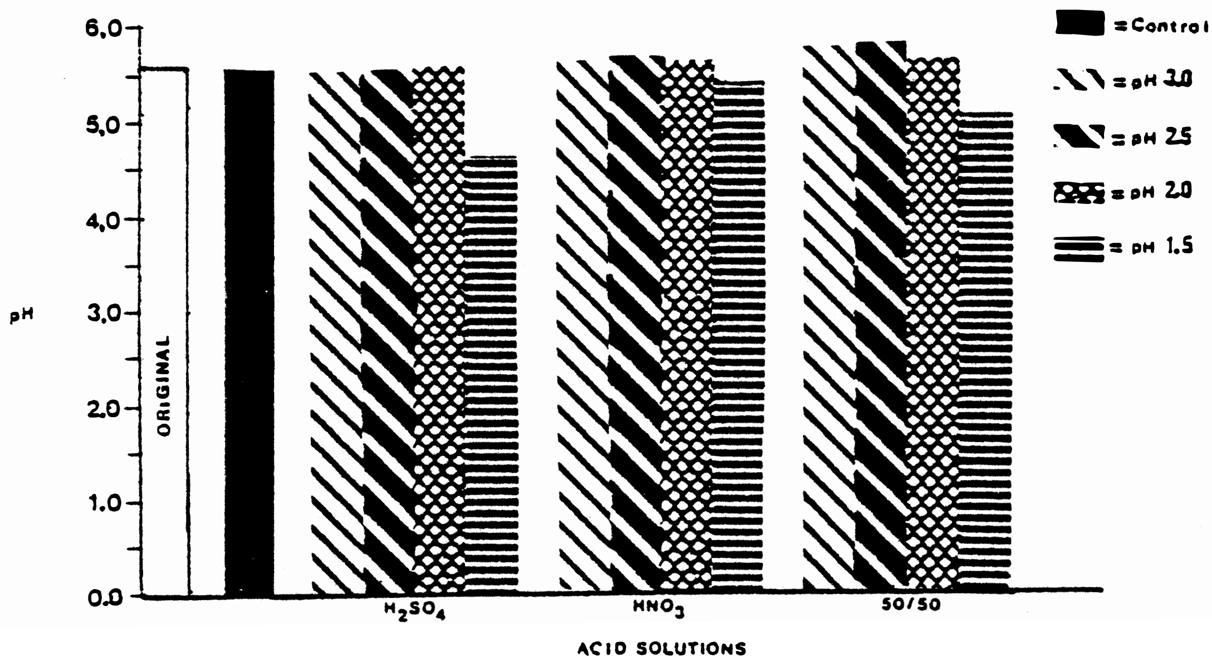


Fig. 35. pH levels for the soil in which the Kentucky Bluegrass variety, Victa, was grown.

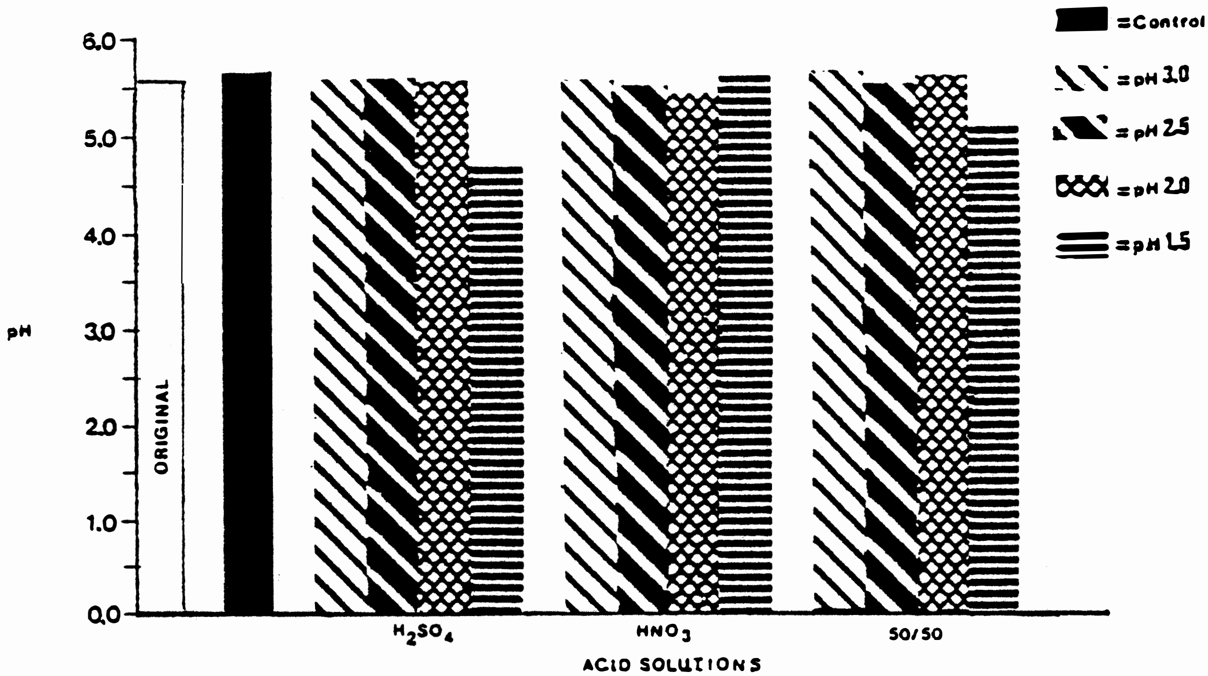


Fig. 36. pH levels for the soil in which the Perennial Ryegrass variety, Manhattan, was grown.

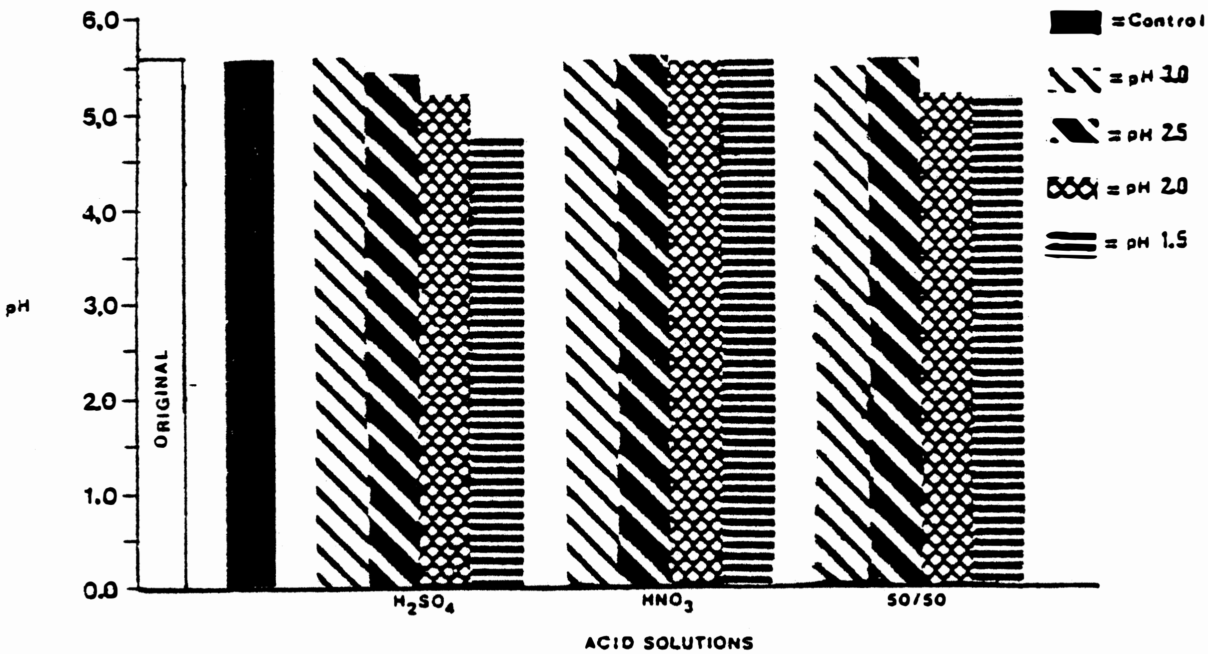


Fig. 37. pH levels for the soil in which the Perennial Ryegrass variety, Pennfine, was grown.

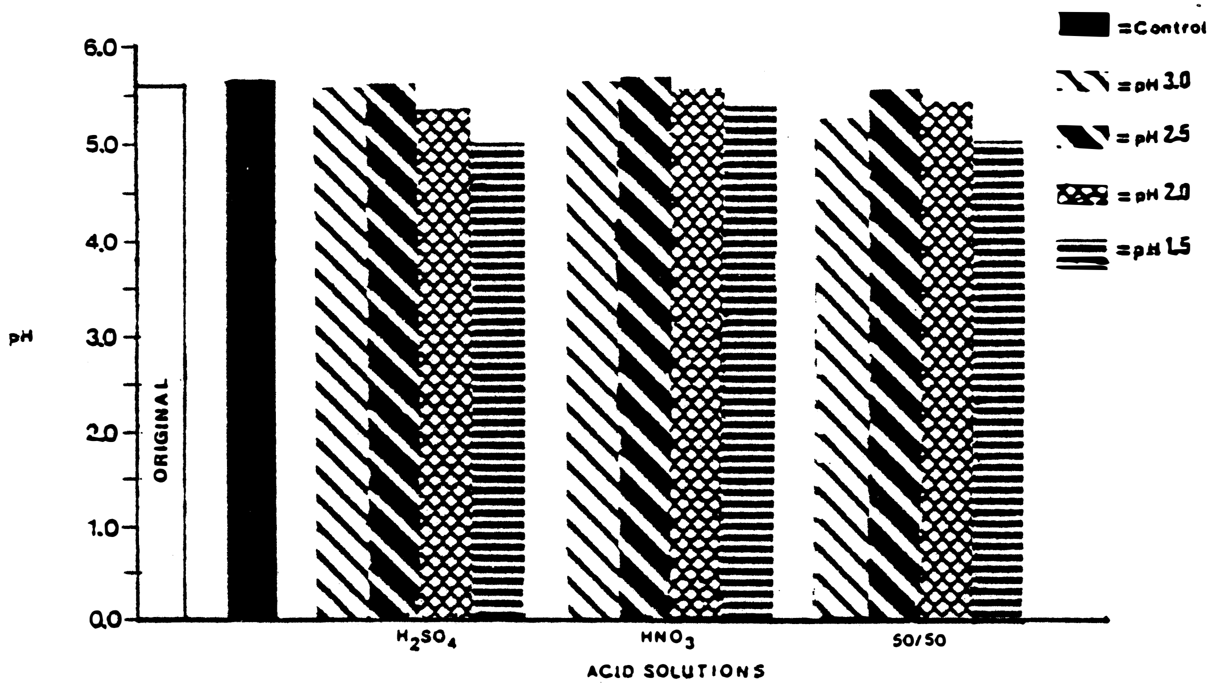


Fig. 38. pH levels for the soil in which the Fine Fescue variety, Biljart, was grown.

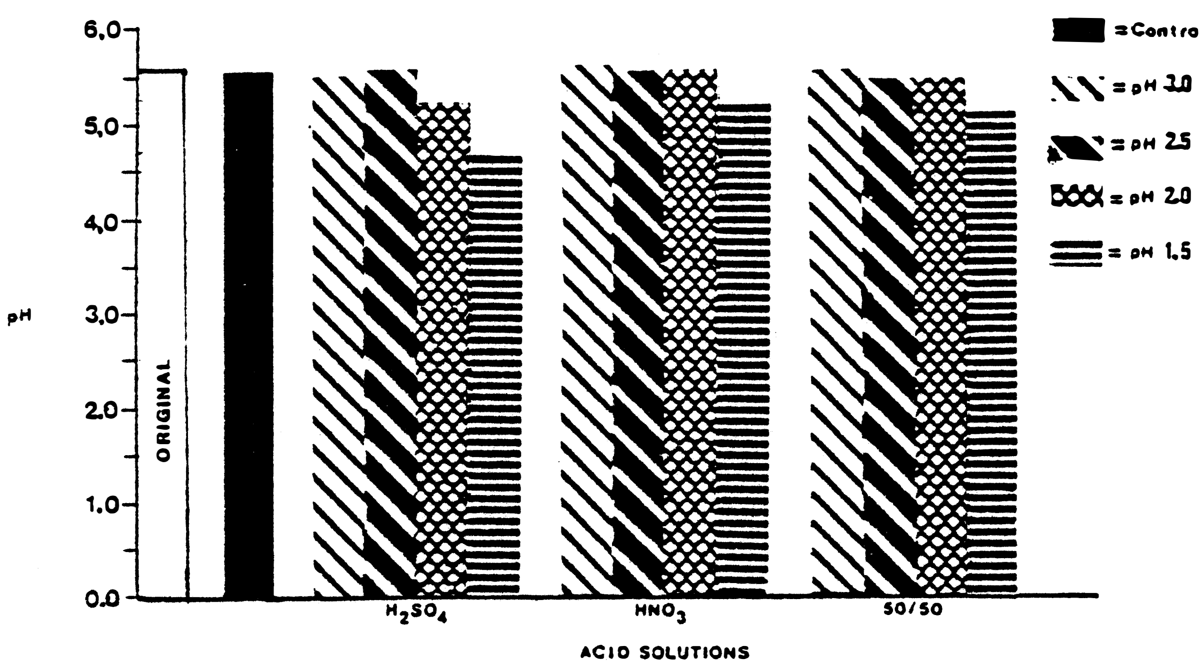


Fig. 39. pH levels for the soil in which the Fine Fescue variety, Pennlawn, was grown.

sequent run. The HNO_3 and $\frac{1}{2}H_2SO_4$-$\frac{1}{2}HNO_3$ treatments showed this decrease for the pH 3.0 and pH 2.5 solutions, but the pH 2.0 and pH 1.5 solutions showed heights equivalent to those found in the first run. Control cups also showed decreased growth for each subsequent run, indicating a change in soil conditions was responsible for height differences rather than direct affects of the acid solutions on the plants.

Damage trends varied (Figs. 4-9), but on the whole indicated increased visual damage with decreased pHs, as was expected. There appeared to be a definite relationship between the amount of growth (height) of these turfgrasses and the amount of visible injury present. When growth was decreased under H_2SO_4 treatments the visual injury also decreased. Plants treated with HNO_3 and $\frac{1}{2}H_2SO_4$-$\frac{1}{2}HNO_3$ solutions which showed increased height also tended to show increased foliar injury. This suggests that rapidly growing turfgrasses are more susceptible to damage than are slower growing plants, possibly because of decreases in cell wall thickness (Beard, 1973).

Results from the chlorophyll extraction experiment showed that 93% of the chlorophyll was removed from the undamaged grasses used in the first run (Table 7). The percent extraction was decreased substantially in the injured plants. Knudson's study (Knudson et al., 1977) did indicate a slight decrease in the percent extraction for damaged plants,

Table 7. Mean percentage of chlorophyll extracted with 95% ethanol from the turfgrasses used in this study.

Turfgrass Variety	% Chlorophyll extracted*
Uninjured	
Biljart	82.9
Baron	93.4
Victa	95.1
Pennfine	95.6
Pennlawn	92.2
Manhattan	96.0
Injured	
Biljart	56.2
Baron	68.7
Victa	49.9
Pennfine	84.7
Pennlawn	72.7
Manhattan	82.0

* Mean value from 3 replicates

though the decrease was much smaller than the decrease seen in this study. Further studies would have to be done to determine the cause of this decrease. With additional work this method could probably attain at least a 95% extraction of chlorophyll for healthy leaves, and slightly less percentages for damaged leaves.

Chlorophyll stability data (Tables 8 & 9) indicated that there was little chlorophyll content change over a week's time for extracts stored in the refrigerator or in the dark at room temperature, when spurious values were not taken into consideration. This change could probably be reduced by minimizing the amount of time the samples are in the light between readings, and by storage under stricter conditions. The chlorophyll extracts which were stored in the light at room temperature showed large decreases in chlorophyll concentrations ranging from 20% to 68%.

Comparisons between damaged and undamaged turfgrass plants (Table 10) showed that in 4 of 6 varieties there was a reduction in chlorophyll A, chlorophyll B, and total chlorophyll in the damaged plants. The other two varieties may not have shown reductions because they seemed less heavily damaged than the others. The chlorophyll A/B ratio showed a decrease in these damaged turfgrasses also. These results seem to be in agreement with Knudson's study (Knudson et al., 1977) which showed that the chlorophyll A/B ratios in bean plants decreased as the total concentration of the

Table 8. Chlorophyll content (in mg/g dry wt.) of the various turfgrass varieties examined. Chlorophyll levels taken one week after storage under various conditions are also given. (First harvest)

Variety	Storage Treatment	Initial Values Chl. A	Initial Values Chl. B	Initial Values Total Chl.	Stored for 1 week Chl. A	Stored for 1 week Chl. B	Stored for 1 week Total Chl.
Biljart	Refrigerated	1.56	.555	2.11	1.43	.544	1.97
	Room temp:						
	- Dark	1.13	.715	1.85	1.38	.410	1.79
	- Light	1.25	.518	1.77	.482	.188	.670
Baron	Refrigerated	2.96	1.13	4.09	-	-	-
	Room temp:						
	- Dark	2.56	1.04	3.60	2.70	1.13	3.83
	- Light	2.71	1.10	3.80	.672	.654	1.33
Victa	Refrigerated	2.33	.992	3.32	2.35	.942	3.29
	Room temp:						
	- Dark	2.59	1.02	3.61	-	-	-
	- Light	2.57	1.01	3.58	.698	.589	1.29
Pennfine	Refrigerated	1.18	.505	1.68	1.20	.516	1.72
	Room temp:						
	- Dark	1.36	.582	1.94	1.49	.686	2.18
	- Light	1.60	.655	2.26	.362	.353	.715
Pennlawn	Refrigerated	1.56	.649	2.21	1.56	.649	2.21
	Room temp:						
	- Dark	1.56	.600	2.16	1.62	.722	2.34
	- Light	1.67	.688	2.35	.504	.395	.899

Table 8. Con't.

Variety	Storage Treatment	Initial Values Chl. A	Initial Values Chl. B	Initial Values Total Chl.	Stored for 1 week Chl. A	Stored for 1 week Chl. B	Stored for 1 week Total Chl.
Manhattan	Refrigerated	1.33	.582	1.91	1.37	.601	1.98
	Room temp:						
	- Dark	2.05	.822	2.87	2.15	.850	3.00
	- Light	1.43	.697	2.13	.508	.452	.960

Table 9. Chlorophyll content (in mg/g dry wt.) of the various acid rain treated turfgrass varieties examined. Chlorophyll levels taken one week after storage under various conditions are also given. (Second harvest)

Variety	Storage Treatment	Initial Values Chl. A	Chl. B	Total Chl.	Stored for 1 week Chl. A	Chl. B	Total Chl.
Biljart (C-26)							
Uninjured	Refrigerated	3.36	1.07	4.43	3.28	.892	4.17
	Room temp:						
	- Dark	4.29	1.75	6.04	4.31	1.33	5.64
	- Light	3.72	1.43	5.15	2.43	1.32	3.75
Injured	Refrigerated	7.71	2.68	10.4	7.30	2.47	9.77
	Room temp:						
	- Dark	4.19	2.05	6.24	4.25	.926	5.17
	- Light	3.29	1.14	4.43	1.95	.956	2.91
Baron							
Uninjured	Refrigerated	6.24	2.76	9.00	5.92	2.02	7.94
	Room temp:						
	- Dark	5.46	2.14	7.60	5.37	1.95	7.32
	- Light	6.05	2.05	8.10	4.06	1.84	5.90
Injured	Refrigerated	7.91	2.71	10.6	7.59	2.89	10.3
	Room temp:						
	- Dark	7.66	3.00	10.7	7.64	2.68	10.3
	- Light	7.95	2.70	10.6	4.54	2.09	6.63

Table 9. Con't.

Variety	Storage Treatment	Initial Values Chl. A	Chl. B	Total Chl.	Stored for 1 week Chl. A	Chl. B	Total Chl.
Pennlawn							
Uninjured	Refrigerated	7.96	2.68	10.6	7.73	2.57	10.3
	Room temp:						
	- Dark	6.66	1.71	8.37	6.67	2.14	8.90
	- Light	7.63	2.93	10.6	4.56	1.93	6.49
Injured	Refrigerated	5.98	2.12	8.10	5.78	2.02	7.80
	Room temp:						
	- Dark	7.43	2.69	10.1	7.41	2.42	9.83
	- Light	5.35	1.83	7.18	3.53	1.58	5.11
Manhattan							
Uninjured	Refrigerated	9.16	3.22	12.4	8.85	3.25	12.1
	Room temp:						
	- Dark	9.19	2.39	11.6	9.19	2.99	12.2
	- Light	7.73	2.71	10.4	5.04	2.40	7.44
Injured	Refrigerated	7.94	2.92	10.9	7.66	2.89	10.6
	Room temp:						
	- Dark	6.35	2.32	8.67	6.31	2.24	8.55
	- Light	7.93	2.53	10.5	4.86	2.31	7.17

Table 9. Con't.

Variety	Storage Treatment	Initial Values Chl. A	Chl. B	Total Chl.	Stored for 1 week Chl. A	Chl. B	Total Chl.
Victa							
Uninjured	Refrigerated	8.13	2.91	11.0	7.70	2.81	10.5
	Room temp:						
	- Dark	7.04	2.50	9.54	7.15	2.35	9.50
	- Light	7.20	2.51	9.71	4.87	2.09	6.96
Injured	Refrigerated	4.88	1.78	6.66	4.75	1.77	6.52
	Room temp:						
	- Dark	6.06	2.29	8.35	5.96	2.05	8.01
	- Light	4.96	2.01	6.97	3.21	1.43	4.64
Pennfine							
Uninjured	Refrigerated	6.80	2.45	9.25	6.56	1.90	8.46
	Room temp:						
	- Dark	8.50	3.41	11.9	8.55	3.19	11.7
	- Light	8.70	3.43	12.1	6.91	2.71	9.62
Injured	Refrigerated	8.18	2.91	11.1	7.89	2.94	10.8
	Room temp:						
	- Dark	7.60	3.09	10.7	7.49	2.83	10.3
	- Light	8.33	3.27	11.6	5.51	2.70	8.21

Table 10. Mean values (in mg/g dry wt.) of chlorophyll A, chlorophyll B, and total chlorophyll from injured and uninjured samples of the turfgrass varieties used in this study. Chlorophyll A/B ratios are also reported.

Turfgrass Varieties		Chl. A	Chl. B	Total Chl.	A/B Ratio
Biljart (C-26)	- uninjured	3.79	1.42	5.21	2.67
	- injured	3.74	1.60	5.34	2.34
Baron	- uninjured	7.84	2.80	10.6	2.82
	- injured	5.92	2.32	8.23	2.55
Victa	- uninjured	7.46	2.64	10.1	2.83
	- injured	5.30	2.03	7.30	2.61
Pennfine	- uninjured	8.00	3.10	11.1	2.59
	- injured	8.04	3.09	11.1	2.60
Pennlawn	- uninjured	7.42	2.44	9.86	3.04
	- injured	6.25	2.21	8.46	2.83
Manhattan	- uninjured	8.69	2.77	11.5	3.14
	- injured	7.41	2.59	10.0	2.86

chlorophyll decreased. Possible explanations given were that chlorophyll A may be more readily degradable than chlorophyll B, or that the synthesis of chlorophyll B was increased (or that of chlorophyll A decreased) relative to uninjured leaves. This study seemed to suggest that it was a degradation of chlorophyll A which caused the decrease since the chlorophyll A levels seemed to be more drastically reduced than the chlorophyll B levels in the damaged leaves (Table 10), though there are probably other factors involved.

The visible damage measurements (Figs. 4-9) showed that a difference in treatments did occur in this study, but this method was only good as a general indication of injury levels, and subject to bias. Height differences proved to be a good indication of soil condition, but really did not directly indicate any actual damage to the plants themselves. The chlorophyll method described in this paper seemed to be the most promising method for injury determination because of it's ease of use and relative sensitivity.

It is difficult to say if acid rain might cause serious effects on turfgrasses in the natural environment. It has been suggested that acid rainfall may act as a "poor man's fertilizer" (Farm Journal Staff, 1981) by adding nutrients to the soil. This study showed that though nitrates were reduced in most of the soil samples, there was still increased growth (in the case of HNO_3 and $\frac{1}{2}H_2SO_4$-$\frac{1}{2}HNO_3$ solutions) over the control. This was probably because the grass plants used the nitrates as fast as they were added to the soil by the acid solutions. This does not necessarily mean that this is advantageous though. For one thing, these rapidly growing turfgrasses suffered more physical (lesion) damage than did the ones recieving less nitrogen. Other affects of increased nitrogen levels and growth rates include the necessity to mow more often, possible changes in the composition of turfgrass communities, and the enhancement of the growth of weeds such as crabgrass and annual bluegrass (Beard, 1973). Another consideration is that other nutrients in the soil beside nitrogen may be removed by the acid rainfall, as was the potassium in this study (Figs. 16-21), and become the limiting factor for plant growth. It should also be noted that sulfuric acid, which is a major component in natural acid rainfall, provides no nitrogen at all and gave no indication of stimulating growth in this experiment (Figs. 10-15).

There did not seem to be any synergistic effects from the $\frac{1}{2}H_2SO_4$-$\frac{1}{2}HNO_3$ solutions used in this study. The height and visible damage measurements showed that these solutions followed the same trends as the HNO_3 solutions, indicating that it was the HNO_3 portion of the mixture expressing itself, while in the pH tests it was the opposite, with the H_2SO_4 portion increasing the acidity of the soil. Also, the $\frac{1}{2}H_2SO_4$-$\frac{1}{2}HNO_3$ solutions effect on potassium levels was intermediate between that of straight HNO_3 and H_2SO_4 solutions.

In summary, this experiment indicated that acid rainfall did have various direct and indirect effects on the six turfgrass varieties studied, raising the possibility that other turfgrasses, wild grasses, and even crop grasses might also be affected. Since this was only a general examination for possible effects it would be desirable to do further, more detailed studies, such as leaching studies on soils exposed to acidic precipitation, foliar leaching studies, examinations of lawns and other large plantings of turfgrasses in areas exposed to natural acidic rainfall, tests on plots of turfgrass grown in the natural environment, and physiological tests on the plants themselves (i.e. carbohydrate tests).

Turfgrass injury may be a relatively minor problem compared to other effects attributed to acid rain, but it has the potential of becoming a major irritation to people who spend much time and money maintaining lawns for landscaping, sporting, or commercial purposes.

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