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The effect of nitrogen :: potassium ratios on nutrient content and low temperature hardiness of perennial ryegrass.

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THE EFFECT OF NITROGEN:POTASSIUM RATIOS ON NUTRIENT CONTENT AND LOW TEMPERATURE HARDINESS OF PERENNIAL RYEGRASS.

A Thesis Presented

By

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DEDICATION

This thesis is dedicated to;

- Carolyn Anne Brooks (my beloved wife) for her support, insight, and encouragement throughout my adult life and academic career.

- Barbara Ann Brooks (my mother) for her sacrifices, inspiration, and love.

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The completion of this study was made possible by the assistance and cooperation of many people. Special appreciation is expressed to DR. KIRK HURTO for serving as Chairman of my thesis committee, and for his encouragement and aid throughout the difficult stages of this study. Appreciation and gratitude is extended to DR. JOSEPH TROLL and PROF. JOHN ZAK for their service and help during this study and for serving as members of my Committee.

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CHAPTER ONE

INTRODUCTION

Perennial ryegrass (Lolium perenne L.) has emerged as an important turfgrass for home lawns, golf courses, athletic fields, and cemeteries. Observations in New England have indicated that perennial ryegrass turfs are damaged during severe winters. Many management factors seem to affect low temperature injury of perennial ryegrass. Among the least understood of these factors is the influence of soil fertility, especially nitrogen and potassium levels. It has been recognized for several years that nitrogen and potassium affect cold hardiness of turfgrass species, even though research concerning nitrogen:potassium ratios effect on winter injury of cool season turfgrass has not been extensive. It is known that nitrogen and potassium are essential growth elements but disagreements remain as to the appropriate amounts required to optimize low temperature survival of turfgrasses.

The purpose of this experiment is to examine the effects of nitrogen:potassium ratios on the direct low temperature kill of perennial ryegrass.

CHAPTER TWO

LITERATURE REVIEW

Perennial ryegrass (Lolium perenne L.) is thought to be one of the first cultivated grasses and is used extensively as a fine turfgrass in the temperate regions of the world. Perennial ryegrass originated in the Mediterranean region of southern Europe, North Africa, and Asia Minor and is adapted to cool, humid regions that have mild winters and cool summers. Perennial ryegrass is a common constituent of seed mixtures used on home lawns, parks, cemeteries, golf courses, and other general use turfgrass areas. Perennial ryegrass is a desirable turfgrass because of its rapid germination rate (usually within 7 days), high shoot density (100-200 shoot/dm^2 at 3.8 cm cutting height), fine texture (leaf width 1-2 mm), uniformity, and wear tolerance, and is adapted to a wide range of soil types. Perennial ryegrass is generally considered to be a short-lived perennial but can persist indefinitely if not subjected to extreme temperature stress. Perennial ryegrass has the poorest low temperature hardiness of all the perennial cool season turfgrass commonly used in the United States (Beard, 1966 & Carroll, 1943). Recent interest by turfgrass breeders and the seed industry has resulted in the release of numerous improved turf-type perennial ryegrass cultivars which exhibit improved potential for use as a permanent turfgrass. Improved cultivars such as 'Manhattan' perennial ryegrass retain the characteristics of rapid establishment rate, high shoot density, texture, and uniformity; and have improved tolerance to low temperature stress.

Optimum temperature for perennial ryegrass growth is dependent on the stage of plant development. Beevers and Cooper (1964) investigated early growth stages (4 to 7 weeks) of perennial ryegrass and observed that plants grown at 25/15 C (day/night) temperatures had higher relative growth rates and dry matter production than did plants grown at 12/12 or 25/25 C temperature regimes. Munata et al (1963; 1966) working with mature perennial ryegrass reported that photosynthesis was optimal at 15 C and decreased slowly at lower temperatures and rapidly at temperatures above 35 C. However, respiration rate increased exponentially with increasing temperatures. Parks and Fischer (1958) reported that mature perennial ryegrass yield was superior at a constant temperature of 20 C than at 10 C or 30 C. Watschke et al (1973) reported perennial ryegrass yields were greater at 23/15 than at 32/25 C temperatures. Valentine and Charles (1979) reported perennial ryegrass had maximum dry-matter yield at a constant 15 C. As the above studies indicate, perennial ryegrass has a temperature-dependent growth rate with optimal growth occurring within the range of 15 to 25 C. This optimal growth range for perennial ryegrass is conducive with the mild summers of New England.

The major factor affecting the persistence of perennial ryegrass in New England are the severe winters. Winter injury of perennial ryegrass can be caused by one or several factors including; direct low temperature kill, alternating freezing and thawing cycles, smothering due to ice layer formation which prevents gas exchange, dessication, and low temperature diseases.

Several workers have examined the low temperature persistence of perennial ryegrass and other cool season turfgrasses. Carroll (1943) reported the lethal soil temperature for Kentucky bluegrass (Poa pratensis L.) and bentgrass (Agrostis spp.) was between -10 and -15 C. Beard (1966) using sod plugs determined that air temperatures of -15 C for 15 hours was lethal to 'Norlea' perennial ryegrass. Thomas and Fayenby (1968) found that a tall fescue (Festuca arundinacea Schred.) ecotype from North Africa had a lethal temperature of -8 C. Cook and Duff (1976) reported that 'Kentucky 31' tall fescue had a similar lethal temperature. Lorenzetti et al (1971) working with different varieties and ecotypes of perennial ryegrass used a freezing test to determine cold tolerance. Decreasing air temperature resulted in 50 percent kill (LT_{50}) of plants held at -8 C for 2 to 4 days. Fuller and Eagles (1978) working with perennial ryegrass seedlings observed a LT_{50} ranging from -5.7 to -12.2 C depending on the method of freezing and cultivar of perennial ryegrass used.

The effects of soil fertility on the direct low temperature kill of various turfgrass have been well documented. (Adams and Twersky, 1960; Carroll and Welton, 1939; Cook and Duff, 1976; Gilbert and Davis, 1971; Goss, 1963; Jung and Kocher, 1974; Juska and Murray, 1974; Keisling, 1979; Kresge, 1974; Kresge and Decker, 1965; Ledeboer and Skogley, 1973; Reeves and McBee, 1972; Reeves et al, 1970; Schwartzkopf, 1972; Smith, 1964; Wilkinson and Duff, 1972; Wilson et al, 1966).

Numerous workers have shown that excessive nitrogen results in reduced low temperature hardiness (Beard and Rieke, 1966; Carroll,

1943; Carroll and Welton, 1939; Gilbert and Davis, 1967; Harrison, 1931; Juska and Hanson, 1967; Miller, 1966). High nitrogen fertilization levels stimulate growth (at the expense of carbohydrate reserves), increase cell size, and reduce cell wall thickness; thereby creating a more succulent plant (Carroll, 1943; Drake et al, 1963; Roberts and Sprague, 1933). Oswalt et al (1959) observed that nitrogen will stimulate shoot growth at the expense of root growth. Increased tolerance to low temperature stress has been reported to be favored by conditions which reduce metabolic activity before low temperature stress conditions occur (Levitt, 1956). Carroll and Welton (1939) in Ohio reported that Kentucky bluegrass fertilized to supply 365 kg N/ha in equal increments in the spring, summer, and fall had less tolerance to low temperature stress than did unfertilized check plots. Carroll (1943) observed that heavy, late fall nitrogen applications increased the low temperature injury of 16 turfgrasses and concluded that low soil temperatures were more injurious to the plant than low air temperatures. Howell and Jung (1965) investigating the seasonal changes in cold resistance of 'Potomac' orchardgrass (Dactylis glopierata L.) at two rates of nitrogen fertilization reported that cold resistance increases rapidly through November, to a maximum in December and January, and decreases slowly through February and quickly through March. Resistance levels on March 30 and September 24 were equal. The lower nitrogen level resulted in less injury than the higher level of nitrogen fertilization. Jung and Kocher (1974) studying winter injury of several cool season turfgrasses noted that winter injury increases when rates

of nitrogen were increased from 0 to 240 kg/ha. Two common-type perennial ryegrass cultivars had an average winter injury of 83% for all N treatments, while 'Norlea' and 'Pennfine' had a winter injury of 33% and 41% respectively for all nitrogen treatments. Mathias et al (1973) conducted studies on perennial ryegrass fertilized with nitrogen at rates of 112, 224, 448, and 672 kg/ha/yr applied in three equal applications and observed no winter injury at air temperatures as low as -22 C. Baker and David (1963) found that high levels of nitrogen (in excess of 336 kg N/yr) promoted winter injury of perennial ryegrass. The above studies indicate that excessive nitrogen fertilization can be detrimental to low temperature hardiness of grasses.

The practice of potassium fertilization for reducing low temperature injury of turfgrass has been advocated in recent years (Beard, 1973; Schwartzkopf, 1972). However, limited research has been conducted on turfgrasses.

Improvements in winter survival and yield of alfalfa (Medicago sativa) were reported when there was 1.25% or more potassium in the herbage (Seay et al, 1949). Robinson et al (1962) reported that potassium fertilization increased survival and yield of orchardgrass (Dactylis glomerata L.). Wang et al (1953) stated that high levels of lime and available potassium markedly promoted winter survival of alfalfa. Jung and Smith (1959) working with alfalfa in sand culture noted that survival after exposure to freezing temperatures increased as the level of elemental potassium increased up to an amount equivalent to 224 kg/ha. Juska and Murray (1973) noted that Arizona common

bermudagrass [Cynodon dactylon (L.) Pers.] averaged 8% winterkill for plots treated with potassium and 27% winterkill for plots not receiving any potassium fertilization; while potassium treatments did not significantly improve winter survival of 'Midiron' bermudagrass, a cultivar selected for its improved tolerance to low temperature stress.

Investigators have advocated the use of balanced nitrogen to potassium ratios to increase the freezing tolerance of grasses (Beard, 1973; Parsens et al, 1953: Schwartzkopf, 1972; Smith, 1964). Winter survival of grasses appears to be favored by a narrow N:K ratio (Smith, 1964). Low clipping height combined with high N and low K fertilization severely reduced the stand density of orchardgrass in Indiana (Griffith and Teel, 1965). Adams and Twersky (1960) observed that low temperature injury of 'Coastal' bermudagrass resulting from high levels of nitrogen fertilization were reduced with increasing ratios of potassium fertilization. In a study by Reeves et al (1970), nitrogen levels did not significantly reduce cold hardiness but did influence the uptake of phosphorous and potassium. High phosphorous to potassium ratios increased freezing damage. Kresge and Decker (1966) reported winter injury of 'Midland' bermudagrass decreased with increasing rates of potassium. A 2.4:1 ratio of applied nitrogen to potassium maximized winter survival. Gilbert and Davis (1971) reported that nitrogen:phosphorous:potassium ratios of 4:1:4 and 5:1:5 improved regrowth of bermudagrass following low temperature stress. Optimum low temperature survival of common Kentucky bluegrass and 'Toronto' creeping bentgrass (Agrostis palustris Huds.) occurred at a ratio of

2:1 nitrogen to potassium (Beard and Rieke, 1966).

MacLeod (1956) examined the nitrogen to potassium interaction in a number of grass species and observed that potassium fertilization increased the storage of carbohydrate reserves at higher rates of nitrogen and that high rates of potassium without nitrogen fertilization were detrimental to the storage of carbohydrates. Several workers have shown a close correlation between low temperature hardiness and carbohydrate levels in the plant (Bula and Smith, 1954; Jung and Smith, 1961). Cook and Duff (1976) postulated that a properly balanced nitrogen to potassium ratio may affect carbohydrate metabolism associated with freezing tolerance.

Potassium fertilization effect on carbohydrate content has been investigated. Eaton (1952) working with sunflowers (Helianthus annus L.) showed that potassium deficiencies decreased the carbohydrate content of the plant. Russel (1938) found similar results with barley (Hordeum vulgare L.). Loustalot et al (1950) working with nitrogen and potassium relations in tung (Aleurites fordii Hemsl.), noted that low potassium levels were associated with a decrease in the rate of photosynthesis and growth.

Among higher plants most members of the families Campanulaceae and Compositae and the subclass Monocotyledonae accumulate fructosans as their reserve carbohydrate (Phillip et al, 1954; Steward, 1966). According to DeCugnac (1931), two groups of perennial grasses can be distinguished by the type of reserve carbohydrates. Warm season grasses including bermudagrass and bahiagrass (Paspalum notatum Flugge.)

store sucrose and starch while cool season grasses including bluegrass, bentgrass, orchardgrass, and ryegrass store fructose. Schluback (1957) postulated that cool season grasses contain a series of condensation products of fructose called fructosans. The degree of fructosan polymerization varies with the grass species.

The predominate carbohydrate present in perennial ryegrass is fructose and its polymeric form fructosan (Baker and Garwood, 1961; Oswalt, Bertrand and Teel, 1959; Sprague and Sullivan, 1950). Laidlaw and Reid (1952) found that fructosans in perennial ryegrass have a chain length of 25 to 30 fructose units. Fructosans are described as being levorotatory, amorphous or microcrystaline, of varying solubility in cold water, and highly soluble in hot water. Fructosans are non-reducing and are readily hydrolyzed by plant enzymes, to fructose by acids. Smith et al (1964) found that water was a suitable extractant only if fructosans predominate, since starch is not readily soluble in water. Fructosans occur in all parts of the grass plant. The amount found in any one part of the plant has been shown to be related to season, effect of defoliation and influences of fertilizers (Colby et al, 1965; Drake et al, 1963). Waite and Boyd (1953, 1958) observed that stems of actively growing grass plants contain higher levels of fructose polymers than do leaves.

MacLeod (1966) concluded that both nitrogen and potassium are required by plants to store carbohydrates and the supply of nitrogen and potassium should not be limited during the hardening and wintering stages. Beard and Daniel (1965) associated winter hardiness in grasses

to changes which include an increase in soluble carbohydrates and a reduction in the level of hydration in the plant tissue. Julander (1945) has shown that resistance of plants to injury during unfavorable conditions has been correlated with carbohydrate reserves in the plant.

CHAPTER THREE

MATERIALS AND METHODS

The influence of nitrogen to potassium ratios on the vegetative growth and development and low temperature hardiness of 'Manhattan' perennial ryegrass (Lolium perenne L.) maintained as a turf was studied in greenhouse and environmental growth chamber experiments.

Experiment 1

The procedure used to study perennial ryegrass response to different nutrient regimes was to grow three plants in semi-conical plastic tubes 4 cm in diameter and 21 cm long containing 165 cc of acid-washed silica sand. Ten-day old seedlings were selected for uniformity from a larger population which were germinated on half-strength Hoagland's solution (Hoagland and Arnon, 1950) in a growth chamber at 20 C and 16/8 (day/night) photoperiod. Seed used in all experiments was obtained from a single lot of certified 'Manhattan' perennial ryegrass.

Four nutrient treatments were applied per replicate using plexiglas boxes measuring 32 x 32 x 21 cm that were sectioned into four water-tight compartments (Figure 1). Each compartment was connected by tygon tubing to 20-liter carboys containing nutrient treatments (Table 1). Nutrient solutions were delivered to each compartment using a modified time-pressure- reservoir system as detailed by Garg et al (1967). Nine semi-conical tubes containing three perennial ryegrass plants were suspended in a rack above each water-tight compartment.

Figure 1. Arrangement of semi-conical tubes containing perennial ryegrass plants on support rack suspended over compartmentalized, plexiglas box.

Table 1. Concentrations of nutrient solutions used in Experiment 1.

N:K Ratio	Nitrogen (ppm)	Potassium (ppm)
1:1	100	100
1:2	100	200
1:3	100	300
2:1	100	50
2:3	100	150
2:5	100	250
3:1	150	50
4:1	200	50

Concentration of Remaining Plant Nutrients

Element	Concentration (ppm)
P	50.00
Ca	80.00
Mg	24.00
S	32.00
B	0.50
Mn	0.50
Zn	0.05
Cu	0.02
Mo	0.01
Fe	1.00

Pressurization of the carboys caused the nutrient solution to flood the respective compartment causing a capillary rise of the nutrient solutions into the sand contained in the semi-conical tubes (Figure 2). Height of solution movement in the tubes, duration, and frequency of nutrient delivery cycles was varied to maintain proper soil moisture content and to prevent wilt.

Plants were grown in the greenhouse at 20 C for five weeks following transplanting. Supplemental lighting using 1500 mamp fluorescent lamps was provided to extend the daylength to 16 hours. Plants were monitored daily and clipped twice weekly to 3.8 cm. Nutrient solutions were changed weekly.

The experimental design used in these experiments was a randomized complete block design with four nutrient treatments per experiment; two experiments were performed and each experiment was duplicated. Data presented are the means of separate experiments.

At the end of the five week growing period, the physiologically mature plants (Draper, 1975) were transferred to an environmental growth chamber and subjected to a hardening process for three weeks. During the first few days the temperature in the growth chamber was gradually lowered to 1 C and the photoperiod was decreased to 8/16 hours (day/night). Beard (1973) stated that air temperature between 4.4 and -1.1 C for a period of 21 to 28 days would produce plants tolerant to low temperature stress.

From each replication three tubes were harvested before freezing, three tubes were harvested after freezing, and three tubes were

Figure 2. Arrangement of plexiglas boxes and nutrient delivery system under supplementary lamps in the greenhouse.

allowed to recover from freezing under greenhouse conditions. Before the freezing period, three tubes were randomly selected from each treatment and root and shoot fresh and dry weights and tiller counts determined. Plant tissue below the root-shoot interface of the crown and leaf tissue 3.8 cm above the root-shoot interface were removed and discarded. The plant shoot tissue 3.8 cm in length from the root-shoot interface constituted the crown tissue. Crown tissue was separated into two groups for further analysis of electrolyte conductivity, minerals, and water-soluble carbohydrates.

To determine the extent of injury due to low temperature stess, leakage of electrolytes from the cells of the crown tissue was measured. Injury to plant cells by low temperature stress has been correlated with electrolyte leakage (Dexter, 1956). A single crown was placed in 18 x 150 mm test tubes containing 10 ml of distilled water and allowed to equilibrate for 24 hours. At the end of the equilibration period, the electrolyte conductivity was measured using a Markson 20 electrolyte conductivity meter. The crown tissue was boiled for 15 minutes at 1.0 kg/cm^2 pressure, which destroyed cell membrane integrity causing electrolytes to be released from the crown tissue and enabling total cell electrolyte conductivity to be measured. The initial reading divided by the final reading x 100 was used to determine the percent electrolyte leakage.

Mineral and carbohydrate analysis were performed on a second group of crowns dried at 70 C, and passed through a 40 mesh Wiley mill. Tissue was stored in an evacuated dessicator at -18 C prior to analysis.

Fructose analysis was performed using the anthnone method (Yemm and Willis, 1954) as modified by Colby et al (1965). Optical densities were measured at 620 nm and fructose concentrations were determined from a standard fructose curve. Total nitrogen was determined using a micro-Kjeldahl test (Stubblefield and DeTurk, 1940). Phosphorous was determined colorimetrically at 882 nm by the reduction of ammonium-molybodiphosphate complex by ascorbic acid (Watanabe and Olsen, 1965). Potassium was determined by emission spectrophotometry and calcium and magnesium were determined by absorption spectrophotometry.

The remaining six tubes were gradually subjected to a low temperature stress of -10 C for 24 hours. From preliminary experiments it was determined that an air temperature of -10 C for a period of 24 hours produced a stress which killed 50% of field-hardened plants (Tables 10 & 11). At the end of the freezing process three tubes were randomly selected and separated into crown tissue, and root and shoot fresh and dry weights, and tiller counts were determined. The plants in the remaining three tubes were allowed to recover after the freezing period for one week by gradually increasing the temperature in the environmental growth chamber to 20 C. The plants were transferred to the greenhouse and after four weeks of regrowth visual and laboratory determinations of hardiness, plant vigor, and quality were made.

Experiment 2

'Manhattan' perennial ryegrass seeds were germinated in flats containing a 80:20 (w/w) mixture of sand and peat. Plants were grown

for 32 weeks on four different N:K nutrient solutions (Table 2). Treatments were replicated six times in a randomized complete block design. Plants were maintained on their respective nutrient solutions, clipped to 3.8 cm and fertilized twice weekly from June to January. The perennial ryegrass plants were allowed to field harden in protective cold frames covered directly above, but open to the environment on all sides. After the onset of low but not lethal temperatures, plants were washed free of the sand/peat mixture and individual plant's roots were removed. The aerial portion of the plant was clipped to 2 cm above the root-shoot interface to remove all expanded leaves. Perennial ryegrass plants were placed in water-tight polyethylene bags and submerged in a circulation freezing bath containing a 1:1 ethylene/glycol: water (v/v) mixture for 6 hours at -10 C. Plants were allowed to thaw at room temperature and transplanted to flats containing expanded vermiculite. Plants were maintained for 4 weeks under greenhouse conditions and fertilized with half-strength Hoagland's (Hoagland and Arnon, 1950) solution. Measurements of the first fully expanded leaf blade and tiller survival were recorded.

Table 2. Concentrations of nutrient solutions used in Experiment 2.

N:K Ratio	Nitrogen (ppm)	Potassium (ppm)
1:1	100	100
2:1	100	50
2:3	100	150
3:1	150	50

Concentration of Remaining Plant Nutrients

Element	Concentration (ppm)
P	50.00
Ca	80.00
Mg	24.00
S	32.00
B	0.50
Mn	0.50
Zn	0.05
Cu	0.02
Mo	0.01
Fe	1.00

CHAPTER FOUR

RESULTS

Experiment 1

The effects of solution levels of N and K on growth and development of perennial ryegrass are summarized in Table 3. Fertilization treatments did not affect tiller numbers per plant, but had a significant effect on plant weight and carbohydrate levels. Maximum fresh weight accumulation within experiment occurred when solution ratio levels of N:K were 100 ppm N and 50 to 150 ppm K. High solution levels of K resulted in plants that were diminutive and chlorotic in appearance (Figure 3). Shoot dry weights increased linearly with increasing solution ratio N:K levels (Figures 4 & 5). A similar linear relationship existed between the ratio of N:K in solution and tissue level of water-soluble carbohydrate (Figures 6 & 7), but was only significant in experiment 1a.

Nutrient uptake of perennial ryegrass growing in different levels of N and K in solution are summarized in Table 4. No linear relationship existed between solution level of N and tissue N content. In experiment 1a, maximum plant N levels occurred with 100 or 200 ppm K and 100 ppm N; while in experiment 1b, maximum accumulation occurred with N:K solution levels of 200 and 50 ppm, respectively.

Phosphorous content of plant tissue was significantly higher in both experiments with low levels of solution K. Tissue P content was correlated to N content (Tables 5 & 6).

Table 3. Vegetative growth and development of 'Manhattan' perennial ryegrass.

Fertilization Treatments N:K	Plant Fresh Weight Total	Plant Dry Weight Shoot	Plant Dry Weight Root	Tillers/Plant	Carbohydrates Shoot
(ppm)	(mg)	(mg)	(mg)		(%)
Experiment 1a					
100:300	190b	69a	36b	6a	11.9b
100:200	200b	63a	40b	6a	14.4b
100:100	270a	77a	53a	7a	15.5b
100:50	270a	85a	52a	7a	18.4a
Experiment 1b					
100:150	590a	103bc	72a	8a	17.3b
100:250	472b	94c	62a	7a	16.1b
150:50	480b	115ab	69a	8a	23.0a
200:50	465b	126a	54a	8a	17.4b

Means within columns followed by unlike letters are significantly different at the 5% level by Duncan's Multiple Range Test for each experiment.

Figure 3. Chlorotic appearance of perennial ryegrass plants growing in solution levels of 200 and 300 ppm K (treatments c and d, respectively).

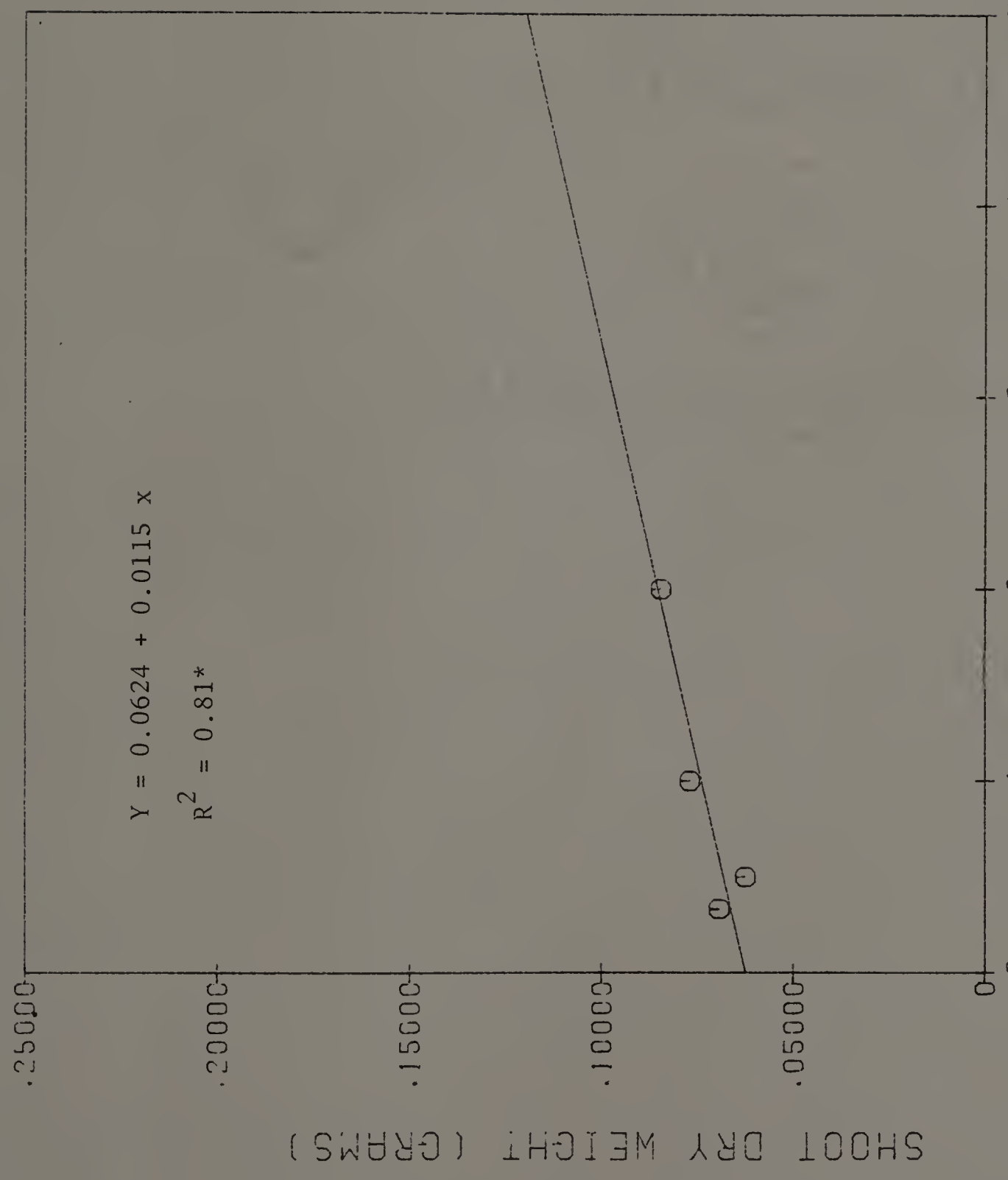

Figure 4. Effect of N:K solution ratios on shoot dry weight (Experiment 1a)

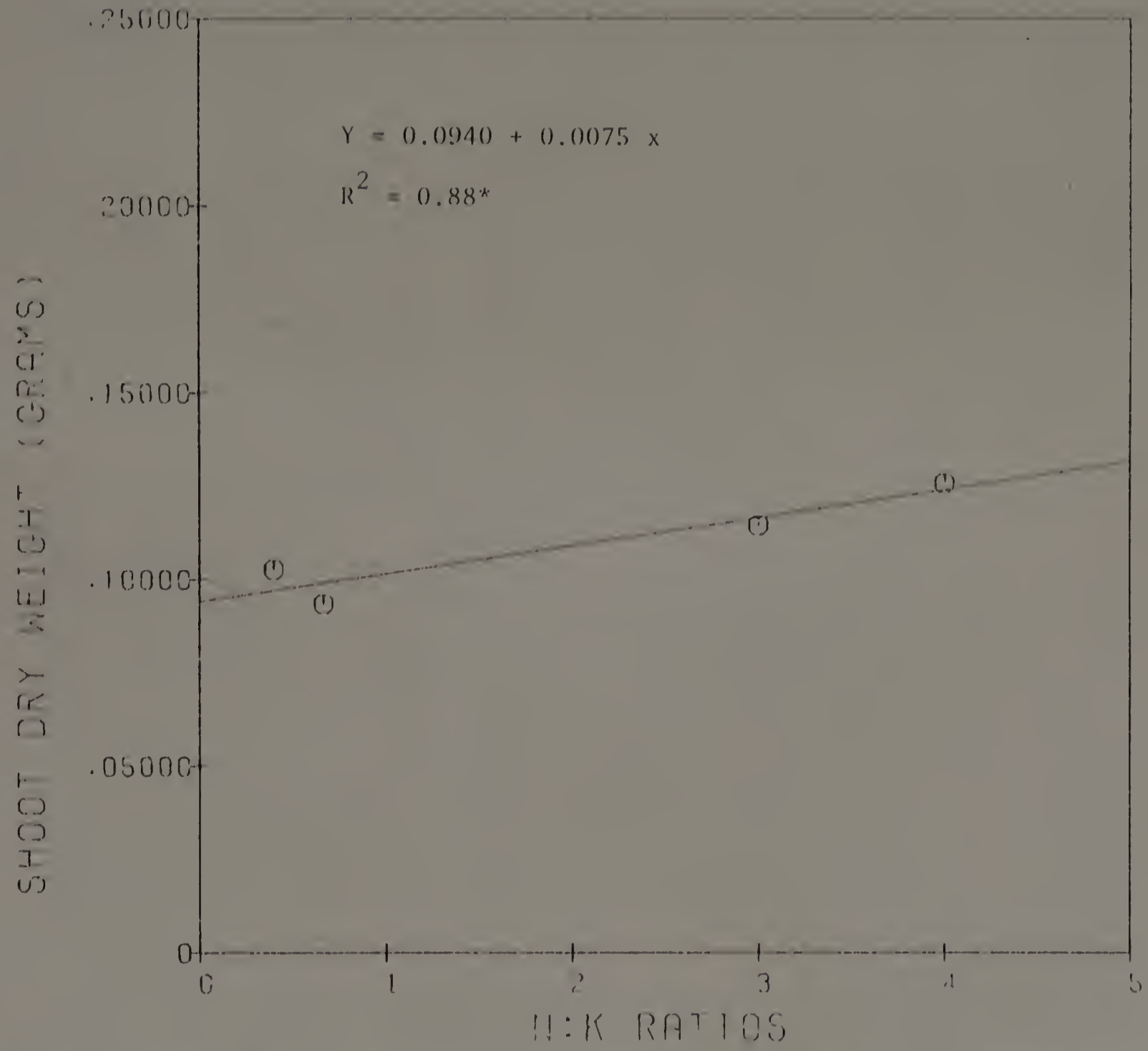

Figure 5. Effect of N:K solution ratios on shoot dry weight (Experiment 1b).

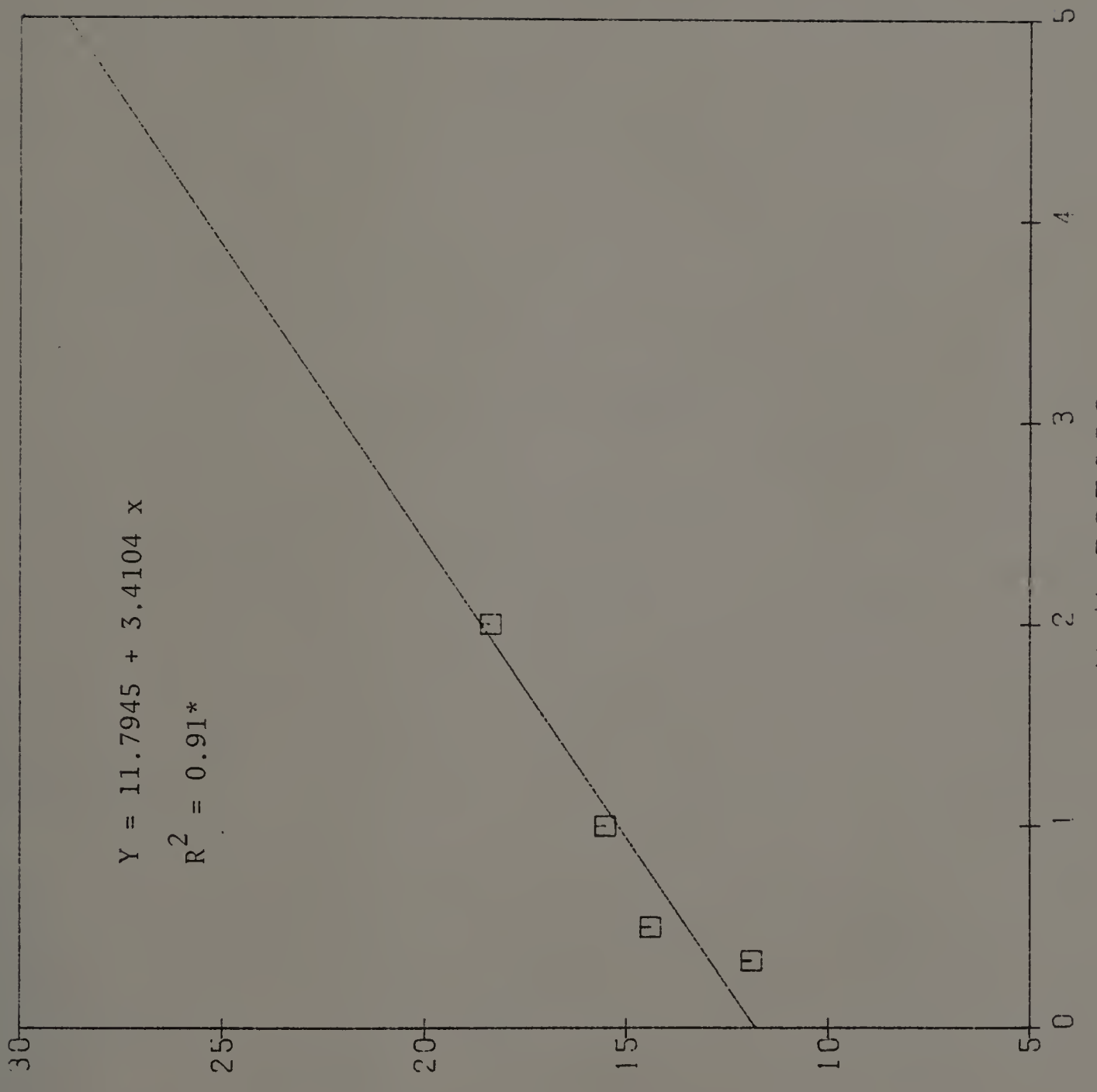

Figure 6. Effect of N:K solution ratios on water soluble carbohydrate levels in crown tissue (Experiment 1a).

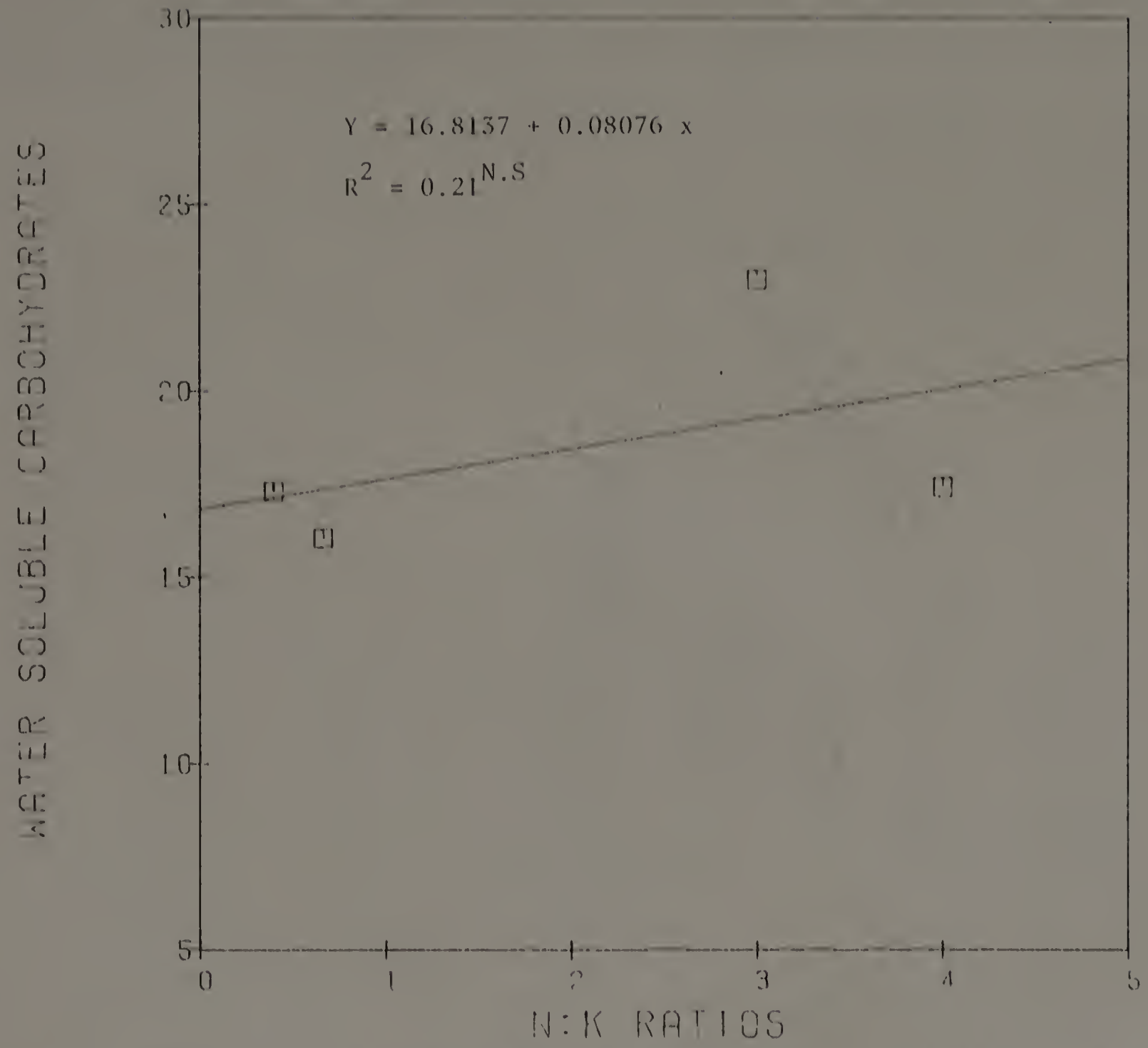

Figure 7. Effect of N:K solution ratios on water soluble carbohydrate levels in crown tissue (Experiment 1b).

Table 4. Nutrient content of 'Manhattan' perennial ryegrass crown tissue.

Fertilization Treatments N:K	N	P	K	Ca	Mg	Ca:Mg
(ppm)	(%)	(%)	(%)	(%)	(%)	(%)
Experiment 1a						
100:300	3.40b	0.35b	2.38a	0.23c	0.43a	0.54b
100:200	3.50ab	0.36b	1.81b	0.25c	0.42a	0.60b
100:100	3.77a	0.45a	1.73b	0.44b	0.59a	0.75b
100:50	3.29b	0.34b	1.03c	0.57a	0.51a	1.12a
Experiment 1b						
100:150	3.46c	0.29b	1.69a	0.28b	0.29b	0.97c
100:250	3.75b	0.29b	1.71a	0.19b	0.24b	0.79c
150:50	3.49c	0.30b	0.89b	0.67a	0.38a	1.76b
200:50	4.03a	0.37a	0.77b	0.73a	0.37a	2.00a

Means within columns followed by unlike letters are significantly different at the 5% level by Duncan's Multiple Range Test for each experiment.

Table 5. Correlation coefficients for Experiment 1a.

	Kill	N	K	CH_2O	P	Ca	Mg	Ca:Mg	TFW	SDW
N	.293 P=.052									
K	.300* P=.048	-.207 P=.127								
CH_2O	-.200 P=.135	-.245 P=.088	-.424* P=.008							
P	.285 P=.057	.595* P=.001	-.148 P=.208	-.063 P=.366						
Ca	-.239 P=.093	.272 P=.066	-.279* P=.001	.435 P=.006	.363* P=.021					
Mg	-.094 P=.304	.170 P=.176	-.398* P=.012	.197 P=.139	.313* P=.040	.692* P=.001				
Ca:Mg	-.308* P=.043	.225 P=.107	-.757* P=.001	.408* P=.010	.263 P=.073	.793* P=.001	.137 P=.226			
TFW	.283 P=.058	.111 P=.272	.228 P=.105	.308* P=.043	.173 P=.171	-.088 P=.315	.053 P=.386	-.140 P=.222		
SDW	-.286 P=.056	-.320 P=.431	-.572* P=.001	.125 P=.247	.041 P=.410	.469* P=.003	.334* P=.031	.359 P=.022	-.258 P=.077	
RDW	.078 P=.335	-.136 P=.229	.305* P=.044	.440* P=.006	.076 P=.339	-.169 P=.177	.121 P=.254	-.315* P=.039	.634* P=.001	-.248 P=.086

*Correlation coefficients are significant at the 5% level.

Table 6. Correlation coefficients for Experiment 1b.

	Kill	N	K	CH_2O	P	Ca	Mg	Ca:Mg	TFW	SDW
N	.173 P=.171									
K	.671* P=.001	.084 P=.324								
CH_2O	-.435* P=.006	-.623* P=.001	-.428* P=.007							
P	.172 P=.172	.450* P=.005	.039 P=.414	-.125 P=.248						
Ca	-.460* P=.004	-.047 P=.398	-.485* P=.002	.153 P=.201	.224 P=.109					
Mg	-.023 P=.449	.115 P=.264	-.026 P=.443	-.068 P=.358	.441* P=.006	.710* P=.001				
Ca:Mg	-.670* P=.001	-.166 P=.181	-.695* P=.001	.295 P=.050	-.213 P=.121	.599* P=.001	-.083 P=.317			
TFW	-.529* P=.001	.082 P=.326	-.448* P=.005	.287 P=.055	-.189 P=.149	.020 P=.457	-.243 P=.089	.322* P=.036		
SDW	-.414* P=.009	.294 P=.051	-.320* P=.037	.193 P=.144	-.174 P=.170	-.073 P=.345	-.284 P=.057	.249 P=.084	.832* P=.001	
RDW	-.567* P=.001	-.044 P=.405	-.444* P=.005	.336* P=.030	-.315* P=.039	.045 P=.402	-.228 P=.104	.314* P=.040	.857* P=.001	.674* P=.001

*Correlation coefficients are significant at the 5% level.

A highly significant linear relationship existed between solution levels of K and tissue K content for both experiments (Figures 8 & 9). Tissue K content was negatively correlated with shoot dry weight, Ca content, and Ca:Mg ratio (Tables 5 & 6) in experiment 1.

Calcium content in the plant tissue was significantly surpressed at K solution levels greater than 150 ppm. A significant linear relationship existed between solution N:K ratios and tissue Ca levels for both experiments (Figures 10 & 11). Tissue Ca content was positively correlated in both experiments with Mg tissue content (Tables 5 & 6).

Tissue Mg levels increased with decreasing solution K levels. No linear relationship existed between nutrient solution levels and Mg tissue content for experiment 1a (Figure 12). However, in experiment 1b a significant linear relationship existed between ratios of N:K in solution and the tissue level of Mg (Figure 13).

Tissue Ca:Mg ratio increased as solution K levels decreased. The highest Ca:Mg ratio was observed at the highest level of supplied N (200 ppm). Regression analysis revealed a significant positive linear relationship between Ca:Mg ratio and N:K ratio (Figures 14 & 15).

Fertilization ratios of N:K had a significant effect on percent tiller kill (Table 7). High solution levels of N or K increased the percentage of tillers killed. Percent kill was positively correlated with K tissue levels, and negatively correlated with water soluble carbohydrates (Tables 5 & 6). Percent electrolyte leakage of stressed crown tissue did not differ significantly among N:K treatments (Table 8).

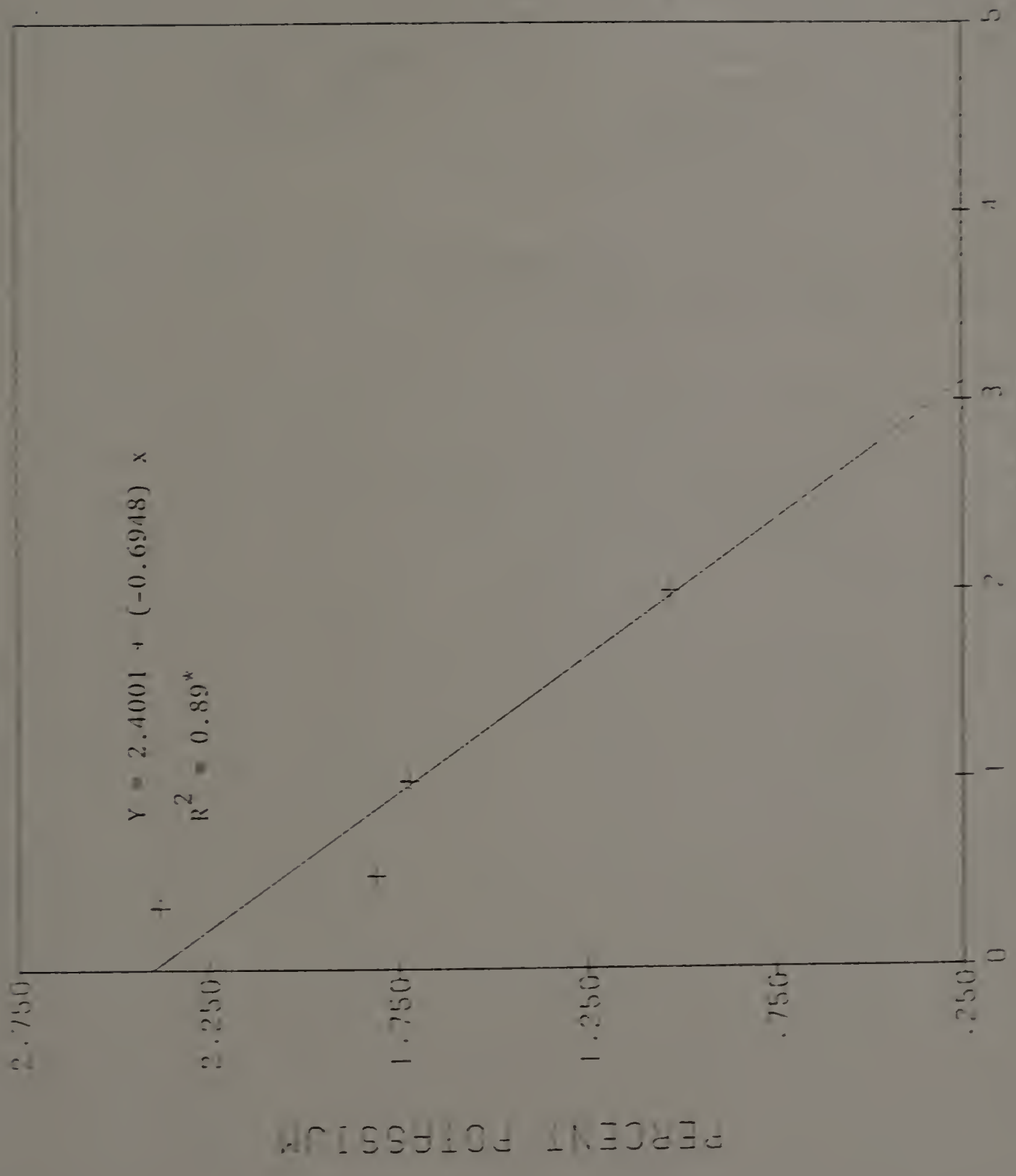

Figure 8. Effect of N:K solution ratios on percent potassium in crown tissue (Experiment 1a).

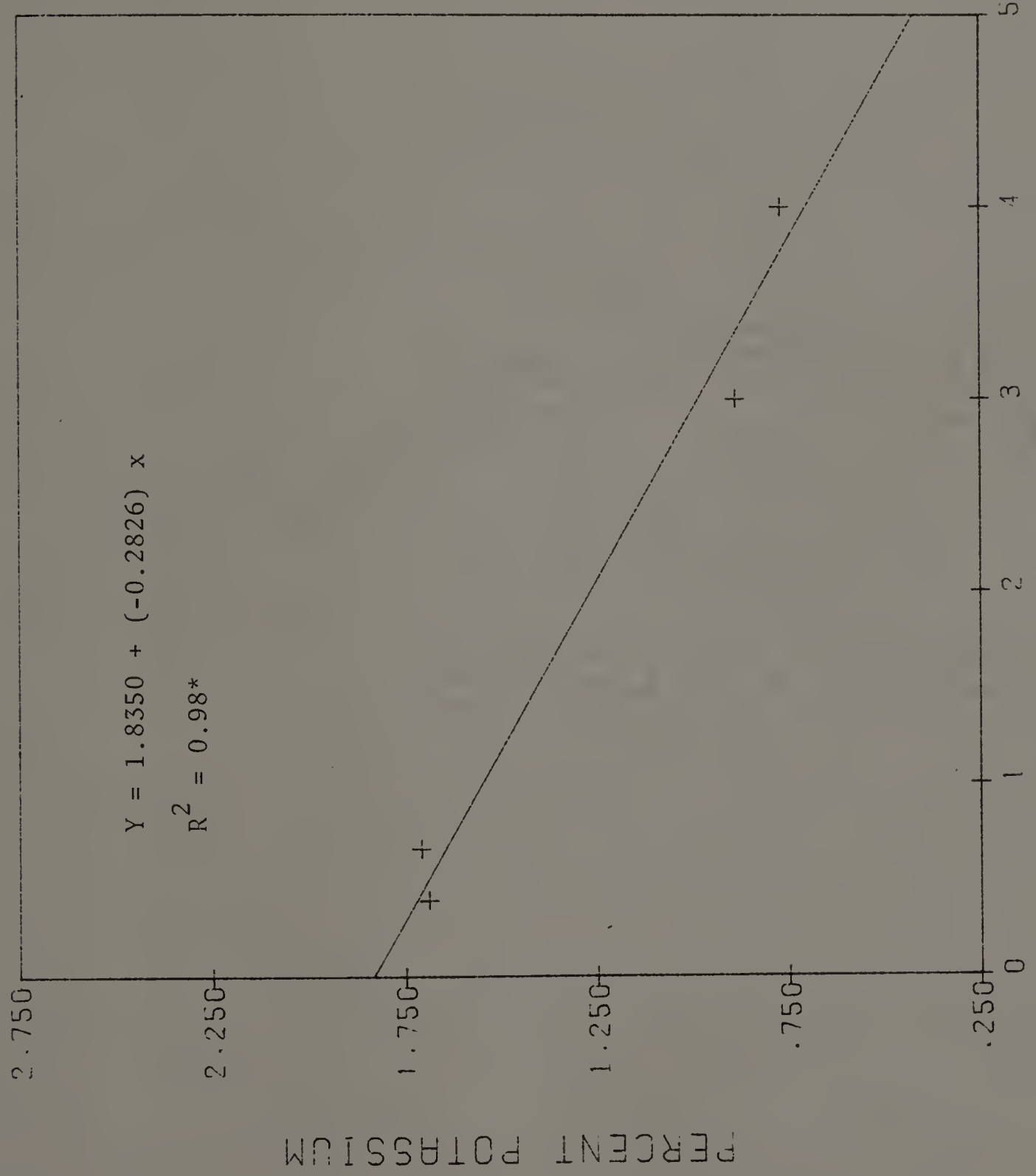

Figure 9. Effect of N:K solution ratios on percent potassium in crown tissue (Experiment 1b).

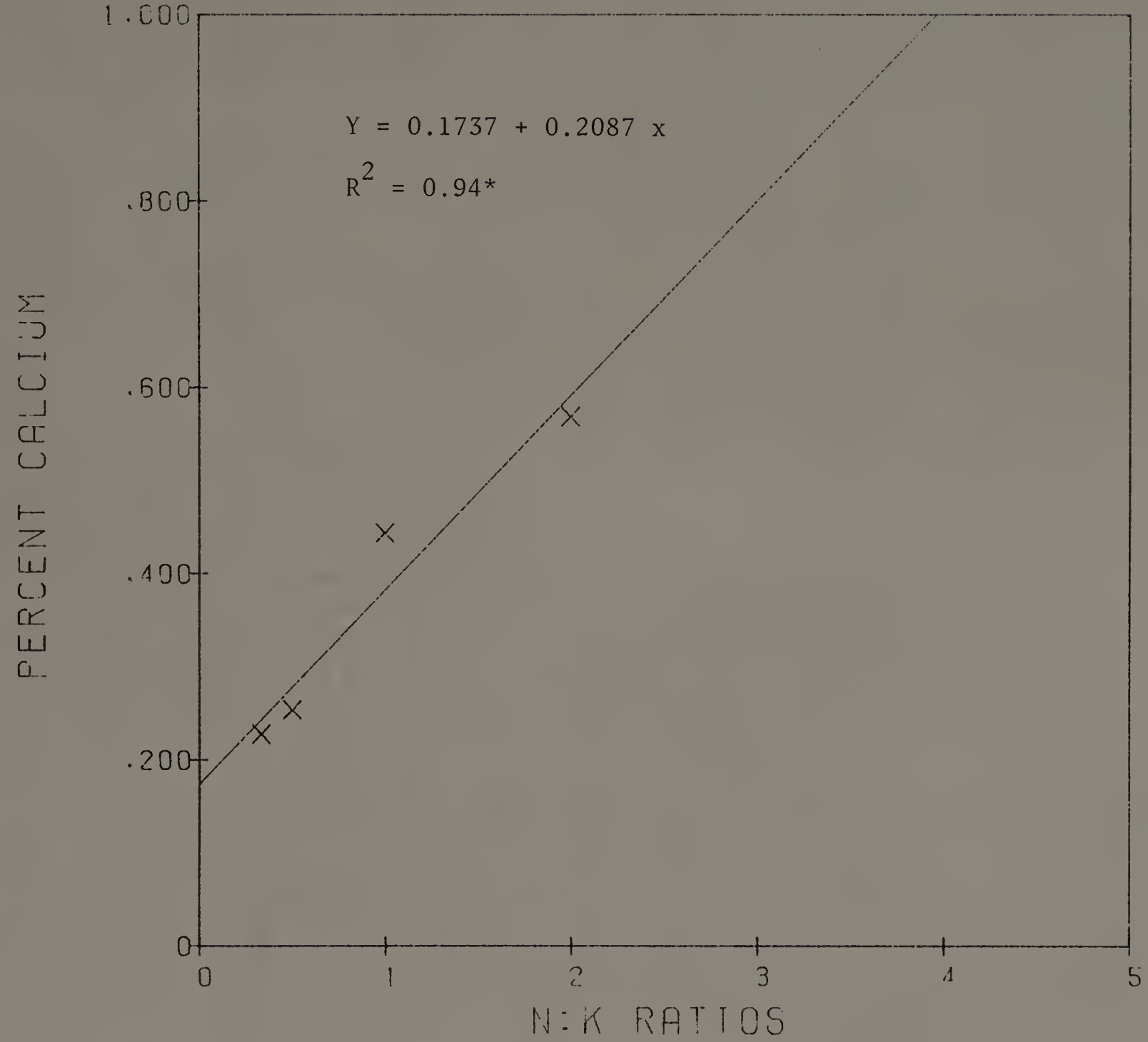

Figure 10. Effect of N:K solution ratios on percent calcium in crown tissue (Experiment 1a).

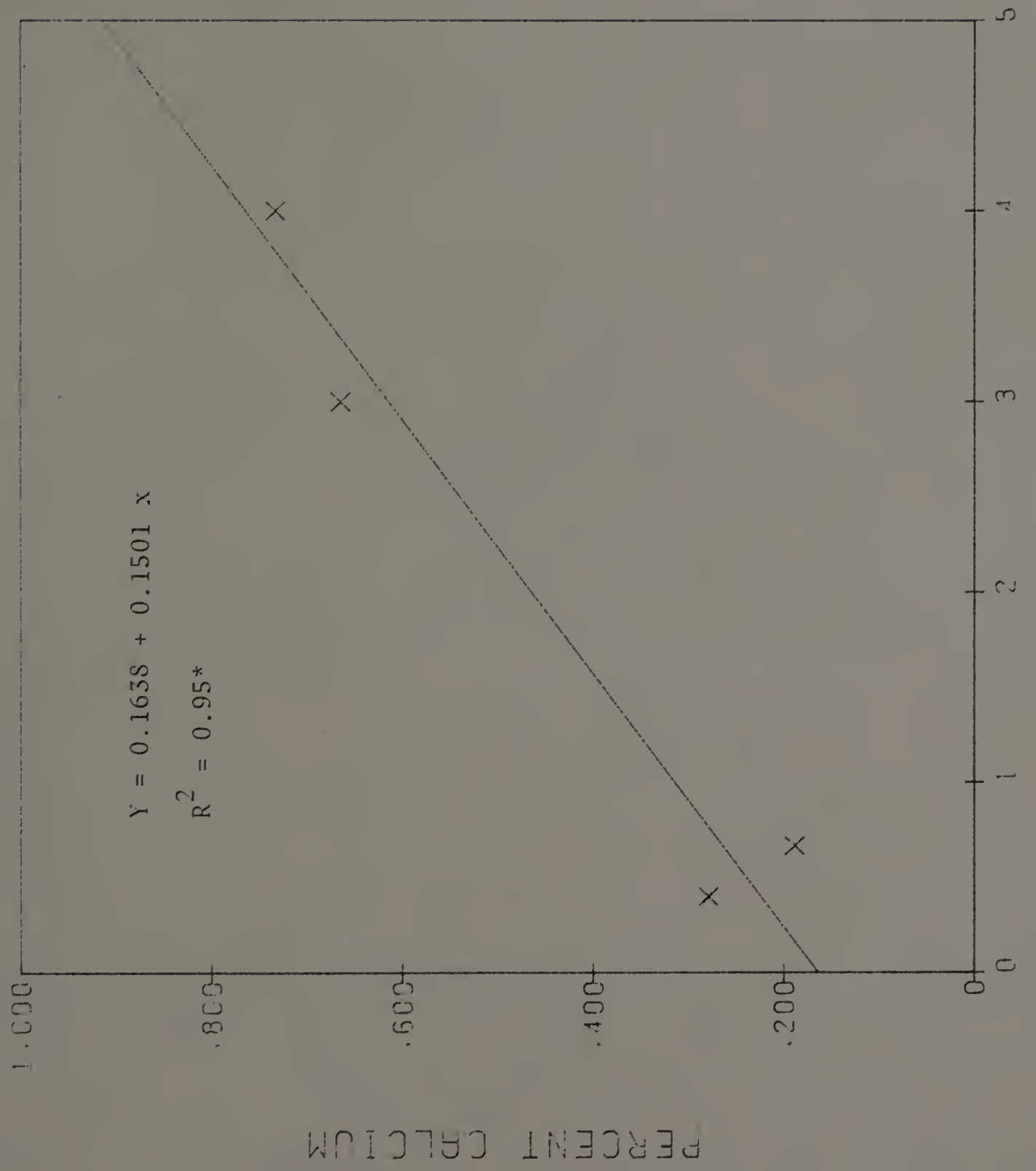

Figure 11. Effect of N:K solution ratios on percent calcium in crown tissue (Experiment 1b).

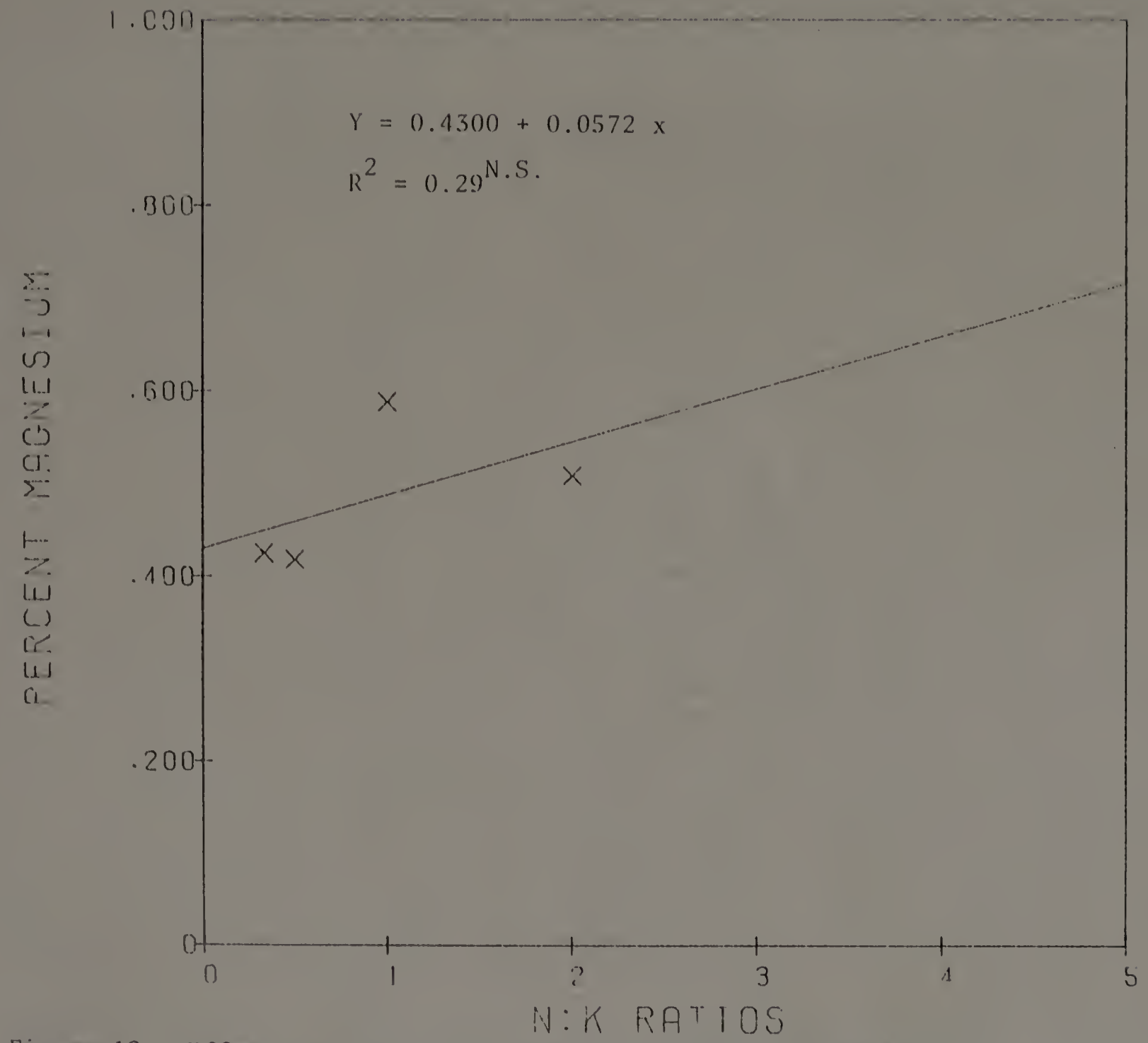

Figure 12. Effect of N:K solution ratios on percent magnesium in crown tissue (Experiment 1a).

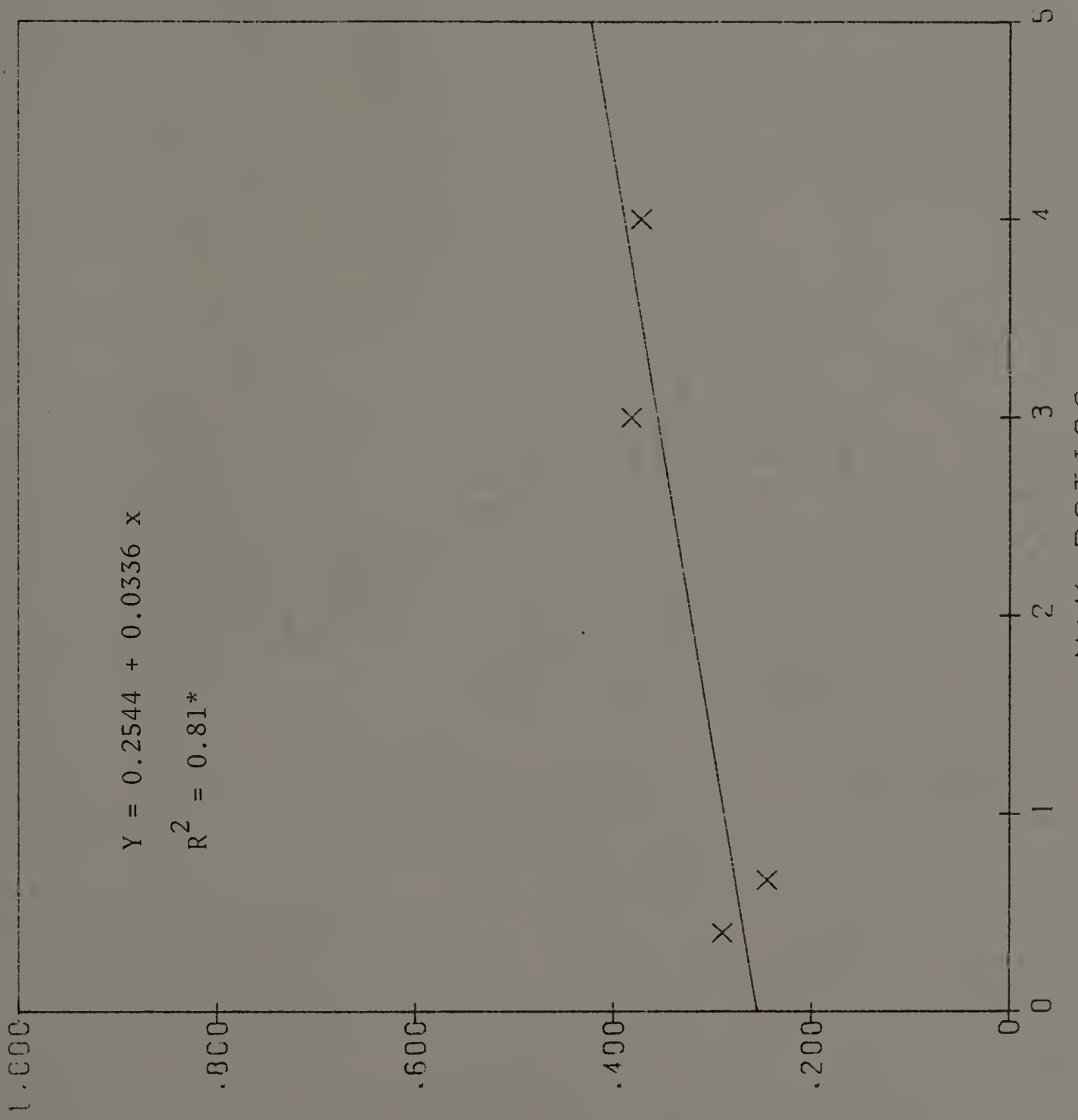

Figure 13. Effect of N:K solution ratios on percent magnesium in crown tissue (Experiment 1b).

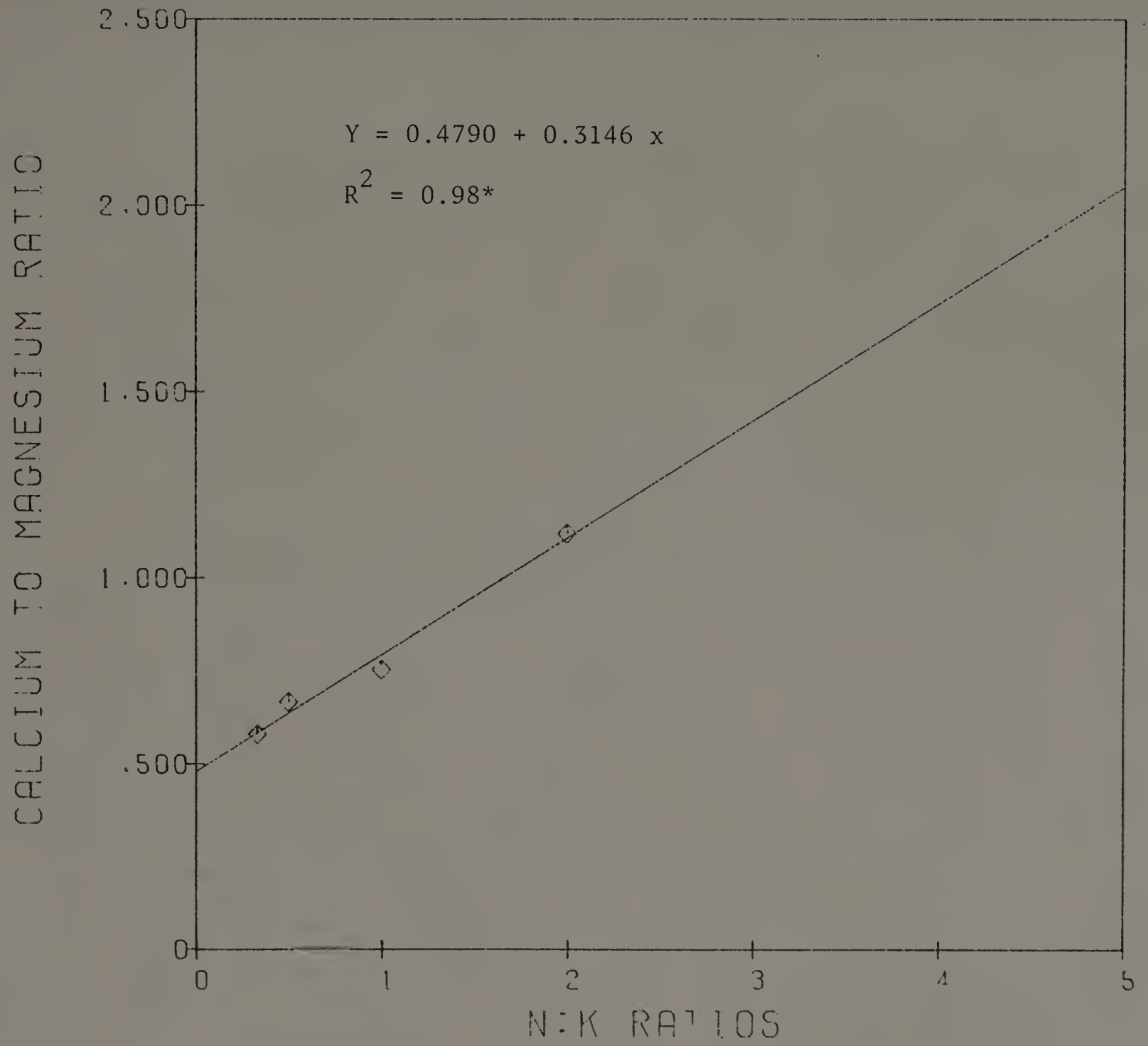

Figure 14. Effect of N:K solution ratios on calcium to magnesium ratio in crown tissue (Experiment 1a).

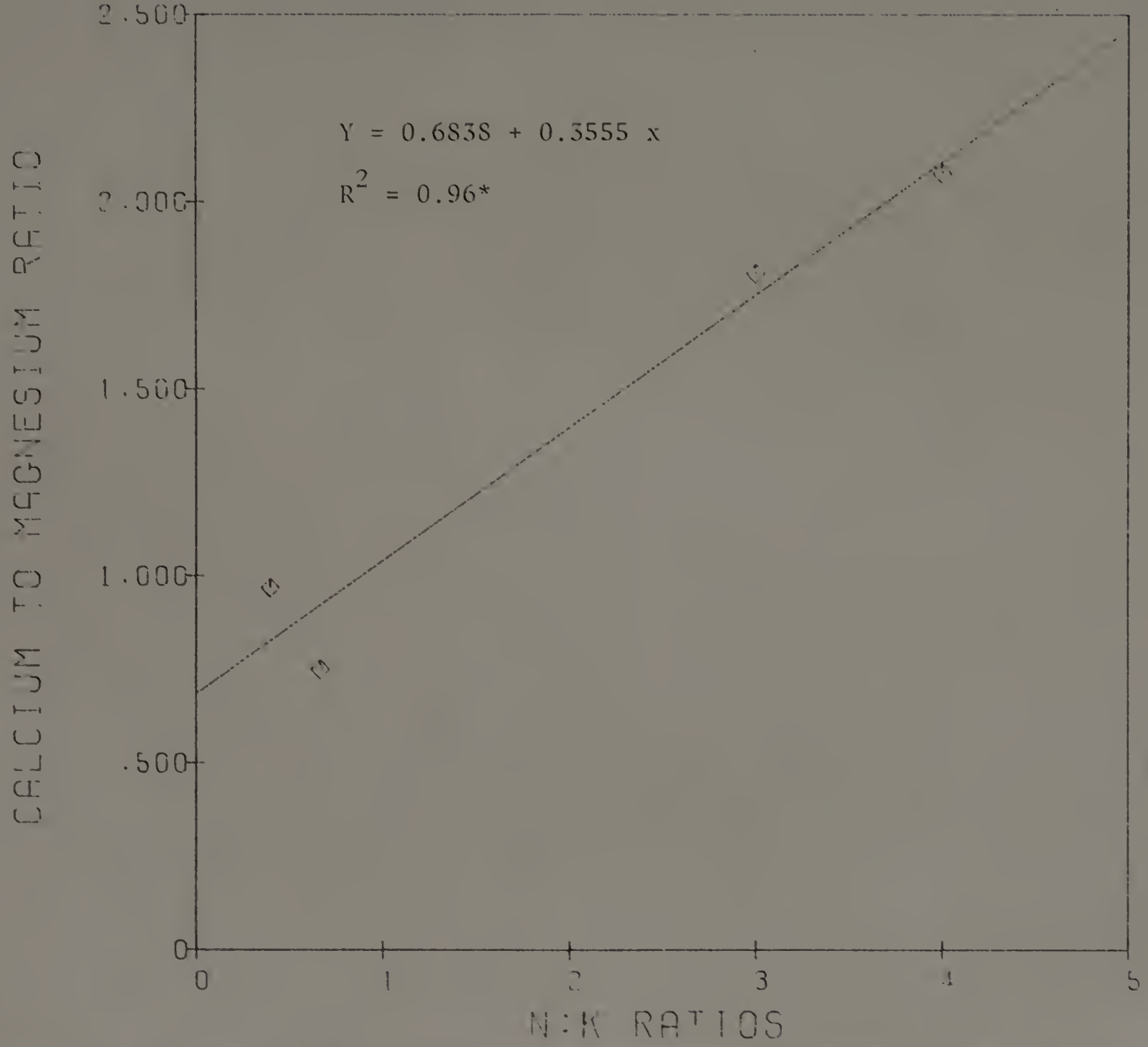

Figure 15. Effect of N:K solution ratios on calcium to magnesium ratio in crown tissue (Experiment 1b).

Table 7. Tiller mortality of 'Manhattan' perennial ryegrass 4 weeks after low temperature stress.

Fertilization Treatments N:K	Tiller Mortality
(ppm)	(%)
Experiment 1a	
100:300	99.7a
100:200	90.0a
100:100	64.8b
100:50	44.7b
Experiment 1b	
100:150	72.7b
100:250	96.8a
150:50	64.9c
200:50	89.3ab

Means within columns followed by unlike letters are significantly different at the 5% level by Duncan's Multiple Range Test for each experiment.

Table 8. Percent electrolyte leakage of stressed crown tissue

Fertilization Treatment N:K	Leakage
(ppm)	(%)
Experiment 1a	
100:300	53.9a
100:200	49.1a
100:100	48.5a
100:50	46.6a
Experiment 1b	
100:150	36.0a
100:250	38.6a
150:50	41.1a
200:50	45.9a

Means within columns followed by unlike letters are significantly different at the 5% level by Duncan's Multiple Range Test for each experiment.

Experiment 2

The effects of N:K ratios on low temperature survival and recuperative vigor of field-hardened perennial ryegrass are summarized in Table 9. Percent kill of plants exposed to low temperature stress was significantly greater in 100:100 and 100:50 ppm N:K solution ratios. Increasing levels of N or K increased the percentage of tillers killed. Leaf elongation of stressed plants increased with increasing N:K ratios (Figure 16).

Table 9. The effects of N:K solution levels on % kill and blade length of the first fully expanded blade after clipping.

Fertilization Treatments N:K	Kill (% of control)	Stressed Plants Elongation
(ppm)	(%)	(cm)
100:50	40bc	5.3ab
100:100	29c	4.2b
100:150	52a	4.1b
150:50	46ab	6.5a

Means within columns followed by unlike letters are significantly different at the 5% level by Duncan's Multiple Range Test for each experiment.

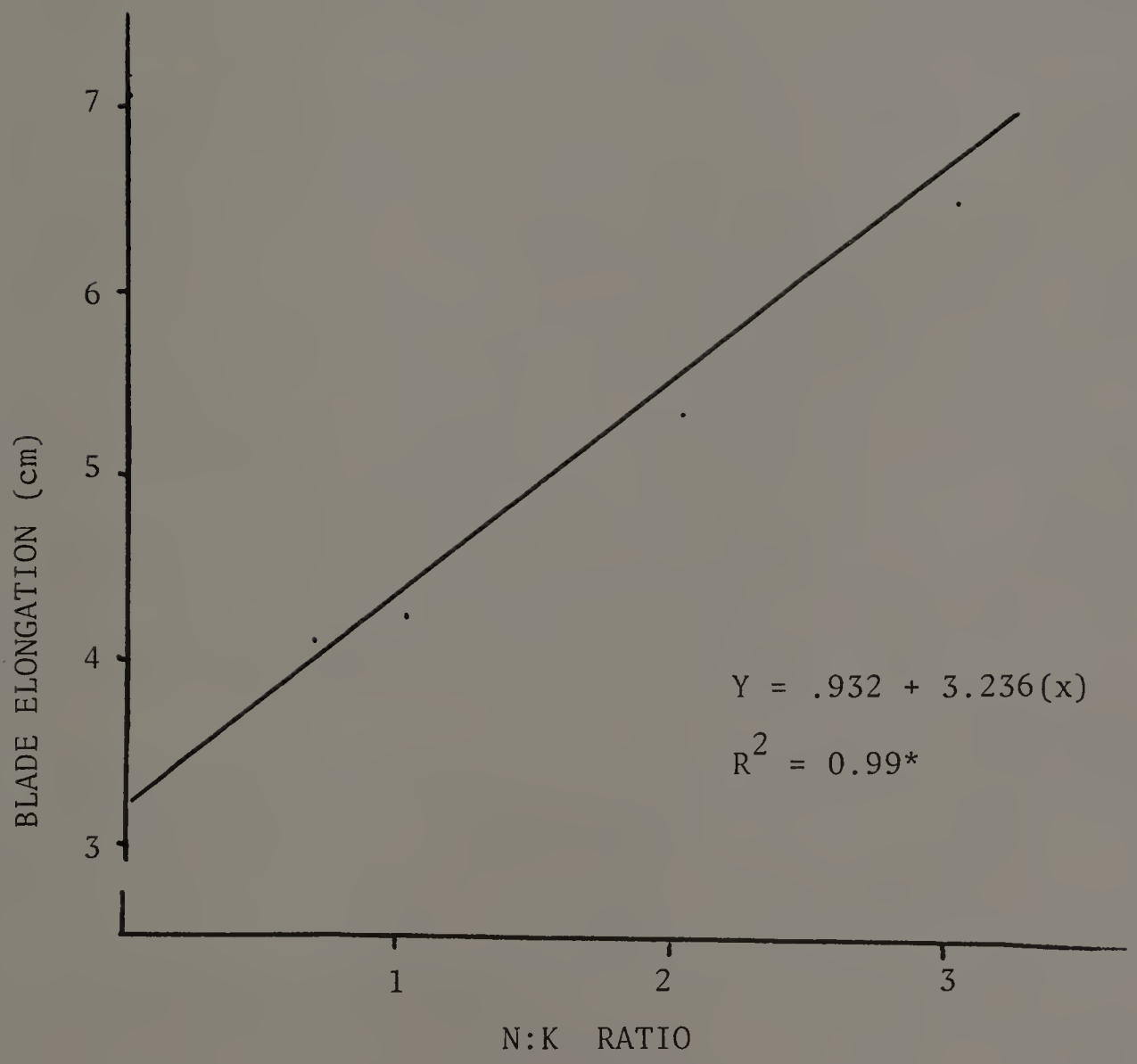

Figure 16. Effect of N:K fertilization ratio on blade elongation of low temperature stressed 'Manhattan' perennial ryegrass.

CHAPTER FIVE

DISCUSSION

Maximum low temperature hardiness of 'Manhattan' perennial ryegrass occurred when solution levels of N and K were in a ratio of 2:1 for experiment 1. Tiller mortality increased as the N:K ratio increased or decreased from 2:1 (Figure 17). High N fertilization rates have been reported to decrease the low temperature hardiness of grass species (Carroll and Welton, 1938). However, this experiment revealed that high rates of K can have the same effect. In a combined analysis of data from nutrient treatments in experiment 1 containing 100 ppm N, increasing levels of K in solution significantly increased tiller mortality (Figure 18).

These findings agree with earlier low temperature stress studies, using different grass species. Beard and Rieke (1966) reported that common Kentucky bluegrass and 'Toronto' creeping bentgrass fertilized with a N:K ratio of 2:1 were more tolerant to low temperature stress. Increasing the ratio of N:K increased low temperature injury. They concluded that, a balanced nutrient relationship rather than a high potassium or low nitrogen level, is essential for maximum low temperature survival. Gilbert and Davis (1971) reported that ratios in which the levels of N and K were approximately equal and 4 to 5 times greater than P, maximized plant regrowth after low temperature stress. In an earlier study, Adams and Twersky (1960) examined the effect of soil fertility on the winter kill of coastal bermudagrass and summarized that a K fertility program should be considered in regions where winter kill may

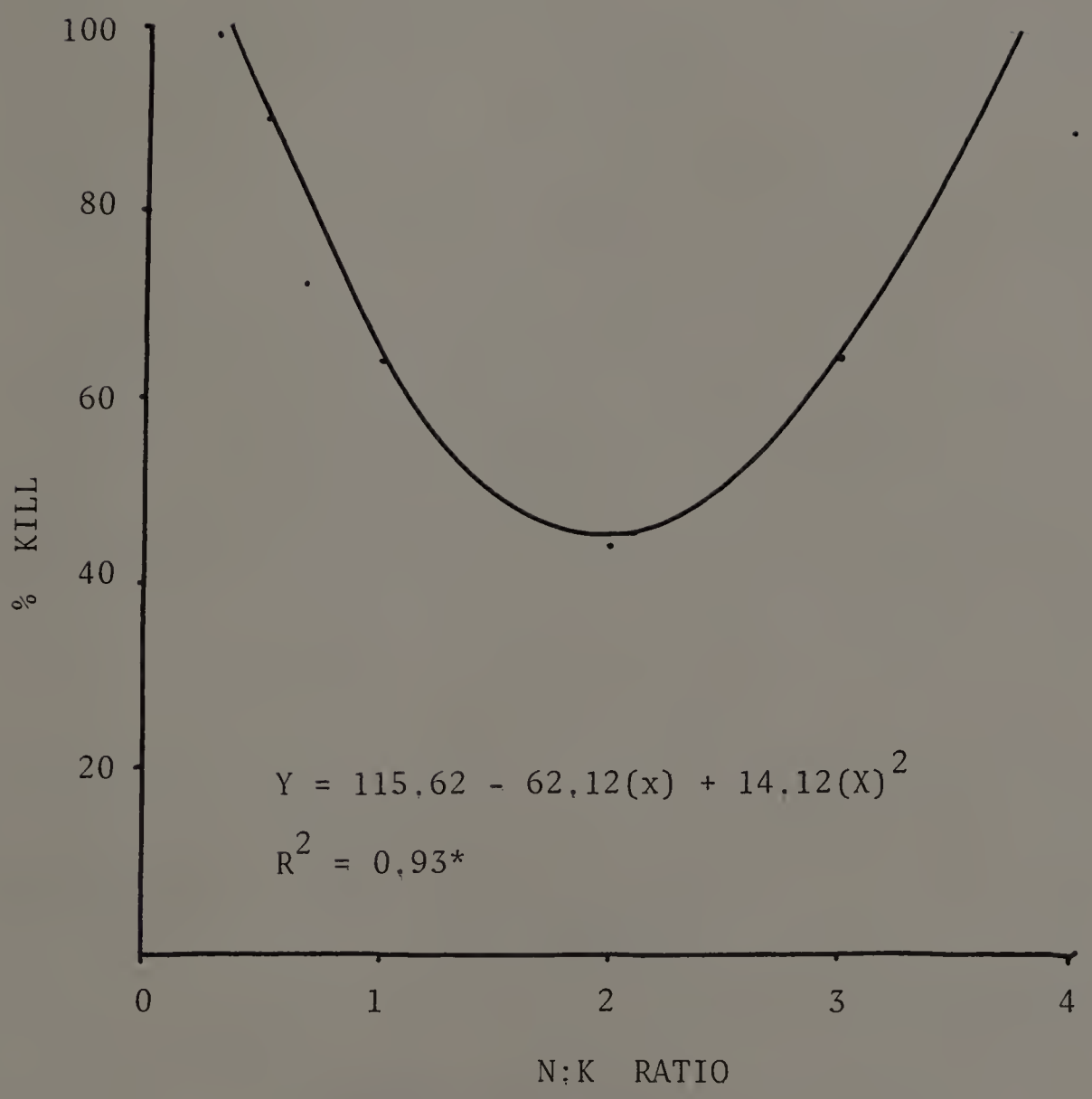

Figure 17. Effect of N:K solution ratios on tiller mortality of 'Manhattan' perennial ryegrass (Experiment 1a and 1b).

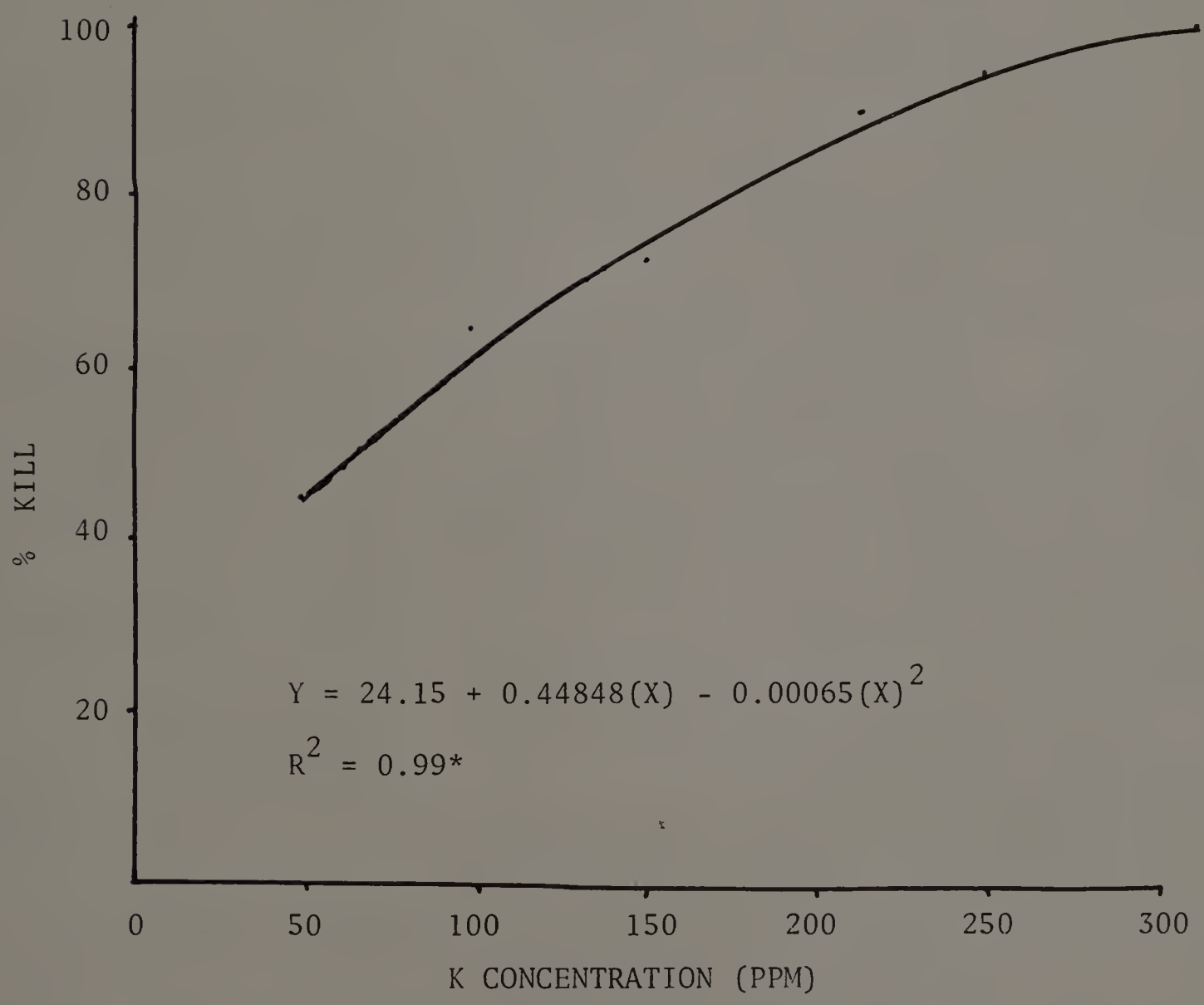

Figure 18. Effect of K solution levels, at content N (100 ppm), on tiller mortality of 'Manhattan' perennial ryegrass (Experiment 1a and 1b).

occur.

Although excessive N fertilization has been reported to cause an increase in low temperature kill, no research can be found on the effects of excess K on low temperature hardiness. From this and other studies it can be concluded that excessive levels of either N or K are detrimental to low temperature survival of cool season turfgrasses. Potassium should be approximately equal to N for optimal low temperature survival of cool season turfgrasses. When tissue K levels are too low or too high, low temperature hardiness may be affected.

The relationship between carbohydrate content and low temperature tolerance has been reported (Dexter, 1956; Levitt, 1956). Olien (1967) stated that carbohydrates prevent ice crystal formation in the cell at low temperatures. The degree of freeze resistance depends on the type and quality of carbohydrates present. Water soluble carbohydrates are in an aqueous solution within the cell thereby increasing the osmotic pressure and depressing the freezing point of the cell. To a certain extent, the greater the amount of water soluble carbohydrates in the cell the greater the freezing point is depressed. This led Trunova (1965) to postulate that carbohydrates may act as a cryoprotectant against ice crystal formation in the plant. Potassium nutrition has been observed by several researchers (Loustalot et al, 1950; Eaton, 1952; and Wall, 1939) to be important to carbohydrate metabolism. Holt and Hilst (1969) and Cook and Duff (1976) found that increasing K levels during the hardening phase decreased water soluble carbohydrates in Kentucky bluegrass and 'Kentucky 31' tall fescue, respectively.

Under the conditions of this experiment, water soluble carbohydrates decreased with increasing solution levels of K in experiment 1a. However, there was no significant linear relationship between water soluble carbohydrates and solution K levels in experiment 1b. This difference in results may be due to the high levels of N used in experiment 1b. Colby et al (1965) reported that high levels of N fertilization caused consistently lower levels of fructosans in orchardgrass. In experiment 1 tiller mortality was negatively correlated with water soluble carbohydrates such that as water soluble carbohydrates increase, the percent tiller mortality decreases. These results suggest that K is influencing tiller mortality by decreasing water soluble carbohydrate levels with higher K levels.

Levels of K lower than N produced plants with greater shoot dry weight in 'Manhattan' perennial ryegrass. A significant reduction in shoot dry weight was observed at 100 ppm N by increasing K up to 300 ppm (Figure 4). Christians et al (1979) observed that maximum tissue production occurred when K levels exceeded that of N for both Kentucky bluegrass and creeping bentgrass. At 96 ppm N tissue dry weight of both turfgrass species increased linearly with added K up to 96 ppm.

MacLeod and Carson (1966) found that high solution levels of N (250 ppm) and low K (50 ppm) decreased yield, increased mortality and significantly altered tissue content of P, K, Ca, and Mg in several grasses. Results from this experiment show that high levels of K also decrease yield, increase tiller mortality and significantly affected tissue content of P, K, Ca, and Mg in 'Manhattan' perennial ryegrass.

Calcium and Mg tissue content decreased with increasing solution levels of K due to cation competition for root absorption sites. Rathman et al (1960) also found that increasing levels of applied K resulted in decreasing levels of Ca in ryegrass. Loue (1963) attributed decreasing levels of Ca and Mg in corn leaf tissue to cation competition as levels of applied K increased. Broyer (1959) had observed similar results in other crops.

Total N in the tissue did not correspond with applied levels of N. However, assimilation of N was positively correlated with P uptake. Nitrogen and P were negatively correlated in both experiments with water soluble carbohydrate levels. Koontz and Vose (1960) postulated that N stimulates P uptake, which increases carbohydrate utilization through oxidative phosphorylation. Potassium tissue levels followed similar trends to those of Robinson, Rhykerd, and Gross (1962) working with orchardgrass where it was noted that increasing rates of applied K result in increasing levels of K in the plant tissue. This is often referred to as luxury consumption of K.

A direct and indirect method of estimating freezing tolerance was used in experiment 1. In the direct method (percent of live versus dead tillers after 4 weeks of recovery) there was significant differences in the percent kill under different N:K solution levels. In the indirect method (measuring the increase in electrolyte content after freezing) solution levels of N and K had no significant effect on percent electrolyte leakage. Cook and Duff (1976) using an indirect method on 'Kentucky 31' tall fescue found similar results.

CHAPTER SIX

SUMMARY AND CONCLUSIONS

Low temperature hardiness of 'Manhattan' perennial ryegrass was affected by solution levels of N and K. Narrow ratios of N:K, where N is equal to or slightly greater than K, produced the least low temperature kill. Whereas, high levels of N and K at low levels of K and N, respectively, increased low temperature kill. Water soluble carbohydrates were inversely related to low temperature kill and K suggesting that water soluble carbohydrates act as a cryoprotectant and enhance low temperature hardiness, while excessive levels of K are detrimental to accumulation of water soluble carbohydrates.

Turfgrass plants must be winter hardy not just low temperature hardy to survive in the field. Winter hardiness of turfgrass is dependent on several stress factors including; heaving, smothering, desiccation, diseases, as well as low temperature stress. Climatic factors, soil conditions, and cultural practices will influence the degree of winter hardiness also. Thus, improving turfgrass winter hardiness requires a consideration of the above factors. This became evident in field studies initiated in 1978. Two cultivars of perennial ryegrass were grown in the field to determine the effects of rates and ratios of N to K at two cutting heights. Complete winter kill of both cultivars were observed in these plots. Ice cover on these plots was extensive during late winter whereas perennial ryegrass plots several meters away that were not covered by ice had less kill. These observations indicate that N:K ratio may not always improve winter survival of perennial ryegrass. More extensive

work needs to be conducted on other factors affecting winter injury of perennial ryegrass to improve its adaptation and use in New England.

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APPENDIX

Preliminary Experiment

Determination of temperature and duration to produce a lethal temperature where 50% of the field-hardened perennial ryegrass (Lolium perenne L.) tillers were killed (LT_{50}) was made. Field-hardened 'Manhattan' perennial ryegrass plants were collected in early December from the South Deerfield Turfgrass Research Plots. Plants were washed free of soil before running experiments and twenty plants were placed in water-tight polyethylene bags and submerged in a circulating freezing bath containing a 50:50 (v/v) mixture of ethylene-glycol and water. Twenty-two polyethylene bags were used in a temperature-course study with a freezing rate of -2 C/hour from 0 to -22 C. Two bags were withdrawn from the freezing bath every two hours and allowed to thaw at room temperature. Twenty plants contained in polyethylene bags at room temperature for the same duration served as controls. Plants were watered as needed to prevent wilt and fertilized weekly with half-strength Hoagland's (1950) solution. Regrowth was evaluated four weeks later as percent survival.

A second group of plants was used to determine the effects of duration of freezing on plant survival. Twenty-four polyethylene bags were immersed in the circulating temperature bath maintained at -10 C. Two bags were removed every hour and allowed to thaw at room temperature, transplanted into flats containing vermiculite, and allowed to regrow in the greenhouse as detailed previously.

Decreasing temperatures (Table 10) and duration (Table 11) resulted in greater tiller injury. Injury was observed at -4 C and was

Table 10. Temperature-course study.

Temperature (C)	% Kill
0	0f
-2	0f
-4	7ef
-6	20de
-8	38cd
-10	52c
-12	71b
-14	100a
-16	100a
-18	100a
-20	100a
-22	100a

Table 11. Time-course study.

Duration at -10 C	% Kill
4	29c
5	31c
6	48b
7	95a

Means within columns followed by unlike letters are significantly different at the 5% level by Duncan's Multiple Range Test for each experiment.

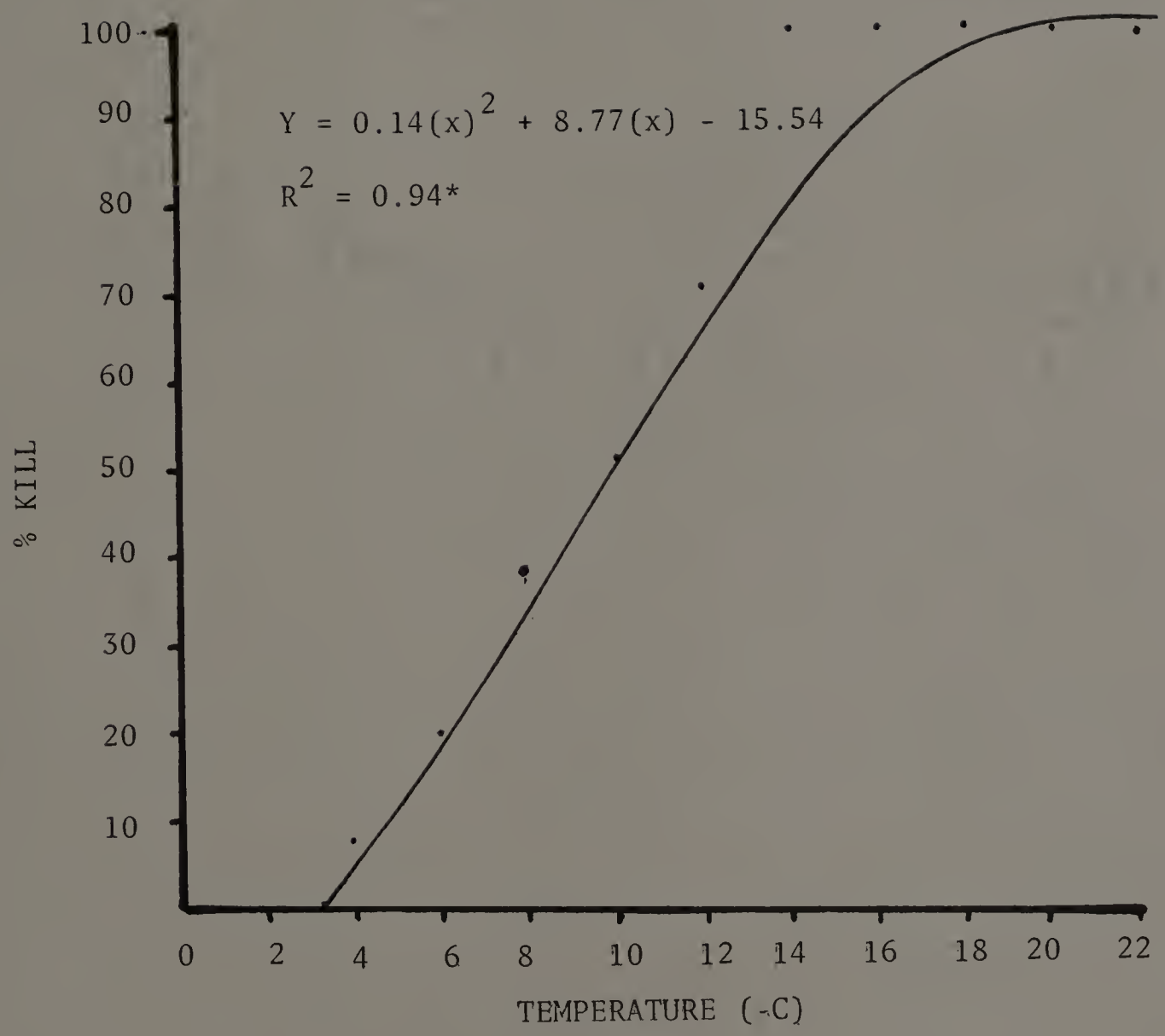

Figure 19. Temperature response curve of 'Manhattan' perennial ryegrass survival.

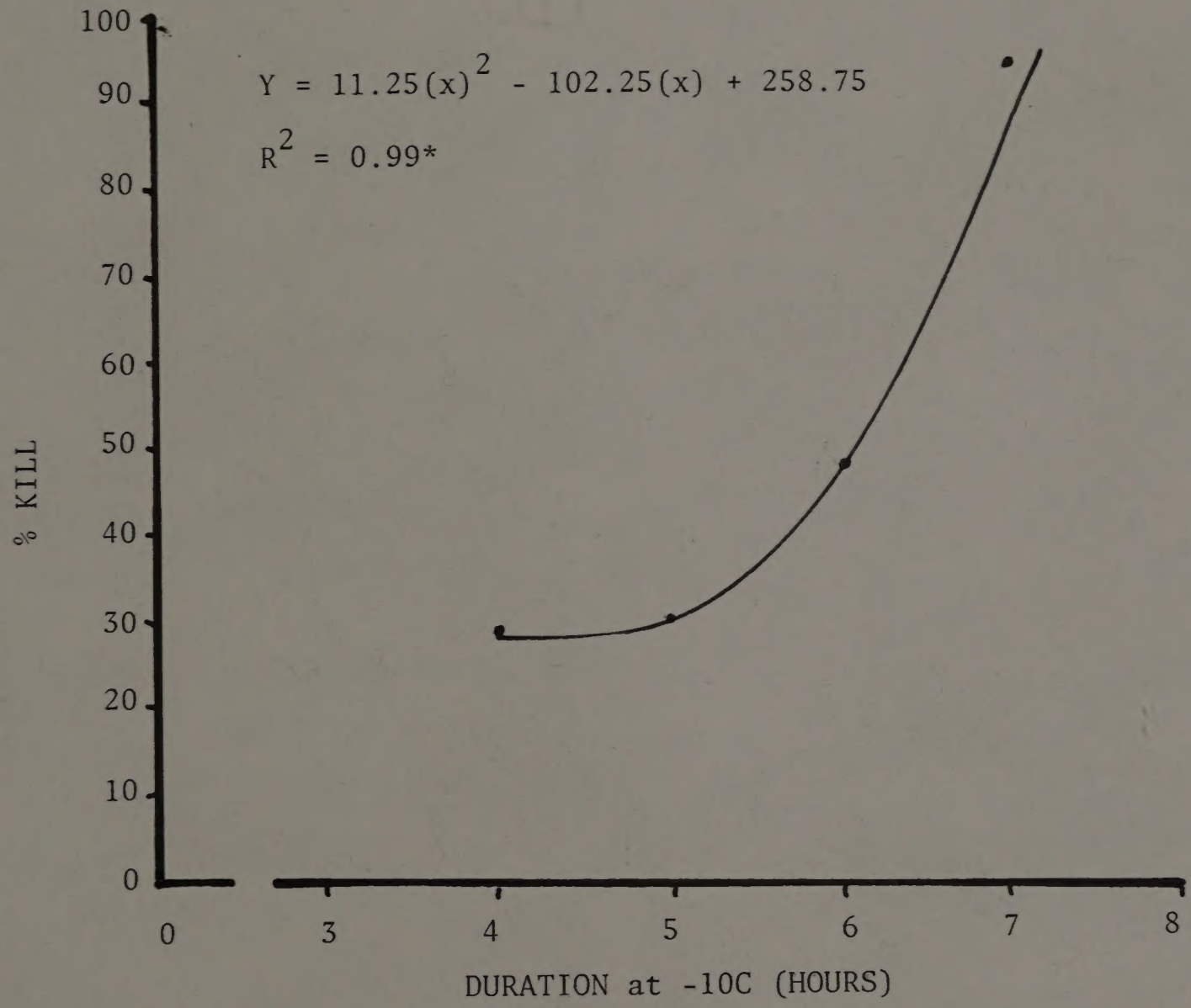

Figure 20. The effect of duration of exposure of 'Manhattan' perennial ryegrass to -10 C on plant survival.

complete at -14 C. Regression analysis studies indicate that the lethal temperature (LT_{50}) resulting in 50% tiller mortality of 'Manhattan' perennial ryegrass was -10 C for 6 hours in a circulating freezing bath (Figure 19 & 20). These results are in agreement with Fuller and Eagles (1978) where they observed a LT_{50} ranging from -5.7 to -12.2 C depending on the cultivar of perennial ryegrass.