EFFECTS OF OSMOTIC STRESS ON CERTAIN METABOLIC COMPONENTS OF WHEAT

By

Raymond E. Meyer

Bachelor of Science

Kansas State University

Manhattan, Kansas

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EFFECTS OF OSMOTIC STRESS ON CERTAIN METABOLIC

COMPONENTS OF WHEAT

Thesis Approved:

Joe R. Gingrich
Thesis Adviser

Charles E Denman

Eddie Basler

Donald C. Abbott

Dean of the Graduate School

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CHAPTER I

INTRODUCTION

Plant growth and development are related to the amount of water available to the plant but more specifically to the amount of water in the plant and the energy with which it is held.

Studies of the effect of moisture stress on plants are often complicated by insufficient definition of the term "growth." Common measures of plant growth, as elongation and changes in fresh and dry weight, are the result of combinations of many biochemical and physiological processes which are not affected to the same degree or at the same rate by moisture stress or changes in the internal water balance of the plant.

To obtain a better understanding of moisture effects on plant growth, a greater emphasis is needed on the effect of moisture stress on basic processes within the plant.

The objective of this study was to investigate the effect of osmotic stress on certain metabolic components of wheat seedlings. Responses studied were changes in respiration, nitrogen content, phosphorus content, amount of soluble carbohydrate, amount of soluble ninhydrin positive material, relative turgidity, and degree of hydration. Osmotic solutions were used to permit a better definition of the particular stress applied on the seedling and a split-root technique was used in an attempt to determine the effect of turgidity on these responses.

CHAPTER II

REVIEW OF LITERATURE

Most of the recent work on the effect of water deficits on physiological processes in plants has been reviewed by Vaadia et al. (1961) and Kramer (1963). Only studies of particular interest to this investigation will be discussed.

The results of many studies using osmotic solutions are at variance to those using soil. While part of this variance has been ascribed to transmission characteristics of the soil, there still seems to be some disagreement among studies reported in the literature as to whether nutrient uptake is affected by soil moisture tension per se or only through its indirect effects on transmission characteristics of the soil.

Danielson and Russell (1957) found that Rb^{86} uptake in corn seedlings was not influenced by osmotic pressure induced by mannitol but was decreased with increasing soil moisture tension. This would indicate that soil characteristics were involved. Dean and Gledhill (1956) ascribed a reduced phosphate absorption in dry soil to alterations in the physiology of the roots. Emmert and Ball (1933) indicated that there was no loss of the ability to absorb nitrate by the plant under stress. A reduced ability to absorb phosphorus resulted in an accumulation of nitrate in the tissue because of reduced tissue formation. Gates (1957) found that in young tomato plants phosphorus percentage was lower under moderate wilting than severe wilting. He ascribed this to a greater and earlier

effect of stress on phosphorus than on dry weight. Hydrolysis of phosphorus compounds might have been a direct effect of stress and probably occurred early in the drying cycle. He stated that in the development of stress, phosphorus metabolism is disturbed before nitrogen and recovers less rapidly upon watering.

Woolley (1963) used osmotic solutions of "Carbowax 4000" in a study on spring wheat. He found an increase in percentage and total nitrogen, a decrease in total phosphorus, but no significant change in dry weight of the shoots with increasing stress. He did not find any significant difference in percent nitrogen of the roots under stress but found a significant decrease in phosphorus percentage.

Sisakyan (1939) indicated that enzymatic hydrolysis was activated by stress but that the degree of water deficit at which hydrolysis is affected is different in drought resistant varieties. This points out a possibility for variation of results reported in the literature. Petrie and Wood (1938) suggested that protein increased with water content but it could not be told whether synthesis was decreased or hydrolysis was increased under a water deficit. Nezgovorova (1957) found that corn and oats under water stress increased in pigments, lipoids, hemicellulose, pectic substances, and cellulose. Carbohydrates, organic acids, and amino acids decreased under water stress. Yoo and Todd (1961) found that soluble RNA, protein, and activity of proteinase decreased with loss of water. Wood and Petrie (1938) indicated that respiration rate rose as the water content fell to a certain level but that further decrease in water content caused the respiration rate to fall. Zholkevich (1958) found an increase in respiration under drought.

Todd et al. (1962) indicated that relative turgidity as measured by the ratio of fresh water content to the imbibed water content was an excellent indicator of the degree of moisture stress in cereal plants.

Gingrich and Russell (1957) found that fresh and dry weights were greater under osmotic stresses than under similar soil moisture stresses. There was no significant effect of an osmotic stress on the dry weight of corn seedlings. This was ascribed to a lack of an effect on the amylolytic enzymes by the osmotic solutions. Gingrich and Russell (1956) indicated also that the plant was most sensitive to soil moisture stresses between 1 and 3 atmospheres. It was suggested that this might be due to transmission characteristics of the soil.

Slatyer (1961) using osmotic substrates composed of KNO_3, NaCl, mannitol, and sucrose found a rapid recovery from wilting in all cases except mannitol. He indicated that the reaction of plants to readily diffusible osmotic substrates would be different than the reaction of plants to slowly absorbed and non-metabolized solutes. Effects of diffusible osmotic substrates are not strictly analagous to the effect of soil water tension, since the osmotic pressure and turgor pressure levels are displaced.

Eaton (1941) grew corn and tomato plants with the roots divided between two or more solutions of unequal ionic concentrations. He indicated that osmotic pressure rather than specific ion effects was involved in the results. Farrar (1959) using a split-root system to study moisture effects defined wilting as, that state of the plant when the diffusion potential of water in a certain root tissue fell below a critical value. Mederski and Wilson (1960) using a split-root system of sand and soil

thought that internal plant water stress should be minimized and, therefore, any effect on top growth of the corn should not be due to internal stress but due to decreased root growth (ion uptake or translocation) rather than loss of turgescence and subsequent effect on physiological processes.

Hagan et al. (1957) showed that dry weight production, photosynthesis, and respiration rates were not affected appreciably until the moisture content in the entire root zone approached the permanent wilting percentage. This would indicate that as long as the plant was receiving water from some portion of the root zone under low stress, certain measures of plant growth would be unaffected. A similar type of system should be approximated by a split-root system. They pointed out that there was no one simple and general relation between soil moisture conditions and all aspects of plant functioning but that some processes are relatively insensitive to increasing stress while others are relatively sensitive.

CHAPTER III

MATERIALS AND METHODS

The following methods were selected and developed from preliminary studies and a perusal of the literature.

Cultural Methods

Triumph, a variety of Hard Red Winter Wheat (Triticum aestivum L.), was used throughout the study. Seeds were soaked in 4% H_2O_2 for 5-10 minutes, aerated in distilled water for 36 hours, and then placed on cheesecloth stretched across a glass rod rack over a pan of water with the ends of the cloth in water. Approximately $\frac{1}{2}$ inch airspace was between the cheesecloth and the water. The seeds were placed in the dark at 25°C. for 4 days and then placed on the benchtop for 1 day. The seedlings were then transferred to containers of nutrient solution in the growth chamber. After 4 days the nutrient solutions were replaced with the appropriate osmotic solutions. Aeration was provided without excessive bubbling.

Containers used were fabricated from plexiglas and consisted of 2 compartments approximately 15 x 6 x 1 inch each (Figure 1). Short lengths of lucite tubing were notched out and placed on the center partition. One plant was placed in each tube, with one root in each side of the container, and stabilized with glass wool (Figure 2). Ten plants per container were used. Containers had well fitted covers and were made completely opaque by painting.

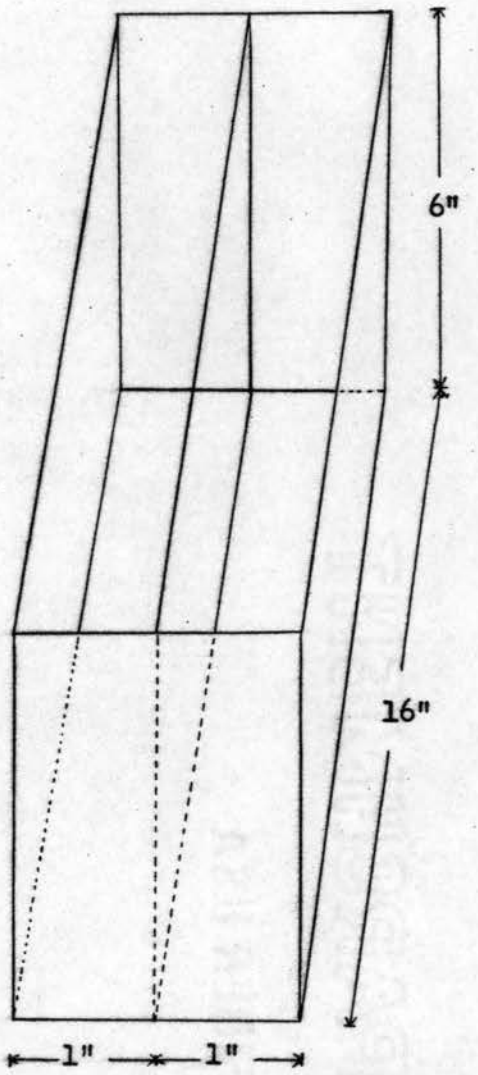

Figure 1. Diagram of container used for growing plants.

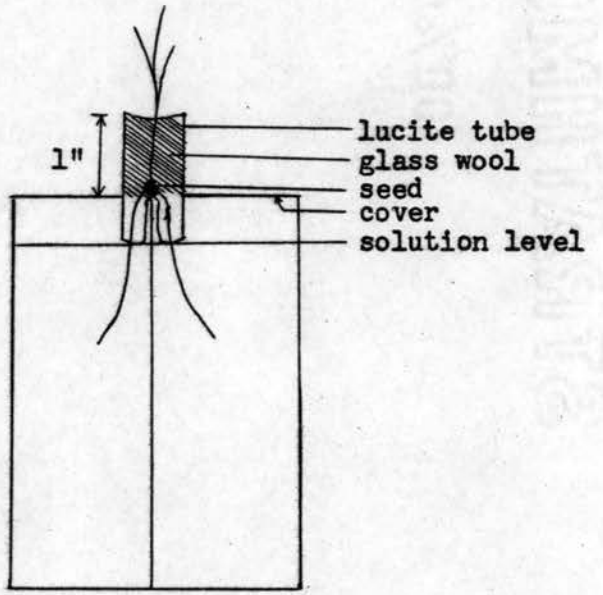

Figure 2. End view of container showing placement of plants.

Growth chamber conditions were a continuous 18°C., a 16 hour light period, and a light intensity of 2500-3500 ft-c depending upon the height of the plants. Relative humidity ranged from 60-70%.

Osmotic solutions were prepared by dissolving the appropriate amount of "Carbowax 6000" in the nutrient solution used by Guinn (1961). Osmotic concentrations were determined cryoscopically. Treatments applied were (0,0), (1,1), (3,3), (9,9), (0,1), (0,3), (0,9); with the numbers designating the bars of osmotic pressure applied to the respective compartments. Nutrient solution was the "0" treatment.

Analytical Methods

Plants were harvested 1, 2, 4, and 5 days after application of the stress. Tubes containing the plants were taken from the container and the roots were removed immediately below the seed and blotted. The tops were cut above the lucite tubing (Figure 2). The shoot and 2 sets of roots were each placed in a 12 x 75 mm. test tube and stoppered.

Plant shoots were weighed to obtain fresh weight, then floated on deionized water for 24 hours and reweighed to obtain the imbibed weight for the relative turgidity determinations. The imbibed leaf tissue and fresh root tissues were dried at 80°C. for 24 hours to obtain the degree of hydration as measured by the percentage of dry matter. The dry matter was digested with concentrated H_2SO_4 and 35% H_2O_2 without having been transferred from the test tubes. After being brought to a known volume, appropriate aliquots were taken for phosphorus and nitrogen determinations. Phosphorus was determined by the method of Shelton and Harper (1941) and nitrogen was determined with a Nessler's solution (Umbreit et al., 1951). Respiration measurements on roots were conducted using a Warburg manometric apparatus and the respective solutions in which the roots were grown.

One plant from each container at each harvest was placed into the tubes and kept in an ice bath for later determinations. These samples were homogenized in 4 ml. of deionized water, using the Servall micro homogenizer. The homogenizer was taken from 0-60,000 rpm. twice in one minute, resulting in a maximum of 20-30 seconds at full speed. The homogenate was filtered through Whatman #42 filter paper under a slight vacuum and appropriate aliquots were used for analysis. All operations were conducted in the cold at 0-5°C. Nitrate determinations were made with the procedure of West and Lyles (1960) and soluble carbohydrates were determined by the anthrone procedure of Carroll et al. (1956). Soluble ninhydrin positive material was determined by a procedure based on that of Moore and Stein (1948). The ninhydrin procedure was assumed to have measured free amino acids and any soluble protein extracted with deionized water.

All determinations were made on 6 plants for the control treatment and on 2 plants for the stress treatments.

CHAPTER IV

RESULTS AND DISCUSSION

Visible effects of the stress treatments were apparent during the experiment. Plants ranged from dark green with the greatest amount of growth in nutrient solution only, to light green with yellow tips and with the least amount of growth for those plants which had 9 bars stress applied to both roots. Plants with a portion of their root system under stress and a portion without stress appeared to be quite comparable to the control plants.

Visible root growth was affected by stress with little growth taking place at the 9 bar stress. Roots under stress were yellow with the intensity of the yellow color positively related to the amount of stress applied. This was clearly apparent between the stressed and nonstressed roots of the same plant. Salim (1962) and Zgurouskaya (1955) reported similar results with roots under a soil moisture stress. The color may be due to formation of a particular pigment under stress (Zgurouskaya, 1955), an accumulation of pigments in general (Nezgovorova, 1957), or a change in state of various compounds.

Data presented in the illustrations are reported as means on the dry weight basis. The treatment with nutrient solution applied to both parts of the root system (0,0) is considered the control.

Shoots were considered as being under uniform stress, when identical osmotic pressures were applied to both parts of the root system. Shoots

were considered as being under nonuniform stress when dissimilar osmotic pressures were applied to the two parts of the root system.

Roots were considered as being under uniform stress when both parts of the root system of a plant were under identical osmotic pressures. Roots were considered as nonstressed and stressed respectively, with a combination of nutrient solution (nonstressed) and an osmotic solution of 1, 3, or 9 bars (stressed) applied to the 2 sets of roots of a plant.

All statements of statistical significance are based on calculated least significant differences at the 95% level of confidence for the appropriate means. (Table IX, page 49).

Turgidity of Shoots

Relative turgidity of the plant tissues at the various times of harvest was measured in two ways. One measure was that of the ratio of the fresh water content to that of the imbibed water content which is generally considered to be "relative turgidity" (Weatherly, 1950). A second measure was the simple ratio of the tissue fresh weight to that of the imbibed tissue weight. Both measures gave the same relative trend and the statistical analysis gave the same significant differences between treatments. The ratio of water contents appeared to spread the experimental points more but did not increase precision. It would appear that the ratio of water contents should vary more as they contain another source of variation in the determination of dry weight.

As seen in Figure 3, the time of harvest had a definite effect on the degree and direction of response of the relative turgidity as determined by the ratio of fresh to imbibed weights. Values for the relative turgidity of plants with differential osmotic stresses applied

to the roots are not shown as they were not significantly different from the control or (0,0) treatment. These values are given in Table I, page 41. At 3 bars stress the relative turgidity continued to increase with time; whereas, at 9 bars it decreased. This might be an indication that between the two values there is a critical value at which relative turgidity would not change or would change very slowly and which might be the maximum point of survival. This may be similar to a critical diffusion potential of the water as defined by Farrar (1959).

Figure 4 shows the response of the relative turgidity to stress after a period of 96 hours. The application of 9 bars stress on both roots was the only treatment resulting in a significant effect. The data obtained on turgidity indicated that the use of split-root systems may aid in keeping the plant tops from undergoing loss of turgidity when stress is applied to only a portion of the root system. This is similar to ideas of Hagan et al. (1957) and Mederski and Wilson (1960).

Hydration of Shoots

As seen in Figure 5, the time of harvest also had a definite effect on the degree and direction of the response of hydration to osmotic stress. A slight increase in percentage of dry matter was indicated with 1 bar of stress 24 hours after application of the stress but none of the effects were significant. This was true whether stress was applied to the entire root system or only to one part. By 48 hours the effects of the osmotic stresses were quite apparent and a lesser effect due to the split application was also indicated. After 120 hours (Figure 5), the difference between the uniform stress and the nonuniform stress was highly significant at the 3 and 9 bar levels with the spread increasing greatly with 9 bars

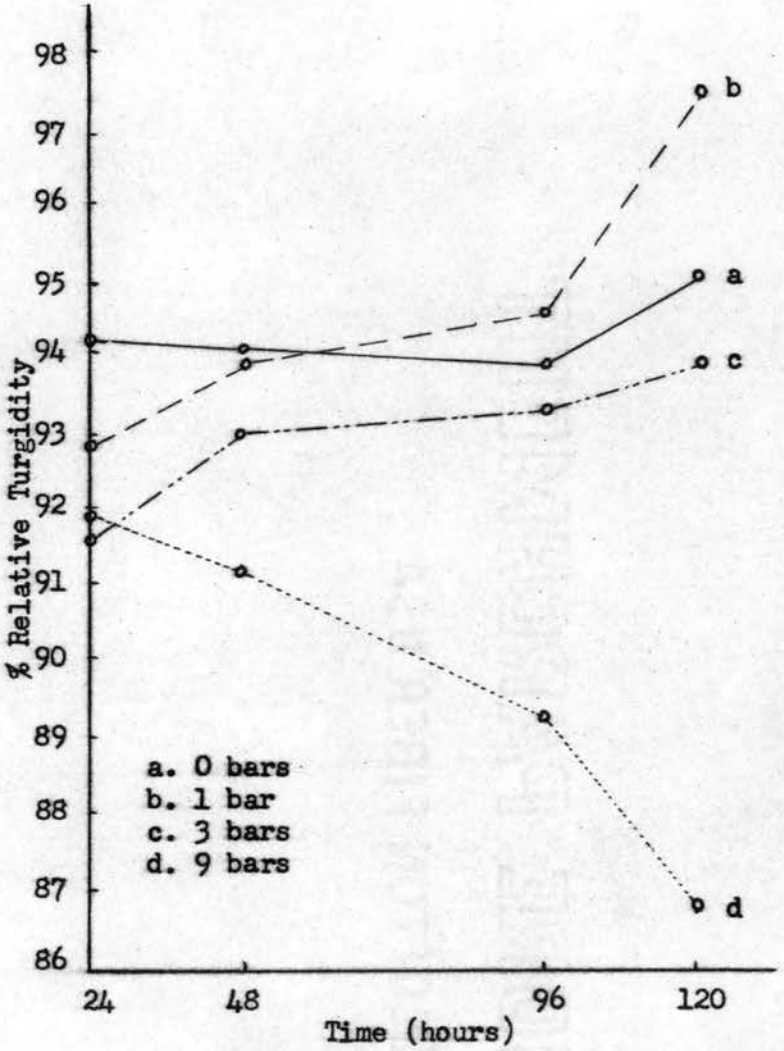

Figure 3. Effect of time of harvest on response of relative turgidity to uniform osmotic pressure.

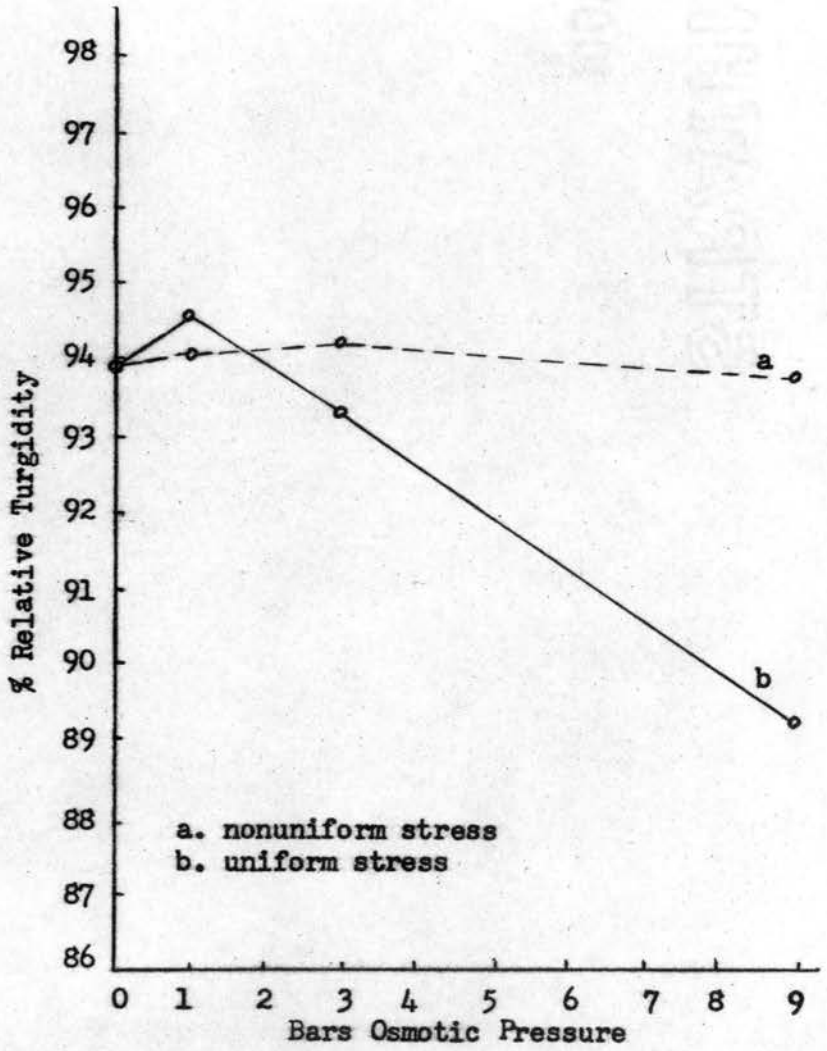

Figure 4. Effect of osmotic pressure on response of relative turgidity after 96 hours.

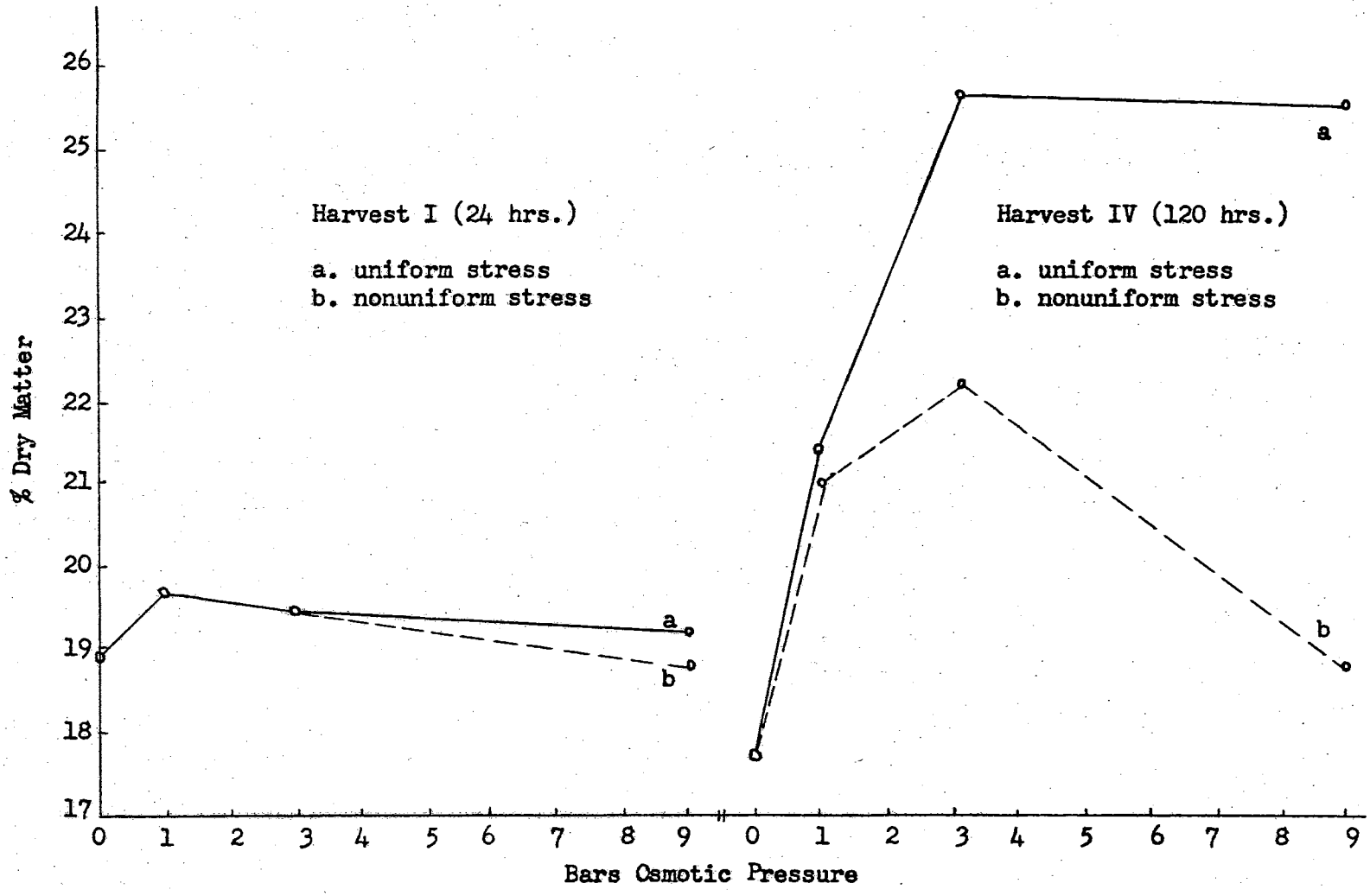

Figure 5. Effect of osmotic pressure on the percent dry matter of the shoots after 24 and 120 hours.

of stress. The data indicates that a split application of stress to roots of the same plant may result in an effect on the shoot but the effect is considerably less than the application of stress to both roots. The fact that a split application of stress caused an effect on the shoot was an indication that the plant was not acting independently of one stressed root. The close agreement between the 9 bar nonuniform stress and the control (Figure 5, line b) indicates that the stressed root may be isolated to some extent at higher stresses. The slowness of response to osmotic stress by dry matter may be a reason for the results of Gingrich and Russell (1957) and Woolley (1963) in obtaining no significant change in the dry weight of shoots with increasing osmotic stress. The split-root data does not support the statement of Hagan et al. (1957), that dry matter production is not affected appreciably until the entire root zone approaches the permanent wilting percentage.

Hydration of Roots

A relatively large response by the roots to osmotic stress was apparent after 24 hours and increased until 120 hours (Figure 6). As indicated in Figure 6, the dry matter percentages for the stressed root of the split treatments were quite similar to those for the uniformly stressed roots. Dry matter percentages for nonstressed roots were not significantly different from the control. Figure 7 indicates that any change after 24 hours is due primarily to a decrease in the percentage of dry matter of the control.

Figure 7 (lines a, b, c, and d) shows that dry matter percentages for the nonstressed roots of the split treatments were not significantly different from the control during the time of the experiment. It was not

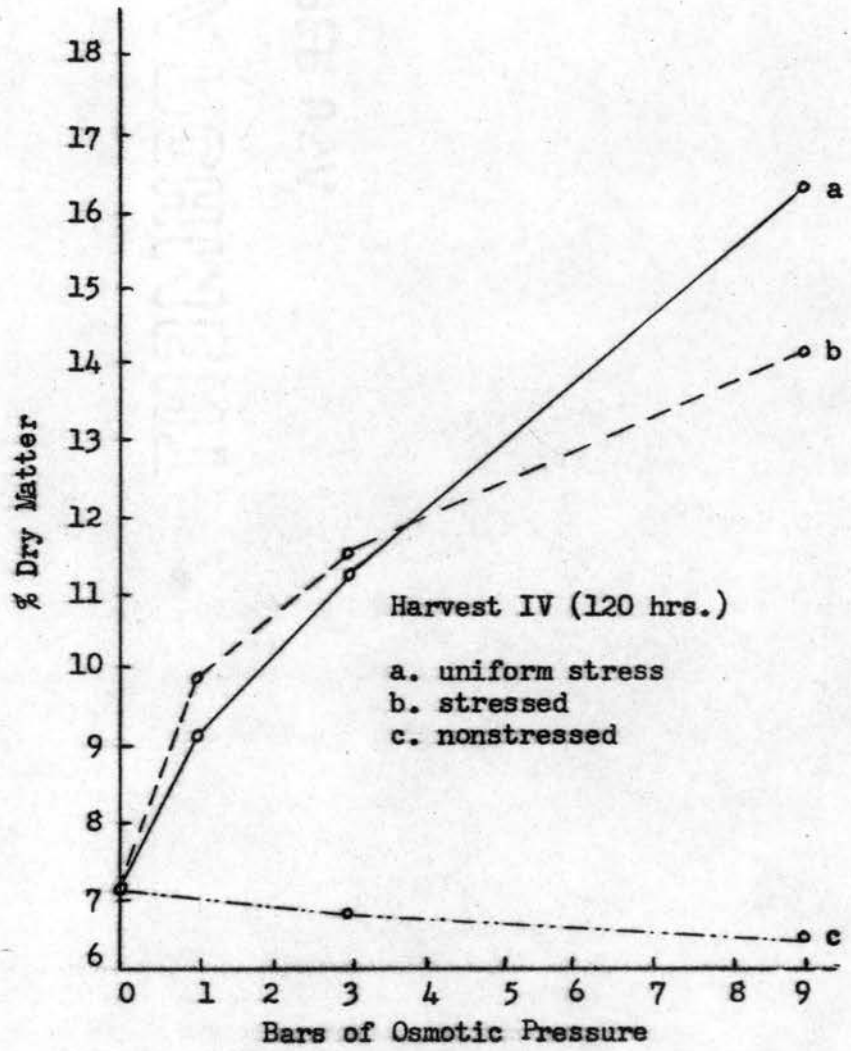

Figure 6. Effect of osmotic pressure on the percent dry matter of the roots after 120 hours.

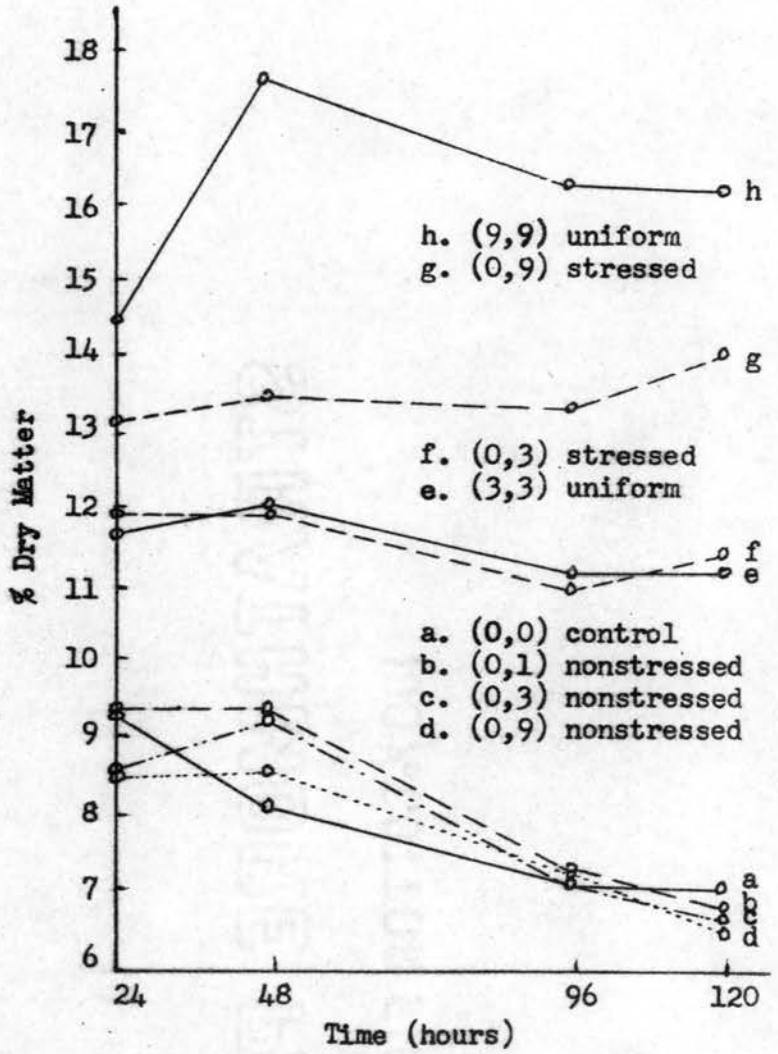

Figure 7. Effect of time of harvest on response of percent dry matter of roots to osmotic pressure.

until the 9 bar stress was reached that there was a significant difference between the stressed root of the split treatment and the uniformly stressed root (lines g and h). This would indicate that at the 9 bar stress there may be either a crossover effect between the stressed and nonstressed roots of the split treatment or a feedback effect from the shoot due to the reduced turgidity with the uniformly stressed roots. The data thus indicates that at the lower stresses and possibly even at the higher stresses the roots may be acting independently.

Nitrogen Content of Shoots

The percentage of nitrogen of the uniformly stressed shoots at the 1 and 3 bar treatments were significantly lower than the control or the 9 bar stress after 48 hours (Figure 8). Woolley (1963) found an increase in percentage of nitrogen with increasing stress. The age of the plant and a different atmospheric environment affecting nitrogen uptake, may explain the difference in the results.

Figure 8 shows, that the plant with the nonuniform stress applied decreased in percent nitrogen when stress was applied to a portion of its root system. This decrease was significant only at 48 hours with 1 bar stress. The response to the nonuniform stress, although not statistically significant, indicated that the nitrogen content of the shoot was probably disturbed regardless of its relative turgidity.

Nitrogen Content of Roots

Figure 9 shows, that 48 hours after application of the stress all of the uniformly applied stresses resulted in nitrogen percentages significantly lower than the control. There was no significant difference between the stressed root of the split application and the roots under

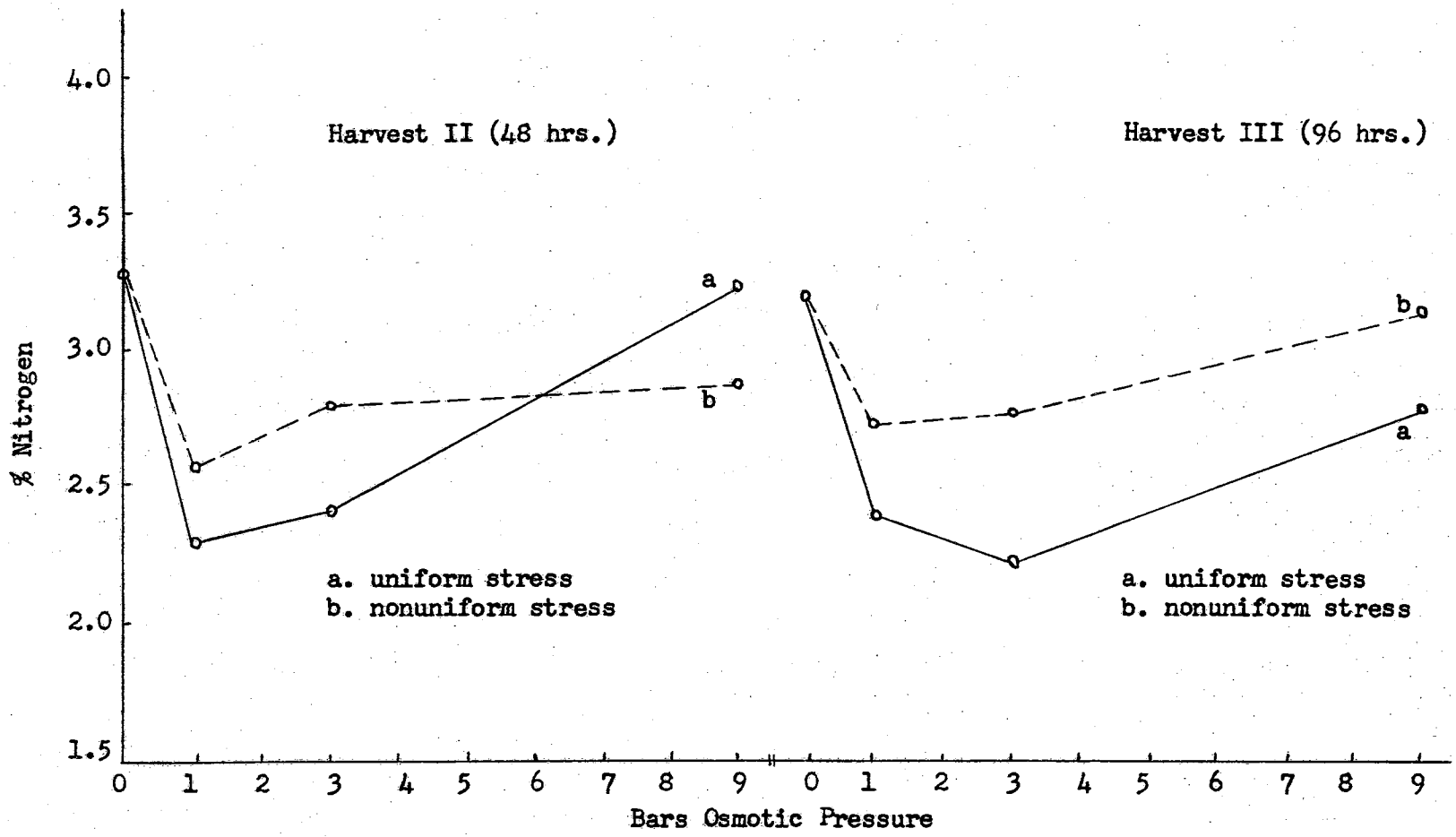

Figure 8. Effect of osmotic pressure on the percent nitrogen of the shoots.

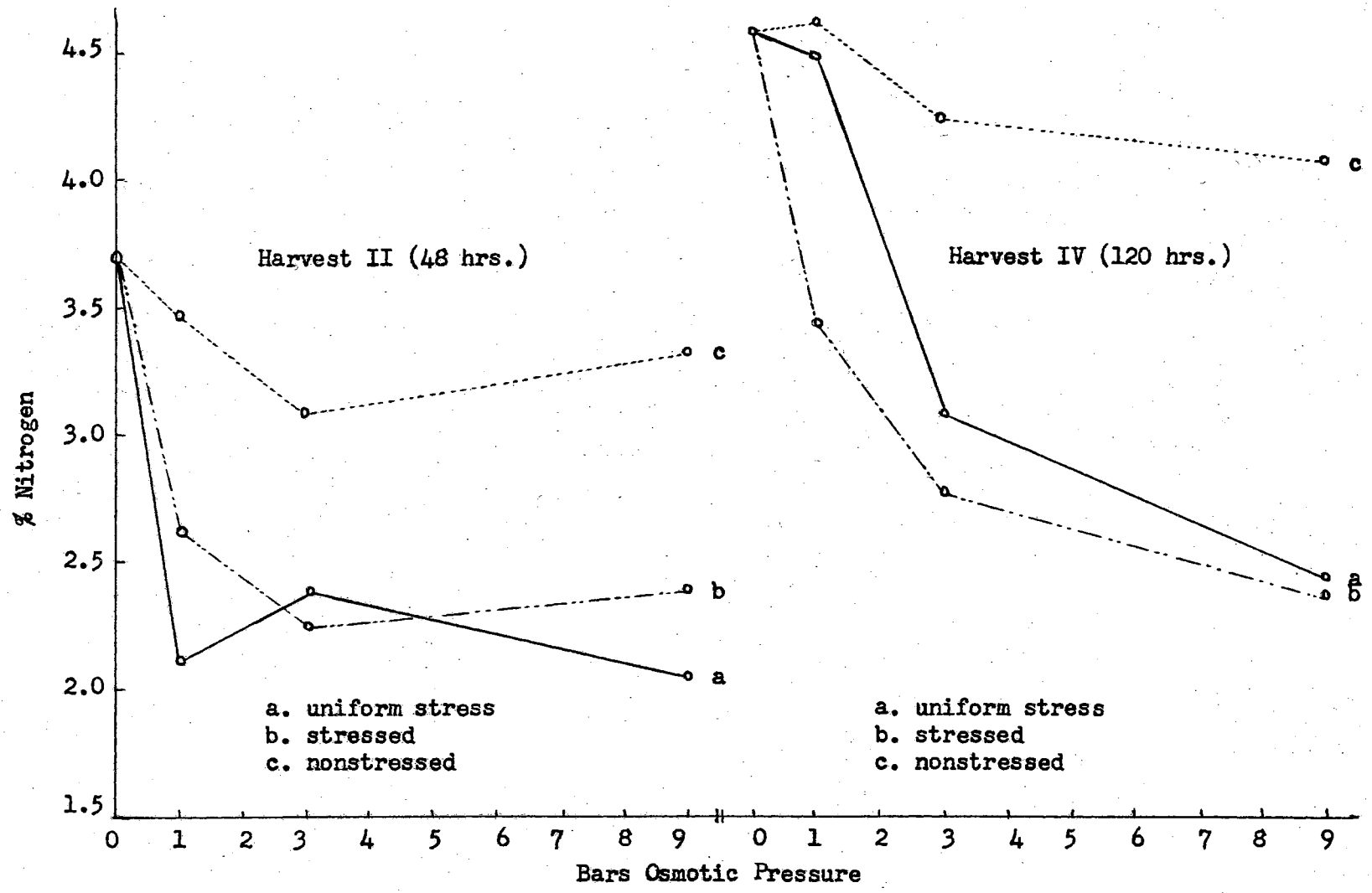

Figure 9. Effect of osmotic pressure on the percent nitrogen of the roots.

uniform stress. The nonstressed root was not significantly different from the control. A comparison of Harvest II with Harvest IV indicated that by 120 hours all the roots had increased in percentage of nitrogen with the 3 and 9 bar levels of the stressed roots remaining significantly different from the control. There was a significant difference between the stressed and nonstressed root of the differentially stressed plants at all levels. This may indicate a possible independence of action between the roots of the nonuniformly stressed plants. The decrease in nitrogen content with increasing stress was the same trend as found by Woolley (1963), who found a non-significant decrease with stress.

The continued increase of nitrogen in the roots, as shown by a comparison of Harvest II and Harvest IV in Figure 9 and in Table III, page 43, may indicate that the roots were still obtaining materials from the endosperm. This appears unlikely as Folkes and Yemm (1958) indicated that in barley the transfer of nitrogen from the endosperm to the embryo is virtually complete after 8 days.

Phosphorus Content of Shoots

Figure 10 shows, that 48 hours after application of the stress the only significant decrease of phosphorus in shoots was at 1 bar stress. At 96 hours all of the uniform stresses resulted in a significant decrease of phosphorus percentage. The reason for the increase in phosphorus content with 3 bars stress at 48 hours is unknown but a similar phenomenon was observed in an earlier experiment. The low level in phosphorus content at an intermediate stress is similar to that observed by Woolley (1963), whose low point was displaced to about 6 bars.

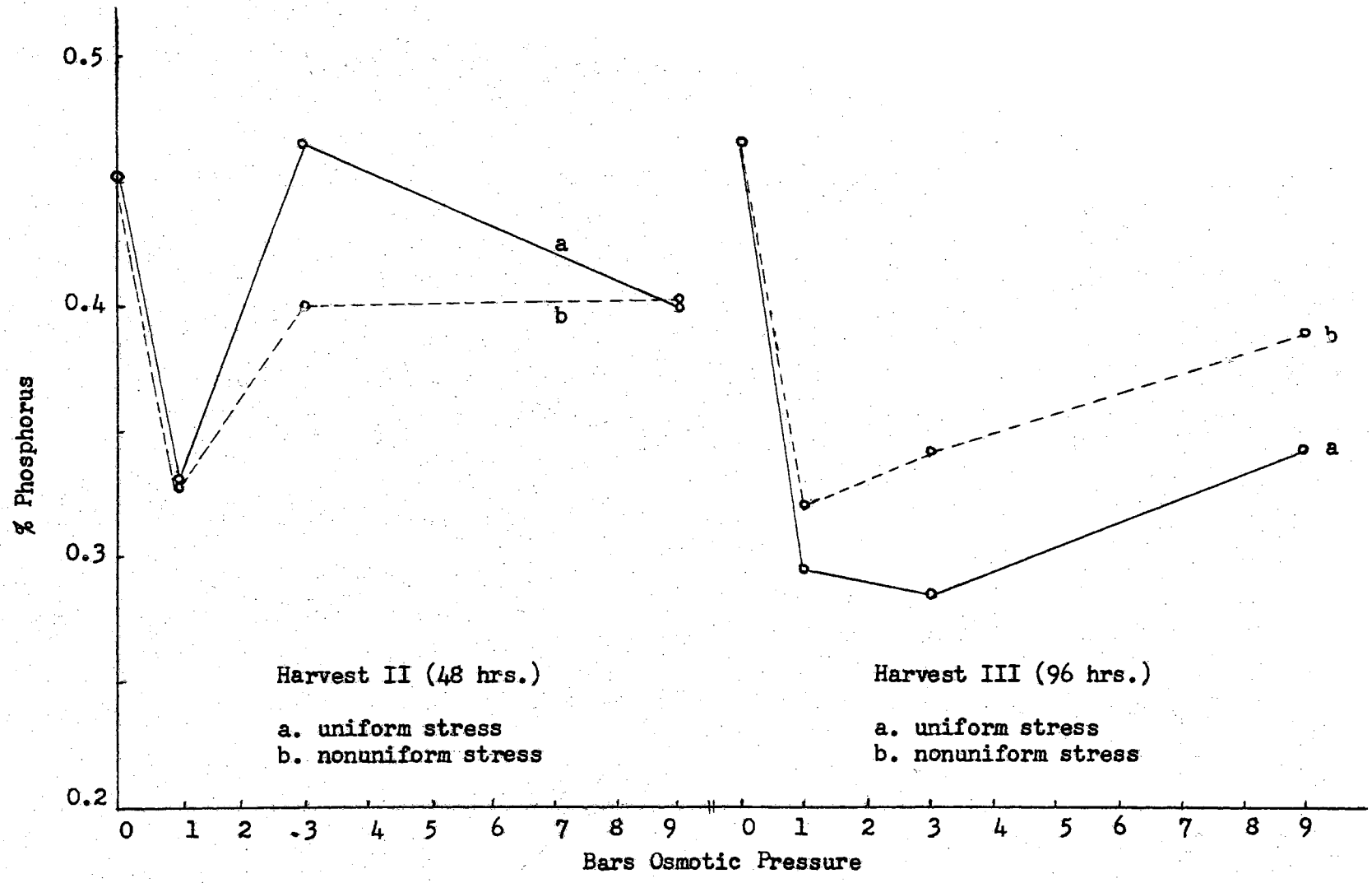

Figure 10. Effect of osmotic pressure on the percent phosphorus of the shoots.

This difference in the point of minimum phosphorus percentage could quite possibly be due to the difference in the length of time the plants were under stress before harvest, atmospheric conditions, or a difference due to variety (Sisakyan, 1939). The shoot with nonuniformly applied stress was also significantly different from the control; indicating that phosphorus content of the shoot was independent of the relative turgidity factor. The data indicated that the plant was not acting independent of a differentially stressed root system. No reason is apparent for the decrease of phosphorus in the shoot with a split treatment; presumably, the one nonstressed root should have been capable of absorbing sufficient phosphorus throughout the experiment for the entire plant.

The data from Woolley (1963), this study, and a preliminary experiment support findings of Gates (1957), that phosphorus was lower under moderate stress than under severe stress; indicating that the effect of stress on phosphorus was greater than on dry weight, and probably was started before the effect on dry weight. Hydrolysis of phosphorus compounds may be a result of stress and probably occurs early upon stress.

Phosphorus Content of Roots

Figure 11 shows the response of phosphorus content of the roots to the various degrees of osmotic stress after 24 hours and 96 hours of stress. The uniform 9 bar stress was significantly lower than the control after 24 hours and all uniform stress treatments were significantly lower at 96 hours. The stressed root of the split treatment was significantly lower than the control at all stresses for both harvests. The difference between the stressed and nonstressed root of the differentially

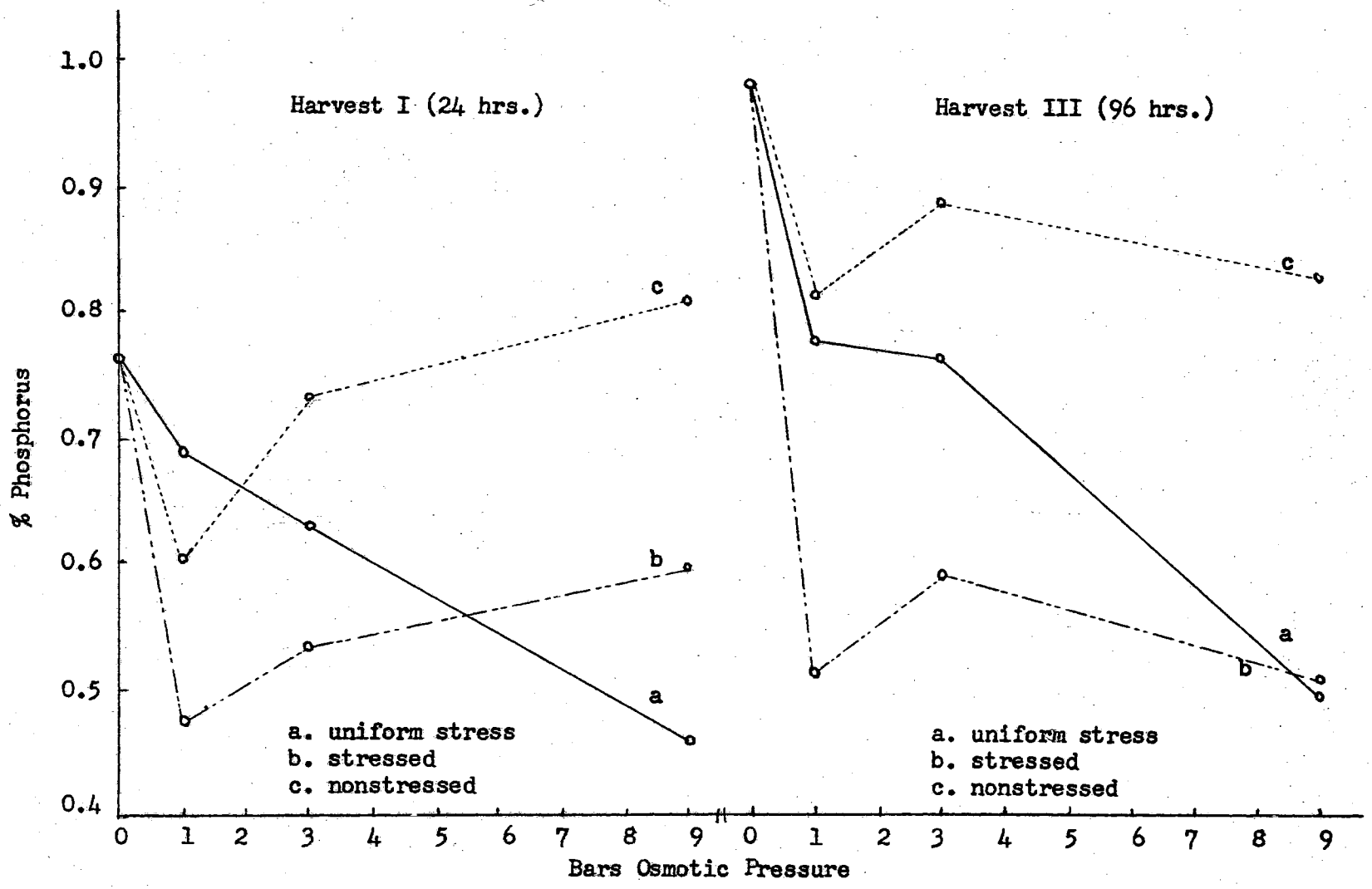

Figure 11. Effect of osmotic pressure on the percent phosphorus of the roots.

stressed plant was significant after 96 hours and both roots showed the greatest reduction at 1 bar stress.

The greatest decrease in phosphorus content being obtained at the highest stress with a uniformly applied stress is in opposition to Woolley (1963), who obtained a low point in phosphorus content at 3 bars. The nonstressed root of the split treatment was also affected to some extent particularly with one bar stress. The stressed root of the split root and roots under uniform stress appeared to be affected differently except at 9 bars of stress.

That phosphorus content of the control roots increased with time; whereas, those at 9 bars remained the same can be seen from a comparison of the 2 harvests in Figure 11 or Table IV, page 44 . Whether this indicated or not that metabolic activity had essentially ceased is unknown. The data seemed to support the conclusions of Dean and Gledhill (1956), that reduced phosphate absorption in dry soil was due to alterations in the physiology of the roots.

Nitrate Content of Shoots

The effects of osmotic stress on the percentage of nitrate in the shoots at 24 and 120 hours are plotted in Figure 12. After 24 hours, only the uniform application of 9 bars stress was significantly different from the control. The trend of response then began to change and after 120 hours, only the uniform application of 1 bar was significantly different from the control; but in a different direction than the initial response. The shoot with the split stress applied maintained the lowest nitrate content with 1 bar of stress applied to one of its roots. The nitrate content of the control remained relatively unchanged during the time of this study;

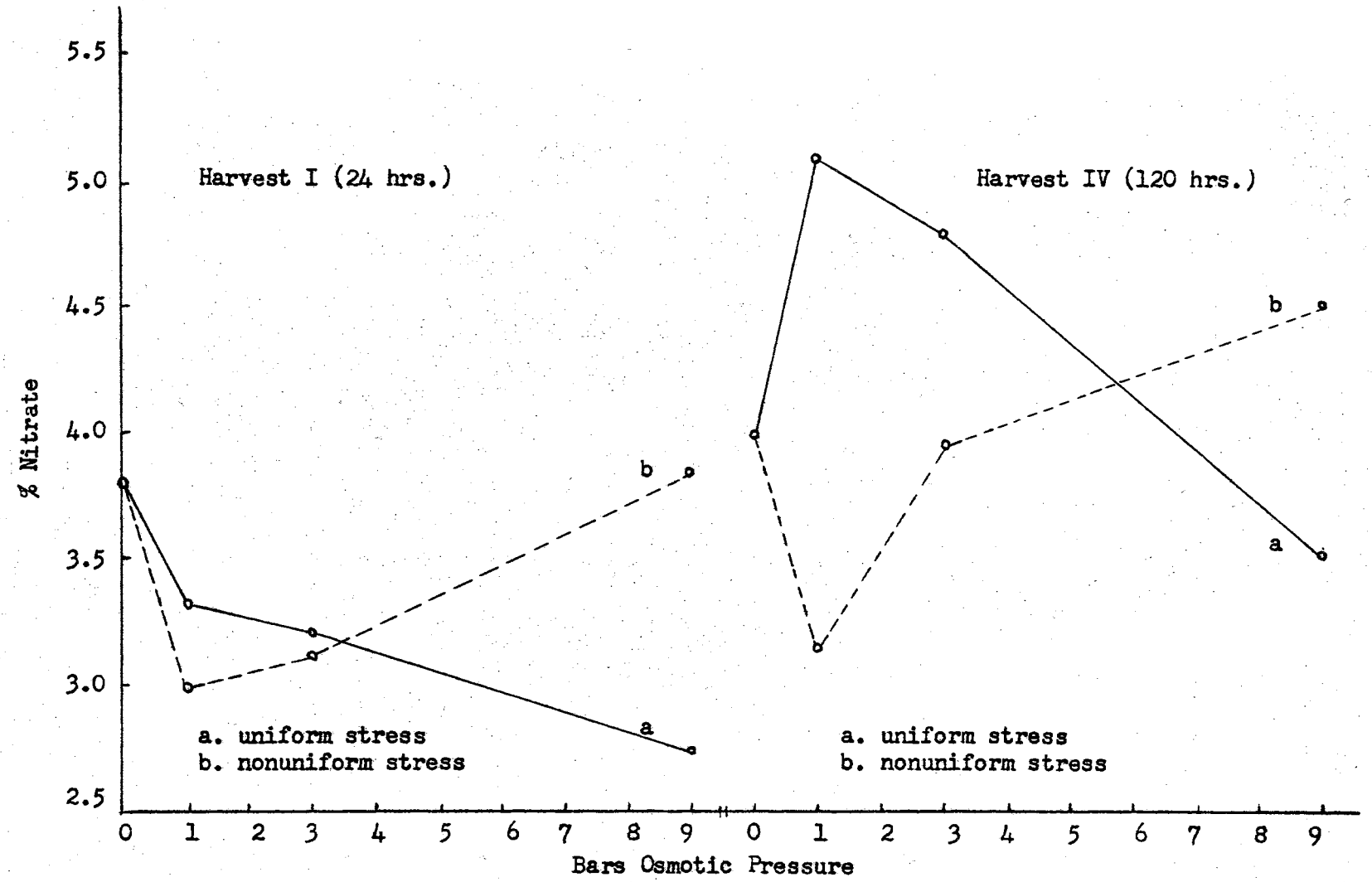

Figure 12. Effect of osmotic pressure on the percent nitrate of the shoots.

whereas, all of the treated plants increased in nitrate content.

Emmert and Bell (1933) indicated that the ability of the plant to absorb nitrate was not impaired under stress but nitrate accumulated in the tissue because of the reduced phosphorus and the subsequent decrease in tissue formation. This study failed to indicate that nitrate accumulates because of less tissue formation since the least growth took place at 9 bars stress where phosphorus was decreased and nitrate did not accumulate.

Nitrate Content of Roots

The effects of an osmotic stress on the nitrate content of the roots after 24 and 120 hours are given in Figure 13. The immediate effect on the uniformly stressed plants appeared to be a great decrease in nitrate content at the 1 bar stress level. A comparison of Harvest I with Harvest IV shows that the nitrate content of the control and 1 bar stressed root increased with time; whereas, those stressed at 3 and 9 bars decreased slightly. After 120 hours all stress treatments, both uniform and non-uniform, showed a significant decrease in nitrate content as compared to the control.

The differentially stressed roots indicated that they were not acting independently, although the nonstressed root was affected significantly less than the stressed root except at the 1 bar level at 24 hours.

A comparison of Figures 12 and 13 indicates a possibility of translocation of nitrate to the shoot under stress. Translocation to the shoot and a possible reduced uptake may account for the low nitrate content of the roots under stress.

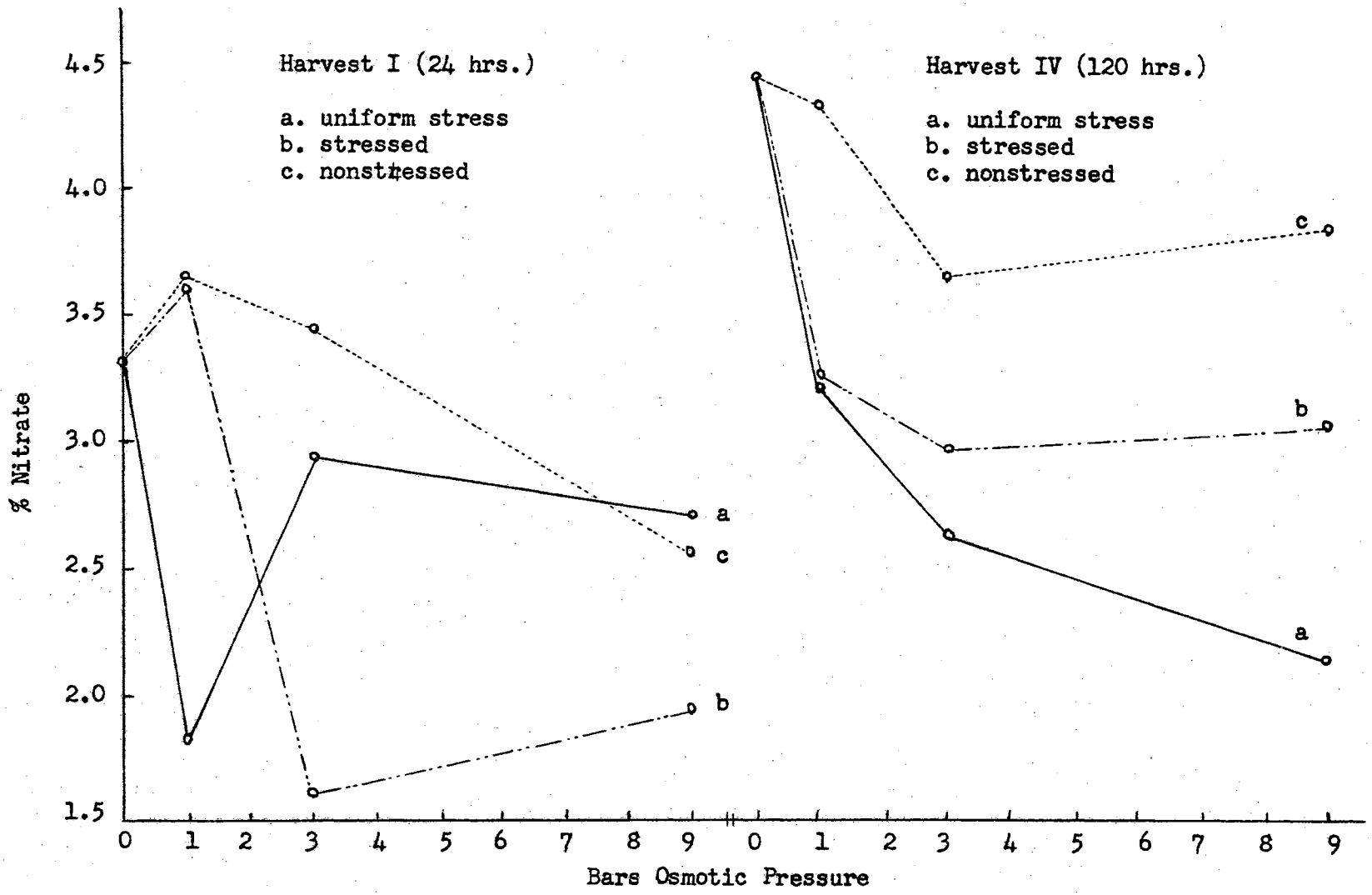

Figure 13. Effect of osmotic pressure on the percent nitrate of the roots.

Soluble Carbohydrate Content of the Shoot

Soluble carbohydrate content of the shoot appeared to be little affected initially, although there was a nonsignificant decrease with 1 and 9 bars uniform stress (Figure 14). After 96 hours, there was a significant increase of carbohydrate at the 3 and 9 bar stress levels for the uniformly stressed conditions. There appeared to be an effect of the 1 bar differentially applied stress level at both the 24 hour and 96 hour harvests but it was not statistically significant.

The results of this study are different from those of Nezgovorova (1957), who obtained a decrease in carbohydrates under stress.

Soluble Carbohydrate Content of Roots

With uniformly stressed plants, there was a significant increase in soluble carbohydrate content at all uniform stress levels after 24 hours (Figure 15). There was also a significant increase in soluble carbohydrate for the nonstressed root of the split treatment at all stress levels but the stressed root of the split application was significant only at the 1 bar level.

Soluble carbohydrate levels in the roots decreased during the time of the experiment and at 120 hours, only the 9 bar treatment of the uniformly stressed plant was significantly different from the control. Both roots of the differentially stressed plant were not different from the control, indicating either that there was free exchange between them or the carbohydrate contents of the roots were determined by the turgidity of the shoot. There would still need to be an explanation for Harvest I. Since carbohydrate accumulated in the roots before it accumulated in the shoots, the indication would be that utilization

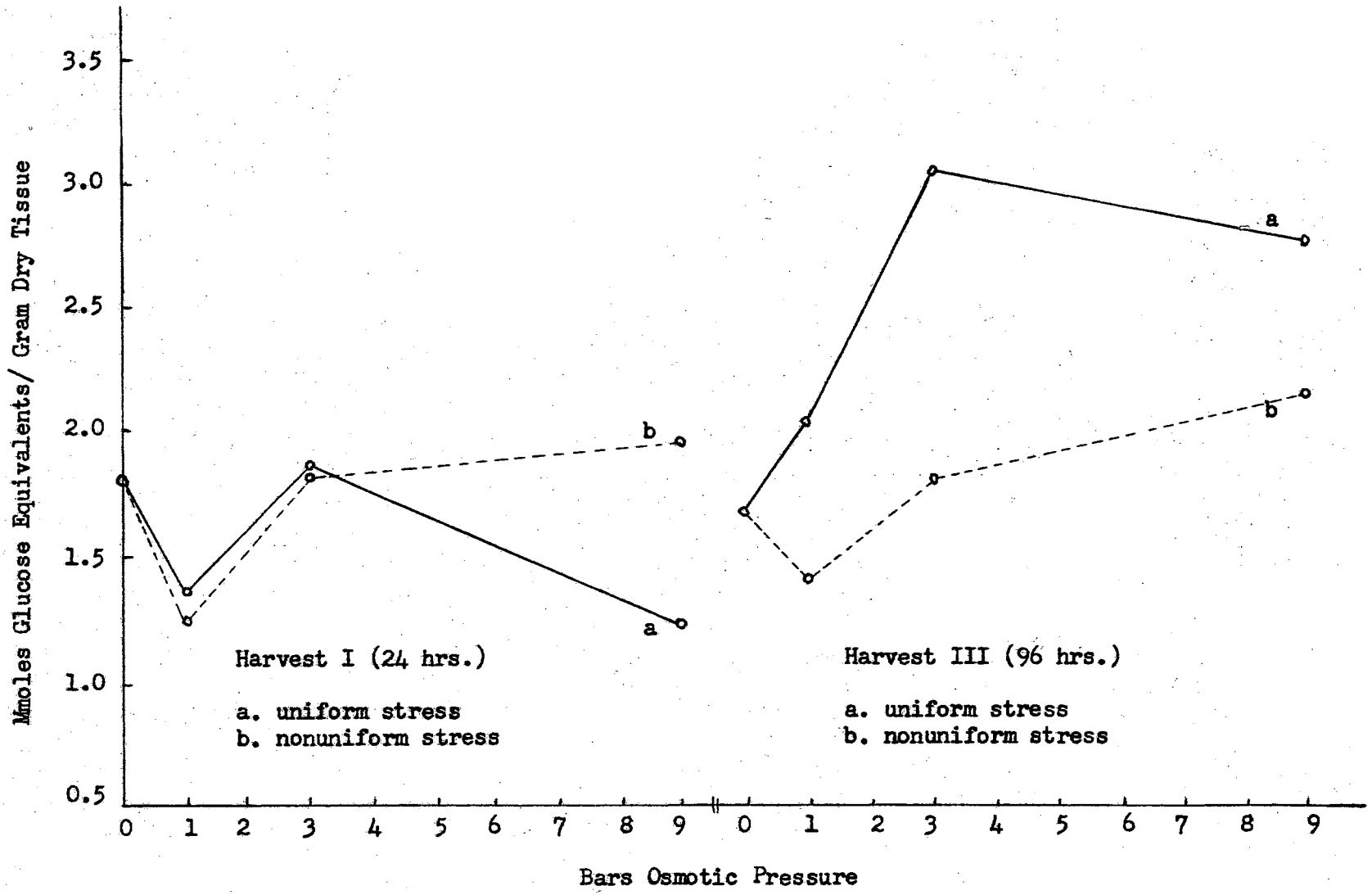

Figure 14. Effect of osmotic pressure on the soluble carbohydrate content of the shoots.

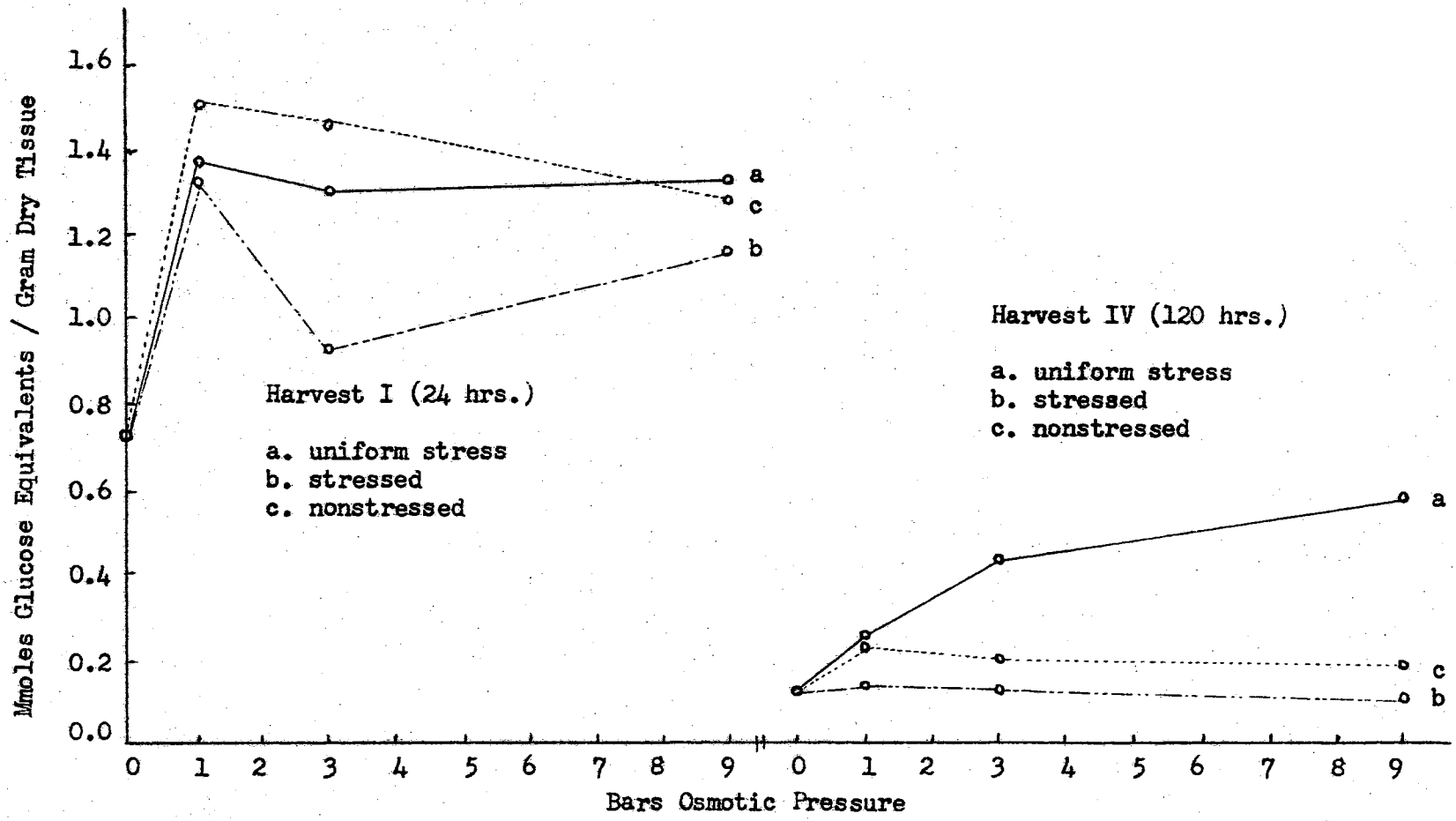

Figure 15. Effect of osmotic pressure on the soluble carbohydrate content of the roots.

of carbohydrate in the root was decreased before the translocation from the shoot was affected. Whether this carbohydrate accumulation was due to the effect on phosphorus metabolism or nitrogen metabolism is unknown. The continued increase of carbohydrate in the shoot would indicate that photosynthesis was not greatly affected by osmotic stress over the period studied.

Soluble Ninhydrin Positive Material in Shoots

As indicated in Figure 16, a uniform osmotic stress resulted in a significantly lower ninhydrin test at the high stresses in 24 hours and after 48 hours all uniformly stressed plants were significantly lower than the control.

The nonuniformly stressed shoot was also different at 24 hours for all levels of stress but at 48 hours only the 1 bar stress resulted in a significant difference and after 96 hours there was no significant effect of the split stress.

The ninhydrin positive material under uniform stress treatments did not change significantly with time,and the content in the differentially stressed plants increased significantly with time; thereby, indicating that in both instances the greatest change took place within the first 24 hours. The tremendous effect at the one bar nonuniform stress indicates that the nitrogen metabolism of the plant was greatly affected by a small increment of stress. The data indicates that the plant was not acting independently of the stress even though stress was applied only to a portion of the root system. There was no apparent reason for the faster recovery of ninhydrin positive material at the higher stress levels in differentially treated plants.

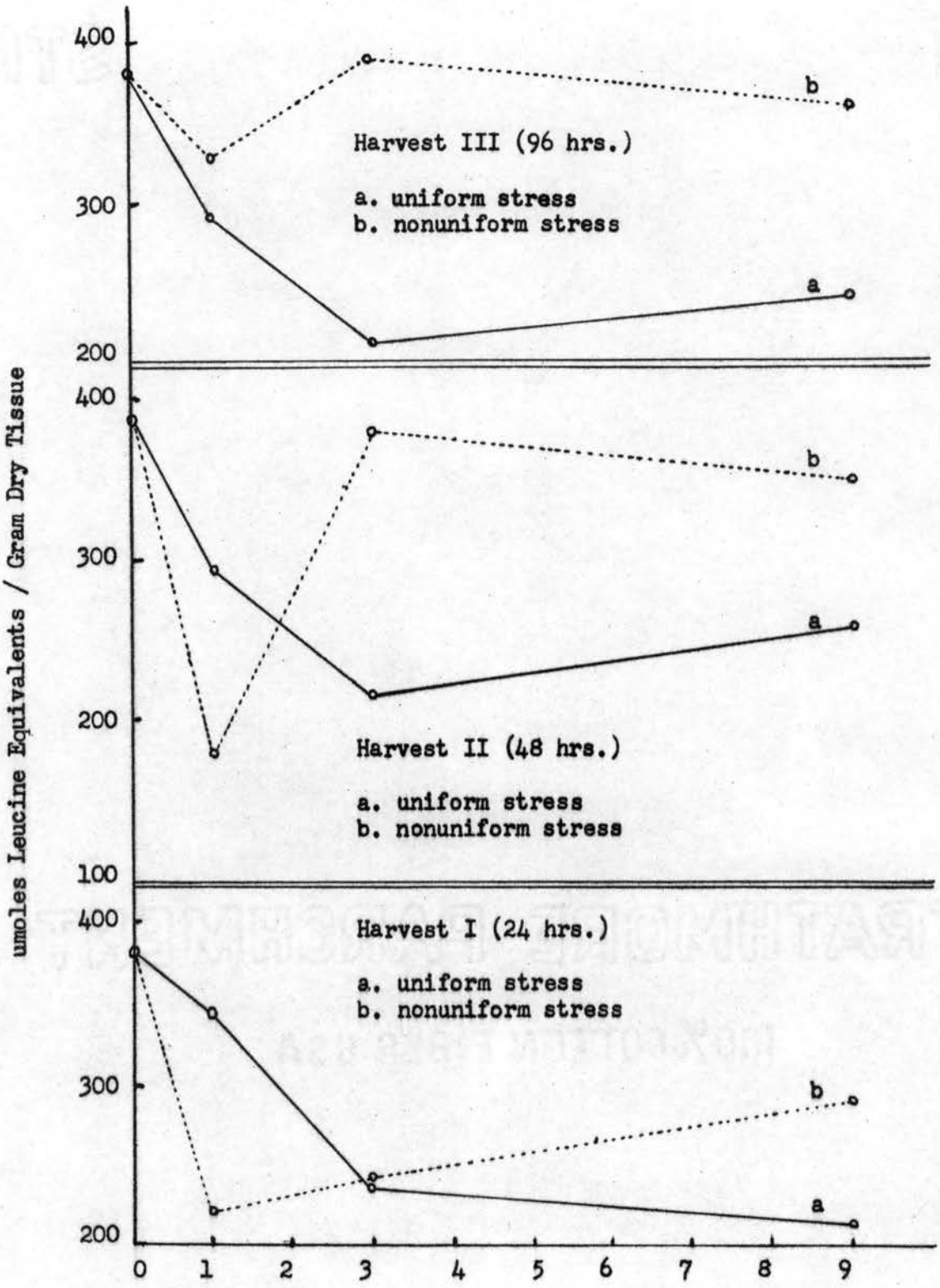

Figure 16. Effect of osmotic pressure on the content of ninhydrin positive material in the shoots.

Soluble Ninhydrin Positive Material in Roots

After 48 hours, all uniform stress treatments were significantly lower than the controls (Figure 17). The stressed and nonstressed roots of the differentially stressed plants were significantly lower than the control at the 1 bar stress level after 48 hours. After 120 hours, the nonstressed root was identical with the control and the stressed root was not significantly different from uniformly stressed roots.

The data indicates that the roots may be acting independently after a period of time but that there is a tremendous initial physiological shock effect.

Oxygen Uptake of Roots

Data for oxygen uptake is given in Table VIII, page 48. The effect of the various levels of osmotic stress on the roots was quite variable in regard to oxygen uptake. There appeared to be a general trend toward an increased uptake with uniformly applied low stress. No consistent conclusions could be drawn from the effects of a differentially applied stress.

The trend of increased respiration under stress agrees with that of Zholkevich (1958) and Wood and Petrie (1938).

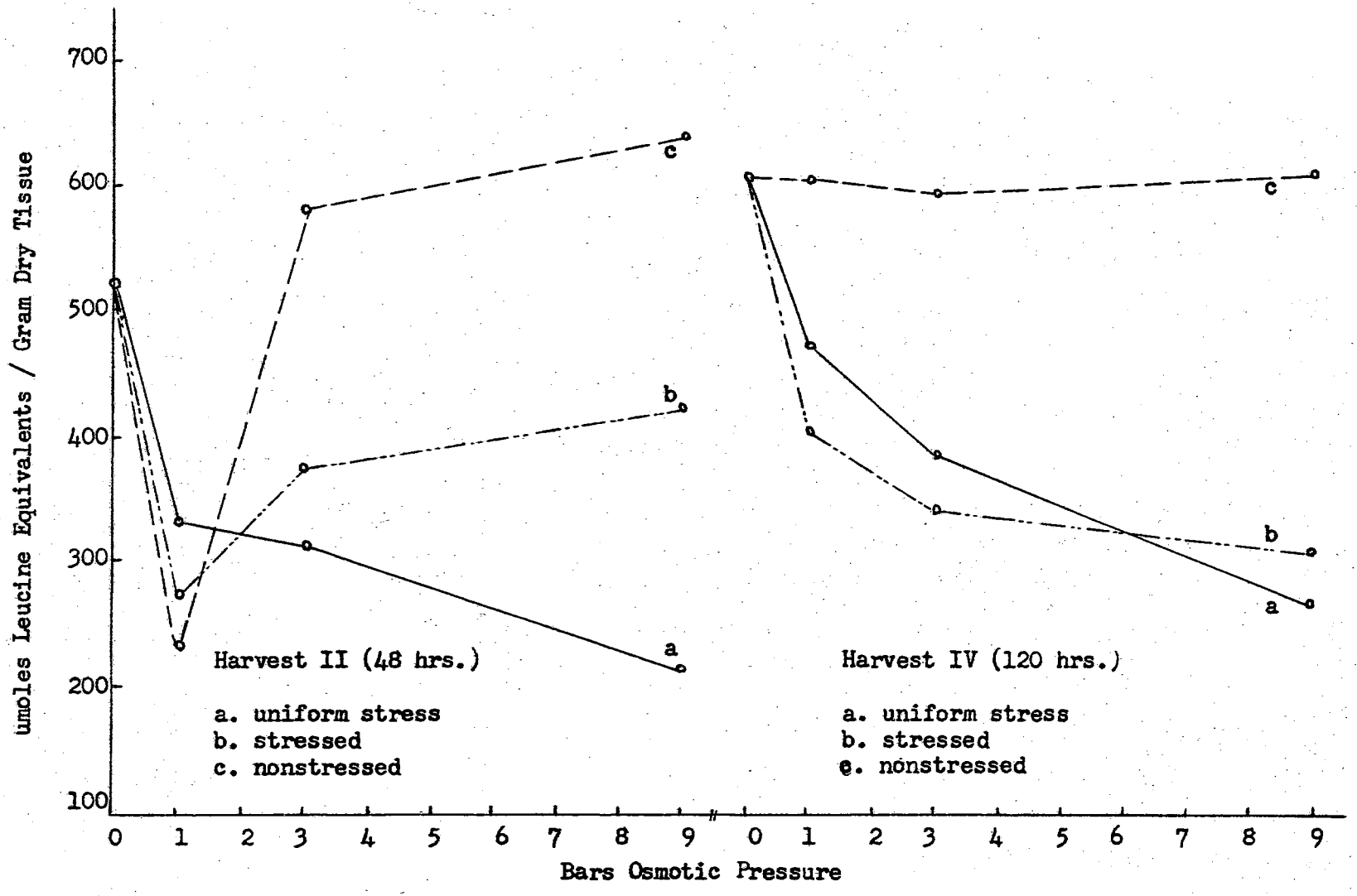

Figure 17. Effect of osmotic pressure on the content of soluble ninhydrin positive material in the roots.

CHAPTER V

SUMMARY AND CONCLUSIONS

Responses of wheat seedlings to applications of various levels of osmotic stress were studied. A split-root technique was used in an attempt to determine the effect of turgidity on these responses.

Relative turgidity as frequently used may be a reasonable indication of the internal water stress of plants but under certain conditions may prove to be misleading in determining the time at which the plant is affected by stress. The data obtained in this study indicates that a water stress on the roots may not affect the plant turgidity under low evaporative conditions until relatively high stresses are obtained. Under these conditions relative turgidity would be a poor measure of plant response.

The increase in percentage of dry matter of the shoots appeared to reflect the osmotic stress applied to the roots after a period of time but responded slowly. Dry matter percentage of the roots increased more quickly in response to a stress and also reflected the amount of stress applied.

Phosphorus and nitrogen percentage were decreased in the entire plant by an osmotic stress. The sharp decrease apparent after 24 hours at the 1 bar stress indicated that uptake of these elements must have decreased immediately after application of the stress with dry matter

production being essentially unchanged.

Nitrate content of the shoot initially was decreased slightly but subsequently increased under low stress, indicating that nitrate translocation to the shoot was not affected until higher stresses were reached. Nitrate decreased in the roots and probably reflected a decreased uptake.

The increase in carbohydrate content under stress indicated that utilization is decreased more than synthesis. This might be a reflection of the phosphorus response to stress.

The immediate and relatively large decrease of the ninhydrin material indicated that the synthesis of these compounds was very sensitive to stress.

The responses to stress would almost certainly be different if the evaporative demand were increased. Time studies would prove to be very helpful in evaluating other studies of this type.

The response of phosphorus and the ninhydrin positive material to stress would indicate that the split-root technique must be used with reservation and a cognizance of the problems involved. A study with radioactive materials would be very interesting.

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APPENDIX

TABLE I

EFFECT OF OSMOTIC PRESSURE ON PERCENT RELATIVE TURGIDITY*

	Time (Hours)	Osmotic Pressure Treatment (Bars)						
		(0,0)	(1,1)	(3,3)	(9,9)	(0,1)	(0,3)	(0,9)
Shoots:								
	24	94.21	92.79	91.56	91.91	93.10	94.20	93.91
	48	94.04	93.91	92.99	91.17	95.69	95.46	93.66
	96	93.97	94.54	93.32	89.21	94.19	94.39	93.72
	120	95.19	97.56	93.94	86.80	94.32	94.00	94.10

*Mean of six plants for (0,0) and mean of two plants for remainder of treatments.

TABLE II

EFFECT OF OSMOTIC PRESSURE ON PERCENT DRY MATTER*

	Time (Hours)	Osmotic Pressure Treatment (Bars)									
		(0,0)	(1,1)	(3,3)	(9,9)	(0,1)		(0,3)		(0,9)	
Shoots:	24	18.90	19.69	19.43	19.18	19.68		19.43		18.79	
	48	20.30	23.74	24.15	22.26	22.21		22.31		21.29	
	96	19.11	20.25	25.06	25.51	21.36		22.21		20.33	
	120	17.75	21.39	25.64	25.49	20.97		22.13		18.78	
Roots:						0	1	0	3	0	9
	24	9.33	10.14	11.68	14.47	9.39	10.90	8.63	11.98	8.59	13.13
	48	8.13	12.13	12.07	17.67	9.43	11.35	9.32	11.91	8.66	13.50
	96	7.19	8.80	11.16	16.31	7.29	10.67	7.16	10.96	7.32	13.33
	120	7.04	9.09	11.17	16.18	6.83	9.80	6.67	11.43	6.43	14.07

*Mean of six plants for (0,0) and mean of two plants for remainder of treatments.

TABLE III

EFFECT OF OSMOTIC PRESSURE ON PERCENT NITROGEN*

Time (Hours)	Osmotic Pressure Treatment (Bars)									
	(0,0)	(1,1)	(3,3)	(9,9)	(0,1)		(0,3)		(0,9)	
Shoots:										
24	3.27	3.13	3.62	2.68	2.94		3.65		3.08	
48	3.31	2.30	2.42	3.24	2.57		2.79		2.84	
96	3.20	2.40	2.23	2.82	2.71		2.80		3.14	
120	3.08	3.17	2.65	3.02	2.58		2.72		2.57	
Roots:					0	1	0	3	0	9
24	2.77	2.84	2.42	1.70	2.63	2.30	3.98	1.98	3.25	2.96
48	3.70	2.12	2.39	2.05	3.48	2.64	3.07	2.25	3.33	2.37
96	3.68	3.01	2.32	2.02	3.45	2.63	3.88	3.38	3.72	2.19
120	4.60	4.50	3.17	2.48	4.65	3.42	4.24	2.76	4.08	2.36

*Mean of six plants for (0,0) and mean of two plants for remainder of treatments.

TABLE IV

EFFECT OF OSMOTIC PRESSURE ON PERCENT PHOSPHORUS*

Time (Hours)	Osmotic Pressure Treatment (Bars)									
	(0,0)	(1,1)	(3,3)	(9,9)	(0,1)		(0,3)		(0,9)	
Shoots:										
24	0.477	0.534	0.461	0.528	0.413		0.450		0.517	
48	0.451	0.330	0.465	0.400	0.328		0.400		0.407	
96	0.468	0.293	0.286	0.348	0.318		0.339		0.380	
120	0.475	0.359	0.274	0.278	0.343		0.367		0.461	
Roots:					0	1	0	3	0	9
24	0.764	0.687	0.629	0.485	0.601	0.476	0.733	0.534	0.809	0.597
48	0.923	0.670	0.572	0.515	0.619	0.444	1.06	0.546	1.09	0.979
96	0.980	0.775	0.765	0.495	0.812	0.514	0.887	0.592	0.829	0.509
120	1.05	1.02	0.680	0.492	1.13	0.695	0.894	0.584	0.954	0.548

*Mean of six plants for (0,0) and mean of two plants for remainder of treatments.

TABLE V

EFFECT OF OSMOTIC PRESSURE ON PERCENT NITRATE*

	Time (Hours)	Osmotic Pressure Treatment (Bars)									
		(0,0)	(1,1)	(3,3)	(9,9)	(0,1)		(0,3)		(0,9)	
Shoots:											
	24	3.80	3.31	3.21	2.72	2.99		3.20		3.84	
	48	4.18	3.81	4.44	4.84	3.51		3.14		3.51	
	96	4.11	4.46	5.17	4.43	4.36		4.81		4.26	
	120	3.99	5.07	4.77	3.51	3.14		3.92		4.50	
Roots:						0	1	0	3	0	9
	24	3.34	1.78	2.95	2.71	3.65	3.60	3.46	1.62	2.57	1.96
	48	3.53	1.51	2.28	1.78	3.07	2.14	1.68	1.71	2.64	2.03
	96	4.55	3.12	2.79	1.83	4.35	3.02	3.82	2.67	3.32	2.72
	120	4.47	3.25	2.64	2.13	4.33	3.23	3.64	2.98	3.84	3.06

*Mean of six plants for (0,0) and mean of two plants for remainder of treatments.

TABLE VI

EFFECT OF OSMOTIC PRESSURE ON THE SOLUBLE CARBOHYDRATE CONTENT EXPRESSED AS MILLIMOLES OF GLUCOSE EQUIVALENTS*

Time (Hours)	Osmotic Pressure Treatment (Bars)									
	(0,0)	(1,1)	(3,3)	(9,9)	(0,1)		(0,3)		(0,9)	
Shoots:										
24	1.80	1.34	1.85	1.20	1.24		1.82		1.99	
48	1.90	1.96	2.31	2.10	2.48		2.17		2.25	
96	1.68	2.02	3.05	2.72	1.39		1.93		2.14	
120	1.41	1.44	2.90	1.83	2.16		1.68		2.12	
Roots:					0	1	0	3	0	9
24	0.733	1.37	1.29	1.32	1.50	1.32	1.45	0.932	1.27	1.16
48	0.342	0.980	0.602	0.682	0.884	0.627	0.591	0.387	0.715	0.372
96	0.217	0.390	0.583	0.734	0.351	0.250	0.270	0.157	0.278	0.235
120	0.123	0.243	0.439	0.581	0.230	0.145	0.199	0.126	0.197	0.112

*Mean of six plants for (0,0) and mean of two plants for remainder of treatments.

TABLE VII

EFFECT OF OSMOTIC PRESSURE ON THE CONTENT OF NINHYDRIN POSITIVE MATERIAL EXPRESSED AS MICROMOLES OF LEUCINE EQUIVALENTS*

Time (Hours)	Osmotic Pressure Treatment (Bars)									
	(0,0)	(1,1)	(3,3)	(9,9)	(0,1)		(0,3)		(0,9)	
Shoots:										
24	382	343	235	213	219		240		289	
48	389	294	217	260	181		380		351	
96	383	292	215	241	329		390		360	
120	436	330	238	285	357		455		356	
Roots:					0	1	0	3	0	9
24	401	439	424	346	339	384	348	211	280	297
48	522	331	313	217	233	273	581	375	643	424
96	650	333	371	307	368	388	576	382	324	394
120	609	474	387	273	606	407	599	344	614	213

*Mean of six plants for (0,0) and mean of two plants for remainder of treatments.

TABLE VIII

EFFECT OF OSMOTIC PRESSURE ON OXYGEN UPTAKE OF ROOTS: MICROLITERS PER HOUR PER GRAM DRY WEIGHT*

	Time (Hours)	Osmotic Pressure Treatment (Bars)									
		(0,0)	(1,1)	(3,3)	(9,9)	(0,1)		(0,3)		(0,9)	
						0	1	0	3	0	9
Roots:											
	24	38	39	34	40	31	38	35	40	34	28
	48	28	33	38	35	27	34	33	32	39	34
	96	22	33	31	23	21	42	28	27	29	22
	120	23	29	37	31	13	24	23	25	25	28

*Mean of six plants for (0,0) and mean of two plants for remainder of treatments.

TABLE IX

CALCULATED LEAST SIGNIFICANT DIFFERENCES AT THE 95% LEVEL OF CONFIDENCE FOR COMPARISON OF MEANS

	Comparison With Control	Comparison of Two Other Means	Comparison of Means of Different Plants	Comparison of Means of Two Roots of Same Plant
Shoots:				
Relative Turgidity	2.1	2.6		
Percent Dry Matter	2.3	2.8		
Percent Nitrogen	0.75	0.80		
Percent Phosphorus	0.11	0.14		
Percent Nitrate	1.2	1.5		
Carbohydrate	1.1	1.4		
Ninhydrin Positive	90	110		
Roots:				
Percent Dry Matter	1.2		1.5	1.8
Percent Nitrogen	1.0		1.2	1.2
Percent Phosphorus	0.21		0.26	0.26
Percent Nitrate	1.1		1.3	1.2
Carbohydrate	0.37		0.46	0.40
Ninhydrin Positive	147		180	159

VITA

Raymond E. Meyer

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF OSMOTIC STRESS ON CERTAIN METABOLIC COMPONENTS OF WHEAT

Major Field: Soil Science

Biographical:

Personal Data: Born at Okarche, Oklahoma, Februaray 21, 1938, the son of William H. and Ann M. Meyer.

Education: Attended grade school in Okarche, Oklahoma, and Girard, Kansas: graduated from Girard High School in 1955; received the Bachelor of Science degree from Kansas State University, with a major in Soils, in June, 1959; completed the requirements for the Doctor of Philosophy degree in May, 1963.

Experience: Reared on farms at Okarche, Oklahoma, and Girard, Kansas; research in Departments of Botany and Agronomy summers of 1960 and 1961; National Defense Education Act Fellow, 1959 to 1962.

Member of: Sigma Xi, American Society of Agronomy, Soil Science Society of America.