CATALASE ACTIVITY AND OXALIC ACID CONTENT IN TWO SALT-STRESSED SPECIES OF ATRIPLEX

by

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CHAPTER I

INTRODUCTION

Salinity is an increasingly important problem in agriculture, especially in arid and semi-arid regions. In these areas, natural leaching by percolating rainfall does not remove excess soluble salts from the root zone. Improper irrigation techniques, using too much or too little water, may also lead to an increase in soil salinity (Hayward and Wadleigh, 1949; Slatyer, 1967). Saline conditions prevail in areas that, because of long growing seasons, high temperatures, high light intensities, and fertile soils, are otherwise well suited to crop production (LaHaye and Epstein, 1969). The increasing need to use water of poor quality, the continuous addition of waste salts to the environment, and the increasing contamination of water sources all lead to gradual soil salinization (Waisel, 1972). Since 95% of the world water sources are saline, salinity is a potential, if not now limiting, factor everywhere. For these reasons, Waisel (1972) states, "The future of plants lies with some group of halophytes." It is therefore urgent that the physiology of salt tolerance be studied.

Plants of the genus Atriplex are both high in protein content and remarkably salt tolerant. As early as 1899, Jaffa proposed that Atriplex species be used as forage plants where plants such as alfalfa

would not grow. In an FAO study, done in 1971 by the Tunisian Institut de Reboisement, it was shown that some species of Atriplex can be grown in levels of salt exceeding that of seawater. They found the protein content to be 15-25%, of which 10-15% of the total protein is lysine, more than twice the lysine found in oats (Franclet and Le Houérou, 1971). For these reasons, Atriplex species are important as forage plants in dry and saline areas (Williams, O.B., 1960, cited by Mozafar, 1969), but high levels of oxalic acid, an organic acid toxic to animals, have proven to be a major problem in their use.

In addition to their value as forage plants, Atriplex species are of interest to plant physiologists. Atriplex has been used extensively to study ionic balance and salt tolerance. Some species of Atriplex are "inefficient" or C_3 plants, fixing carbon into 3-carbon compounds by the Calvin cycle and having photorespiration. Other Atriplex species are C_4 or "efficient" plants, without photorespiration (Johnson and Hatch, 1968; Osmond, Troughton, and Goodchild, 1969; Welkie and Caldwell, 1970). The study of Atriplex thus provides the opportunity for comparing, in two closely related species, the features and relative merits of C_3 and C_4 photosynthetic pathways and photorespiration (Milthorpe, 1970).

Two salt tolerant species of Atriplex were used in this study: Atriplex halimus, a perennial Mediterranean shrub (Franclet and Le Houérou, 1971), and A. hortensis, an annual which was introduced from Europe as a garden herb (Nobs and Hagar, 1973-74). A. halimus is a C_4 plant, and A. hortensis is a C_3 plant (Welkie and Caldwell, 1970). Experiments were designed to subject plants of each species to different

levels of salinity and measure the relation of salinity to oxalic acid production and to catalase activity. Since catalase is involved in oxalic acid production, and since levels of oxalic acid have a great effect on the use of Atriplex, it was hypothesized that there might be a correlation between levels of catalase activity and levels of oxalic acid in species of Atriplex.

CHAPTER II

LITERATURE REVIEW

Salt Tolerance

The responses of plants grown in saline soils vary enormously. The limit of salt tolerance for a given species is an intangible concept since there is no abrupt concentration at which death occurs (Eaton, 1942). Tolerance may be measured as (1) the ability of a plant to survive on saline soils, (2) the yield on saline soils as compared to the yield of other crops on saline soils, or (3) the relative performance on saline and non-saline soils (Hayward and Wadleigh, 1949). All crops do not react in the same way to salt, and factors such as high temperatures may reduce the salt tolerance of some plants (Magistad et al., 1943; Hayward and Bernstein, 1958). Nieman (1962), in his study on the effect of salt on 12 crop plants, reported responses varying from death of the plant to increased fresh weight.

The effects of salinity may vary depending on the stage of development of the plant. There is no correlation between salt tolerance at germination and in later growth and development (Hayward and Wadleigh, 1949). Sometimes seeds will not germinate on saline sites which will support established crops of the same species; for example, seeds from the genus Atriplex are more salt-sensitive during germination than other plants and less salt-sensitive after germination (Bernstein and Hayward, 1958). Beadle (1952) found that chloride ions inhibit the germination of Atriplex and hypothesized that this might be a mechanism which

prevents germination during periods when there is too little moisture for successful establishment of plants.

Salts in saline soils are composed of a mixture of chloride, sulphate, sodium, magnesium, and calcium ions, with sodium chloride being the principal salt form (Williams, M., 1960; Waisel, 1972). Salts may affect plants in two ways: (1) osmotically, i.e., low osmotic potential may limit the water available to the plant; and (2) by the toxicity of different ions in the media. Several researchers have found that the total salt in the media seems to be a more important factor than ionic composition, or kinds of salt (Magistad et al., 1943; Bernstein and Hayward, 1958; Nieman, 1962). Others have found differing reactions with different salts. Increasing strengths of Hoagland's solution reduce the dry weight of Atriplex halimus more than KCl or NaCl at the same external osmotic potential (Mozafar, 1969). Calcium ions may protect plants from sodium or magnesium damage (Hayward and Wadleigh, 1949; LaHaye and Epstein, 1969), while calcium chloride decreases growth unless potassium or magnesium are also increased (Greenway, 1968).

For a plant to be salt tolerant, it must have (1) the ability to increase the osmotic concentration of tissue fluids, (2) the capacity to regulate the intake of ions, and (3) the ability to resist the deleterious effects of accumulated ions (Hayward and Wadleigh, 1949; van den Berg, 1952, cited by Slatyer). Plants may extrude harmful accumulated excess salts by special salt glands or to vesiculated hairs. Other plants may release excess salts through the cuticle, in the guttation fluid, or by retransport via the phloem back to the roots

(Waisel, 1972).

Some plants balance cation uptake with organic acid production as well as by chloride absorption. Osmond (1963) found an inverse relationship between chloride tolerance and organic acid production. He postulated that the production of organic acids may be important in maintaining ionic balance.

Oxalic Acid Production

Oxalic acid was found to be produced in response to an increase in soil salinity and excess cation uptake (Osmond, 1963). The sodium and potassium content of Atriplex inflata and A. spongiosa was found to be largely balanced by oxalate production, but there was no relation between calcium concentration and oxalate production (Osmond, 1967). Osmond postulated that the role of oxalate in ion balance in Atriplex is similar to that of malate in many plants. He also found that oxalate production was severely depressed at reduced light intensities, which would indicate that oxalate is produced directly from some photosynthetic product (Osmond, 1963).

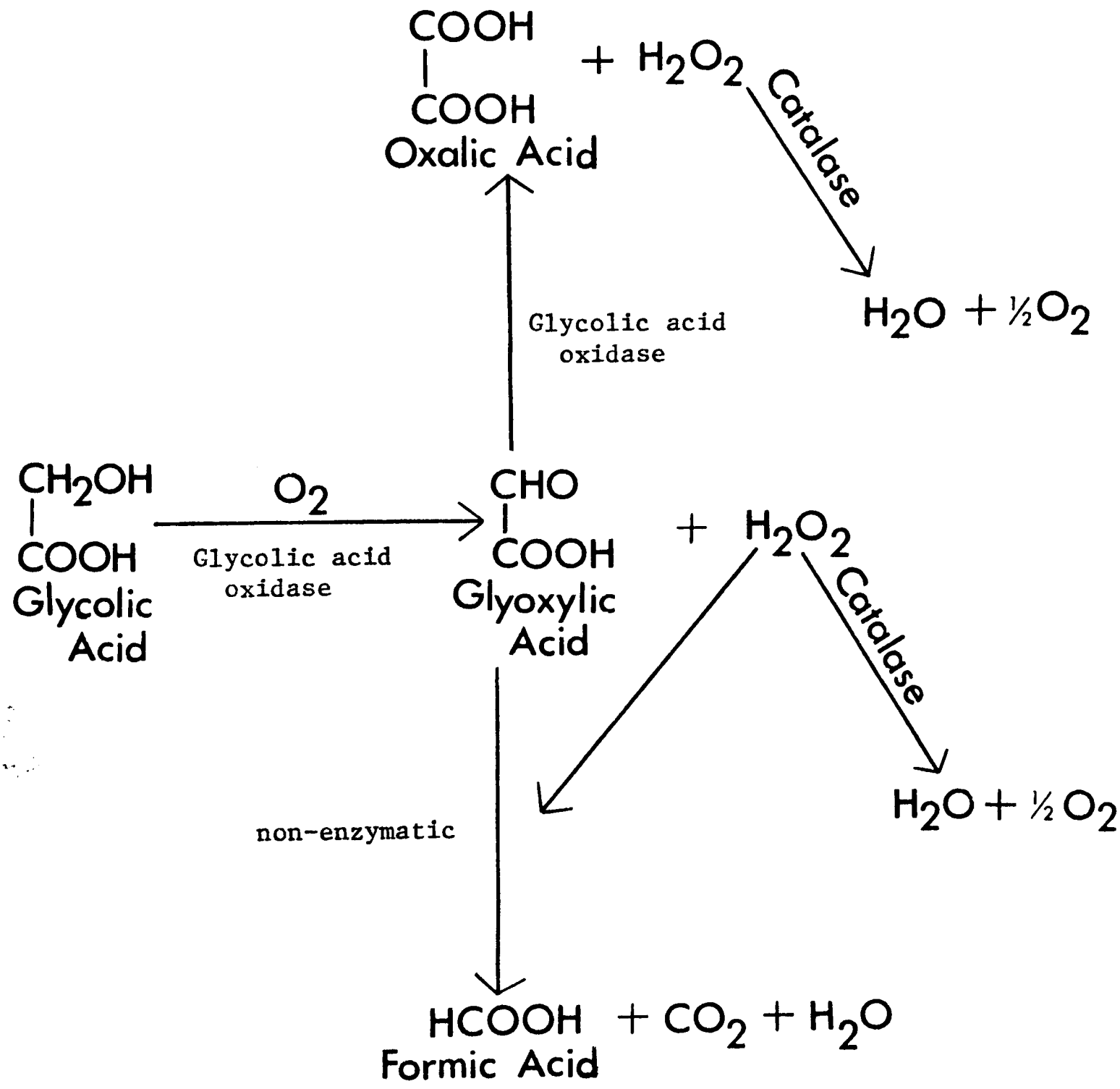
Williams (1960), approaching the problem from the other direction, reported that cations are needed with large amounts of oxalate to maintain the cation-anion balance. In his studies with Atriplex vesicaria, he found that soluble oxalates usually occur as sodium salts. He reported that plants supplemented with sodium chloride were both markedly larger and contained more oxalates than plants not so supplemented. He concluded that sodium appears to be necessary for the nutrition of this species and that it is used primarily to form salts of oxalic acid.

Osmond and Avadhani (1968) used radioactive tracers to demonstrate that, in Atriplex, oxalic acid is produced via the glyoxylate cycle. Labeled glyoxylate fed to A. spongiosa leaves produced a higher proportion of labeled oxalate than other substrates, and this level of oxalate steadily increased during the experiment, even if levels of other products declined. Kenten and Mann (1952) showed that in a Nicotiana tabacum extract, hydrogen peroxide is formed during oxidations of glycolic, lactic, and glyoxylic acids. In the presence of catalase, glycolate is oxidized to glyoxylate, which is further oxidized to oxalate. In the absence of catalase, the hydrogen peroxide formed reacts non-enzymatically with glyoxylate to give formate and carbon dioxide (Figure 1). Glycolate biosynthesis occurs in the chloroplast, and glycolate is oxidized to glyoxylate in the peroxisome (Kisaki and Tolbert, 1969). Since part of this sequence takes place in the peroxisome, which always contains catalase, it is unlikely that formic acid would ever accumulate in appreciable quantities.

Catalase

Catalase is a heme enzyme with four iron atoms per molecule attached to protein and chelated to protoporphyrin IX (Scandalios, 1969). The catalytic center is at the iron atoms, where the Fe^{+2} performs the function of breaking a 2-electron oxidation into two energetically easier steps. These intermediate steps are easier than with Fe^{+2} alone (Saunders et al., 1964). Catalase seems to be present in all organisms (Saunders et al., 1964; Lehninger, 1970).

Figure 1. Pathway of oxalate synthesis.


COOH
COOH
Oxalic Acid
+ H2O2
Catalase
H2O + ½O2
Glycolic acid oxidase
CH2OH
COOH
Glycolic Acid
O2
Glycolic acid oxidase
CHO
COOH
Glyoxylic Acid
+ H2O2
Catalase
H2O + ½ O2
non-enzymatic
HCOOH + CO2 + H2O
Formic Acid

Despite the universal presence of this enzyme, its exact biochemical role or roles are not known with certainty (Lehninger, 1970). Roles other than the decomposition of hydrogen peroxide have been suggested as functions of this enzyme. Catalase can also be used to catalyze coupled oxidations of several alcohols to aldehydes using the H_2O_2 formed in a primary oxidation (Keilin and Hartree, 1945).

Catalase in maize exists as a tetramer, and freezing dissociates the units which may recombine to produce hybrid forms (Scandalios, 1965). Six distinct electrophoretic variants (isozymes) of catalase exist in maize endosperm, while in other tissues of maize the presence and concentration of these six isozymes vary (Scandalios, 1969). Scandalios (1969) suggested that the distribution of the catalase isozymes might give clues as to their function. He also suggested that the catalase system offers good possibilities for the study of developmental genetics in higher organisms.

Most of the catalase in leaves is located in the peroxisomes and not in the chloroplast (Tolbert et al., 1968, 1969), although peroxisomes are often found appressed to the chloroplasts (Frederick and Newcomb, 1969a,b; Kelley, 1970). C_3 plants have more peroxisomes than C_4 plants (Frederick and Newcomb, 1969a,b).

Salt Tolerance of Enzymes

Salinity affects the development and metabolism of plants in different ways. The number of mitochondria in developing leaves increases in plants treated with sodium chloride (Siew and Klein, 1968). Sodium chloride increases oxidative phosphorylation in leaves, which

may lead to changes in the ADP:ATP ratio and thus affect regulation of metabolic pathways (Livne and Levin, 1967). Sodium causes increased ATP synthesis, perhaps as a result of energy-free sodium transport into the vacuole (Jennings, 1968).

The effects of salinity on specific enzymes vary. Weimberg (1970) studied enzyme levels in pea seedlings grown on saline media and found that specific activities of enzymes were the same in saline and non-saline plants. He found that the electrophoretic pattern of malate dehydrogenase was not changed by salt. For peroxidase, the electrophoretic pattern was altered--two of the five isozymes migrated more slowly.

Flowers (1972a,b) compared the effect of sodium chloride on enzyme activity in halophytes and non-halophytes. Enzyme activity was, in general, affected by sodium chloride equally in salt-tolerant and salt-sensitive species. The effect of NaCl varied with pH; for example, with peroxidase there was a severe depression of activity at low pH values and no significant effect at a higher pH. Flowers (1972a) concluded that salt tolerance in the higher plants involves a spatial separation in the plant cell such that the enzymes are not subjected to high concentrations of salt in vivo. Greenway and Osmond (1972) found no difference in enzyme response to salts with enzymes from very salt-sensitive and very salt-tolerant plants, although each enzyme studied showed a different pattern of sensitivity to salt. Activity of malate dehydrogenase and glucose-6-phosphate dehydrogenase was stimulated by low concentrations of NaCl. High concentrations of NaCl were invariably

inhibitory. Aspartate transaminase activity was depressed by salt, even with low concentrations.

These results conflict with those of Porath and Poljakoff-Mayber (1964), who found that salt stimulated glucose-6-phosphate dehydrogenase activity and reduced malic dehydrogenase activity. There was a difference in the effects of Na_2SO_4 and NaCl, which indicates that the effect of salinity on respiration is not purely osmotic, but of a more complicated nature. Kalir and Poljakoff-Mayber (1975) found that low concentrations of NaCl stimulated malic dehydrogenase activity, and higher concentrations (above 0.24 M) depressed activity. They postulated that NaCl induced conformational changes in the enzyme.

Reports on the response of catalase to salinity are contradictory. Pokrovskaia (1957, cited by Mozafar, 1969) reported that catalase activity increased in all plants as the result of salt treatment. Mozafar (1969) found that initial catalase activity was reduced as a result of saline culture solutions, but after aging for two hours, catalase activity increased. Mozafar hypothesized that catalase in saline-treated plants is bound to some membrane or temporarily inactivated.

Atriplex halimus and A. hortensis

Atriplex halimus is a Mediterranean shrub which has been called "ultraresistant" to salinity (Grillot, 1954). Atriplex hortensis is a European species which has been introduced into the western United States and now grows abundantly in salt marshes of the San Francisco Bay (Nobs and Hagar, 1974). Black (1960) reported that Atriplex species have a high degree of selectivity for the sodium ion, and he

thought sodium uptake to be osmoregulatory rather than nutritional.

An element may be considered essential if (1) a deficiency of it makes it impossible for the plant to complete its life cycle; (2) a deficiency is specific to the element in question and can be prevented only with this element; and (3) the element is directly involved in the nutrition of the plant apart from its effects in correcting some unfavorable condition of the environment (Arnon, 1939). On the basis of these requirements, sodium is an essential micronutrient for some species of Atriplex (Brownell and Wood, 1957; Brownell, 1965). When all sodium (even atmospheric) is carefully excluded from Atriplex vesicaria, death of the plant occurs in 35 days. Brownell (1968) found that only Australian species of Atriplex required sodium for survival. Other species of Atriplex gave positive responses to sodium but did not develop deficiency symptoms without salt. Card, Mahall, and Troughton (1974) found that foreshore dune halophytes responded to salt but that salt marsh halophytes did not. Several researchers have reported a positive response of Atriplex halimus to sodium (Blumenthal-Goldschmidt and Poljakoff-Mayber, 1968; Mozafar, 1969; Gale and Poljakoff-Mayber, 1970; Gale, Naaman, and Poljakoff-Mayber, 1970; Kaplan and Gale, 1972). A. halimus requires 4 atm. NaCl (about 0.1 M) for optimal growth (Blumenthal-Goldschmidt and Poljakoff-Mayber, 1968). A. hortensis also shows more rapid growth when supplied with a sodium salt (Brownell, 1968).

The ability of plants to absorb high levels of salts may be due to two processes of cation uptake: one metabolically dependent process operative at low concentrations, and one "luxury" process, perhaps

passive, operative at high concentrations (Osmond, 1966). Salt tolerance in Atriplex may be partly a result of the high protein and low non-protein nitrogen content: the proteins may bond with the ions in the plant cells, thus reducing the osmolarity and overall effectiveness of the ions (Chatterton, Goodin, and Duncan, 1971).

In non-halophytes, high humidity appears to reduce growth inhibition in salt-treated plants (O'Leary, 1971; Nieman and Poulson, 1967). Gale, Naaman, and Poljakoff-Mayber (1970) studied the effect of humidity and salinity on A. halimus. They found that in dry air A. halimus grew better in 0.12 M NaCl solutions. In conditions of high humidity, growth was greatest in the non-saline controls and decreased as salinity increased. NaCl appears to improve the water balance of A. halimus by reducing the water loss from the leaves in a manner which only slightly reduces the rate of photosynthesis per unit leaf area (Kaplan and Gale, 1972). There is a decrease in the water requirement of Atriplex when treated with salt (Bernstein and Hayward, 1958).

In Atriplex plants, excess salts are secreted to vesicular bladders against a concentration gradient. By accumulating salt from the leaf tissue in epidermal bladders, a build-up of high salinity in the mesophyll cells is avoided (Osmond et al., 1969; Mozafar and Goodin, 1970; Pallaghy, 1970; Greenway and Osmond, 1972). This system is similar to salt glands found in other halophytes (Lüttge, 1971). At high temperatures, these bladders collapse and a highly reflective salt crust forms on the leaves (Osmond et al., 1969). This crust may reflect nearly 60% of the heat at high temperatures (Mooney, Björkman, and Troughton, 1974). The vesiculated hairs continue developing and collapsing throughout the life of the plant (Black, 1954).

CHAPTER III

MATERIALS AND METHODS

Germination, Growth, and Treatments of Plants

Seeds of Atriplex halimus and A. hortensis were germinated on a cheesecloth-covered wire screen set in an aluminum tray containing 0.1 mM KH_2PO_4, 0.1 mM $MgSO_4$, and 0.1 mM $Ca(NO_3)_2$. As the solution evaporated, distilled water was added to keep the level of the solution at the screen. When the seedlings had reached a height of 2-3 inches (10-14 days after germination for A. halimus, 7-10 days for A. hortensis), seedlings were transferred to full-strength Hoagland's solution (Hoagland and Arnon, 1950) in 250 ml Erlenmeyer flasks wrapped in aluminum foil to retard algal growth. The hypocotl of each seedling was wrapped in non-absorbent cotton to hold the seedling in place in the flask. Sodium chloride was added to the solutions gradually, in increments of 0.1 M NaCl every three days. Final levels of NaCl included 0 (the control), 0.1, 0.2, 0.3, 0.4, and 0.5 M, giving six different treatments for each of the two species used. All solutions were changed weekly or more often to maintain the concentration of NaCl and nutrients. Between solution changes, distilled water was added as needed to maintain the level of solution in the flask.

Plants were kept in a growth chamber at 25-26° C with 16 hours of light per day for 3-4 weeks. As plants outgrew the growth chamber, they were transferred to a constant light, constant temperature (26° C)

room. In each experiment plants aged 6-9 weeks were used. An effort was made to use leaves of approximately the same size and age in all experiments. After three months or as leaf material was used in an experiment, plants were weighed, dried for 48 hours in an oven at 80° C, and reweighed. The weight of leaf tissue used in experiments was added to the weight of the rest of the plant. Fresh and dry weights for leaves, whole shoot, and root of each plant were recorded.

Catalase Determinations

Two methods were used to determine catalase activity. In the first, catalase activity was measured by measuring O_2 produced from decomposition of H_2O_2 (Machlis and Torrey, 1956). Three grams of fresh plant material were ground in 15 ml distilled water in a mortar. The suspension was allowed to settle and was decanted into a small beaker set in an ice bath. One ml of this enzyme solution was then added to a 25 ml test tube containing 7 ml Sorenson's phosphate buffer at pH's ranging from 6 to 8. Inside the large test tube, a 10 ml test tube containing 5 ml of 3% H_2O_2 was placed. For measurement of oxygen released from the hydrogen peroxide, the large test tube was closed with a one-holed rubber stopper with a glass tube outlet. Rubber tubing ran from this outlet to a 10 ml graduated cylinder filled with water and inverted in a water-filled beaker. At zero time the test tubes were inverted to mix the enzyme-buffer solution and the hydrogen peroxide. The tubes were shaken gently for 4 minutes, and the volume of gas in the graduated cylinder was recorded. The procedure was repeated for each salt treatment using buffers with pH values of 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2,

7.4, 7.6, 7.8, and 8.0. The enzyme solution was kept on an ice bath, and after four hours activity at pH 7.0 was remeasured.

In the second method, catalase activity was measured spectrophotometrically by observing the breakdown of H_2O_2 by measuring the decrease in light absorption of peroxide solutions in the ultraviolet region. In this region oxygen, water, and catalase do not absorb light. Beers and Sizer (1952), who first described this method, called it the first really quantitative method for measurement of catalase activity. In these determinations, a modification of this technique, as described by Lück (1965), was used.

Enzyme solutions were prepared by homogenizing a known fresh weight of leaf tissue (1.5-3.0 g) with 2-3 ml phosphate buffer (pH 7.0) in a glass homogenizer. The homogenate was centrifuged for 10 minutes at 3000 x g and the supernatant was used in the assay. Peroxide buffer solutions were prepared by diluting 0.08 ml 30% H_2O_2 to 50 ml with phosphate buffer. Fresh H_2O_2-buffer solutions were prepared each day, using buffers of pH 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, and 8.0. The disappearance of H_2O_2 was measured on a Beckman DB Ultraviolet Spectrophotometer at 240 nm. Enzyme solution was added with a microsyringe to 3 ml of the H_2O_2-buffer solution, and the time required for an increase in per cent transmission from 35 to 40% was recorded. Measurements were read against a control cuvette containing enzyme solution and H_2O_2-free buffer. Catalase activity in each treatment for both species of Atriplex was measured at intervals of 0.2 pH units over a pH range of 6.0 to 8.0. Activity of catalase was calculated by

the following equation:

$$\text{catalase units} = \frac{321 \times v}{t \times ml \times g};$$

where 321 is a constant (Lück, 1965),

v = volume of water in the homogenate (ml),

t = time in seconds,

ml = ml enzyme solution added, and

g = g fresh weight tissue in the homogenate.

One unit is the amount of enzyme which liberates half the peroxide oxygen from a hydrogen peroxide solution of any concentration in 100 seconds at 25° C. Leaf tissue was taken and the assays were made between 11:00 A.M. and 2:00 P.M. over a period of 7 days.

In a second part of the experiment, enzyme solutions were kept in an ice bath for 3-4 hours and then catalase activity was remeasured.

In a third part of the experiment, 0.1 M NaCl was added to the H_2O_2-buffer solutions, and catalase activity in the control and 0.2 M NaCl treatments for both Atriplex halimus and A. hortensis was measured over a pH range of 6.0 to 8.0.

Oxalic Acid Determinations

The oxalate in the leaves of A. halimus and A. hortensis was determined according to Palmer's modification (1955) of the method described by Baker (1952). Twenty-five 1 cm leaf discs were dried for 24 hours in an oven at 80° C. The discs were ground to a powder in a mortar and placed in a 25 ml test tube to which was added 5 ml of 1 N HCl. The tube was then placed in a boiling water bath, and the oxalate extracted

for 30 minutes. The tube was cooled, and the solution transferred to a 10 ml volumetric flask and made to volume with 1 N HCl. The mixture was then shaken and left to stand overnight. Insoluble material was removed by filtration through dry Whatman No. 1 filter paper, which gave a clear yellow extract. A 5 ml aliquot was pipetted into a 15 ml tapered centrifuge tube and 5 ml phosphotungstate solution (40 ml syrupy phosphoric acid added to 24 g sodium tungstate dissolved in water and diluted to 1 liter) was added as a deproteinizing agent. After 6 hours the tubes were centrifuged at 1500 x g for 10 minutes, and a 5 ml aliquot of the supernatant was transferred to a 15 ml tapered centrifuge tube for precipitation of the calcium oxalate. A 6 N ammonium hydroxide solution was added to the solution drop by drop until a small quantity of phosphotungstate had precipitated. Three ml calcium chloride-acetate buffer solution (25 g anhydrous $CaCl_2$ dissolved in 500 ml 50% [v/v] acetic acid, and added to 330 g sodium acetate trihydrate dissolved in water to make 500 ml) were then added. The pH of this buffer is 4.5, at which oxalic acid produces an insoluble Ca^{++} salt, but no other organic acids produce insoluble salts. The tube was then shaken and refrigerated for 24 hours to allow the calcium oxalate crystals to form. The precipitate was centrifuged at 1500 x g for 10 minutes, the supernatant decanted, and the sediment washed with 5 ml of 5% acetic acid saturated with calcium oxalate (to prevent loss of the salt by solution in the acetic acid). This solution was centrifuged at 1500 x g for 10 minutes and the supernatant decanted. The calcium oxalate was dissolved in 5 ml of 4 N H_2SO_4, heated in a boiling water bath, and titrated hot with 0.01 N potassium permanganate to a faint pink end-point to determine the meq of oxalate present.

CHAPTER IV

RESULTS AND DISCUSSION

Effect of NaCl on the Growth of A. halimus and A. hortensis

For both Atriplex halimus and A. hortensis, fresh weights of the whole plant were greatest in plants grown in 0.1 M NaCl. A. hortensis plants grown in 0.2 M NaCl were larger than those grown without salt. At nigher concentrations of NaCl (0.3, 0.4, and 0.5 M), plants decreased in size and total dry weight as the concentration of salt increased (Figures 2 and 3). At 0.4 and 0.5 M NaCl, plants of both species were stunted and yellow.

Plants of the same age were used in each experiment, but plants could not be placed directly in solutions of high NaCl concentration. The NaCl concentration of the solutions was slowly increased in 0.1 M units every three days. This meant that plants in the 0.1 M NaCl treatment reached their final level of NaCl 12 days before plants in the 0.5 M treatment were brought to their final NaCl concentration.

When shoot and root growth were measured separately, the results were somewhat different. Shoot growth followed overall growth; i.e., tops of A. hortensis plants were larger in the 0.1 and 0.2 M NaCl treatments than in the controls, and tops of A. halimus plants grown in 0.1 M NaCl were larger than the controls, whereas higher concentrations of NaCl retarded growth in both species (Figures 4 and 5). Fresh weights of roots were obtained by drying the roots with a paper towel. Since

Figure 2. Fresh and dry weights of A. hortensis (whole plant). Mean of 4-6 plants for each treatment (f.w.); 6-9 plants for each treatment (d.w.).

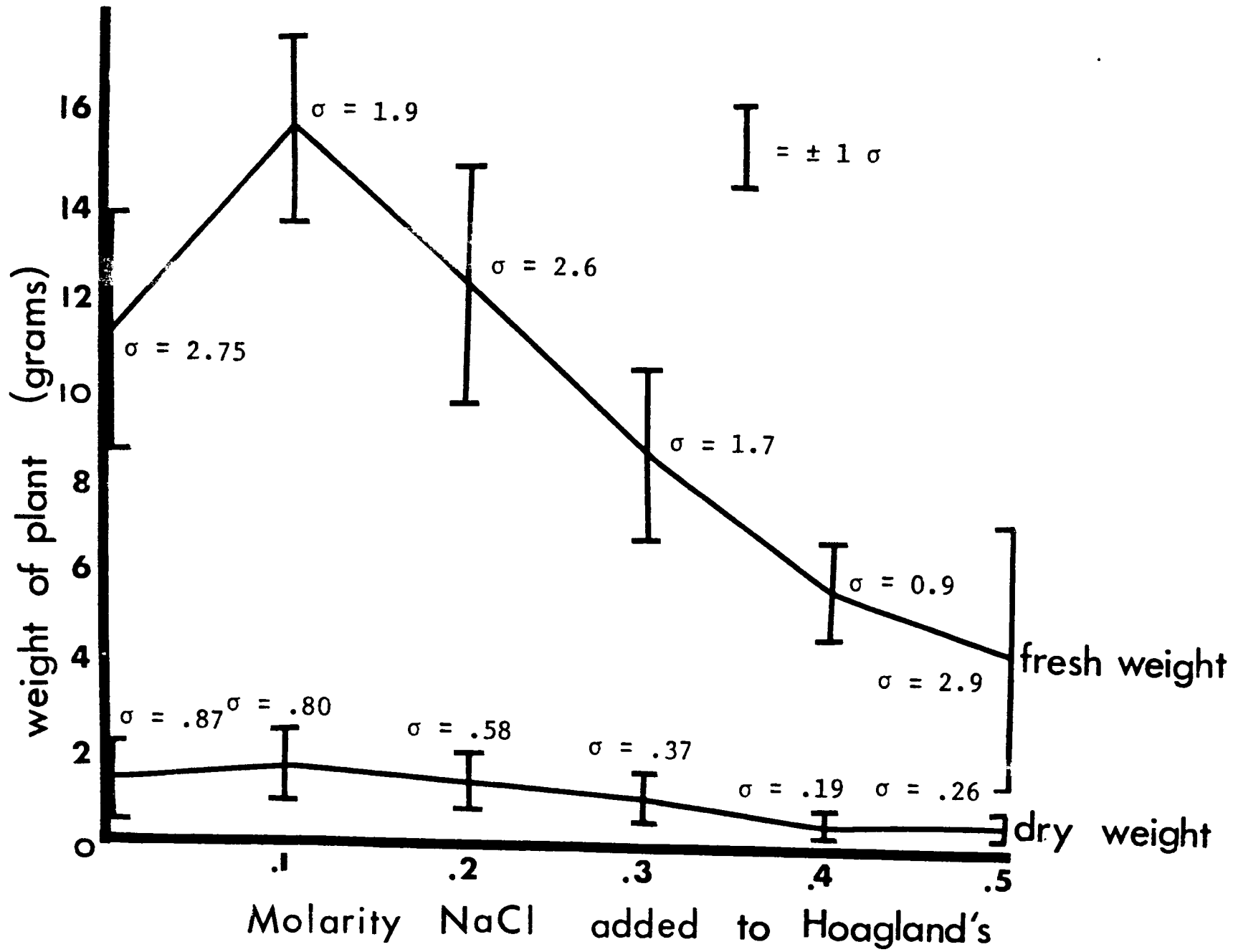

16
14
12
10
8
6
4
2
0
weight of plant (grams)
σ = 1.9
σ = 2.6
σ = 1.7
σ = 0.9
σ = 2.9
σ = 2.75
σ = .87
σ = .80
σ = .58
σ = .37
σ = .19
σ = .26
= ± 1 σ
fresh weight
dry weight
.1
.2
.3
.4
.5
Molarity NaCl added to Hoagland's

Figure 3. Fresh and dry weights of A. halimus (whole plant). Mean of 3-6 plants for each treatment (f.w.); 6-9 plants for each treatment (d.w.).

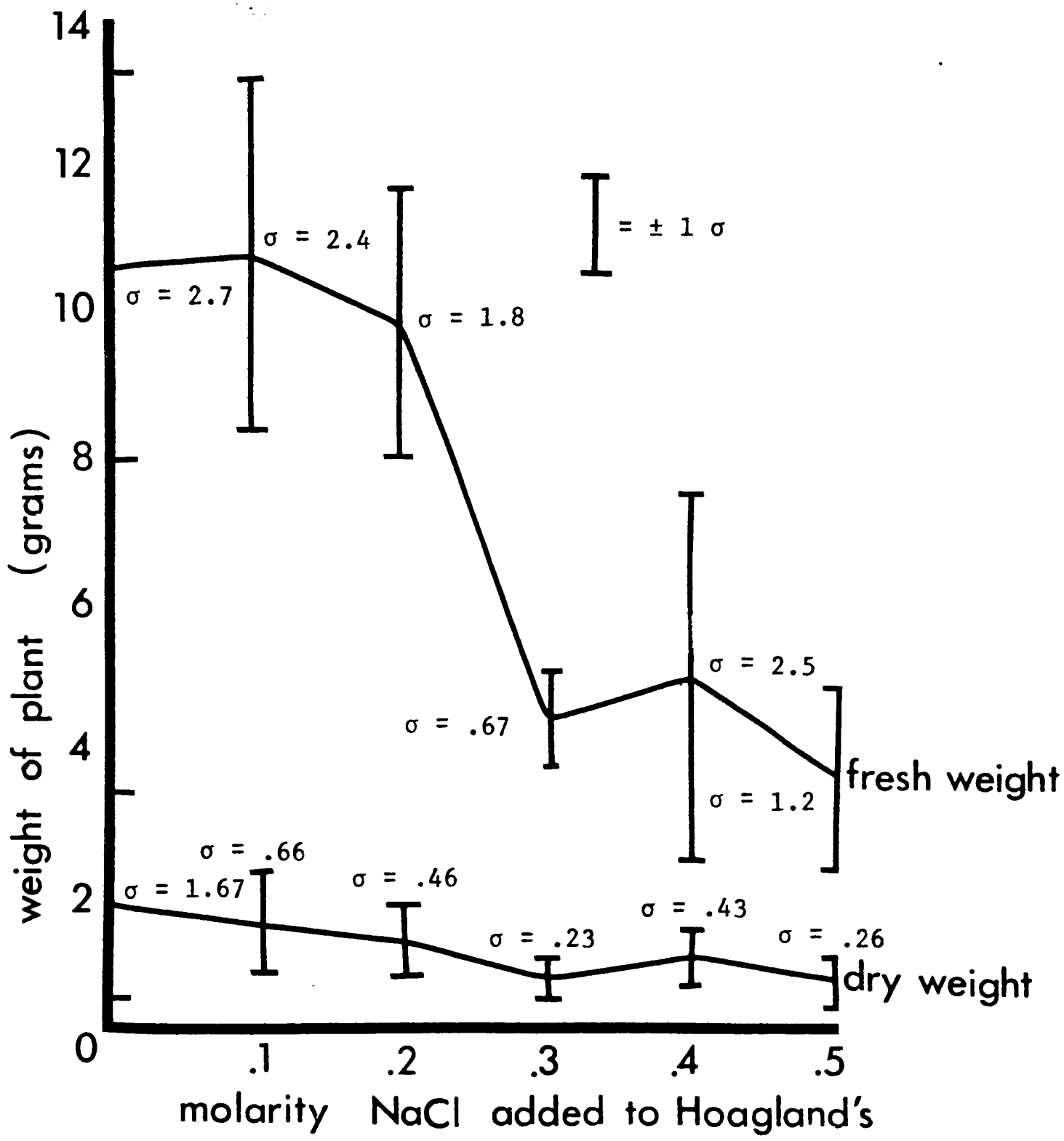
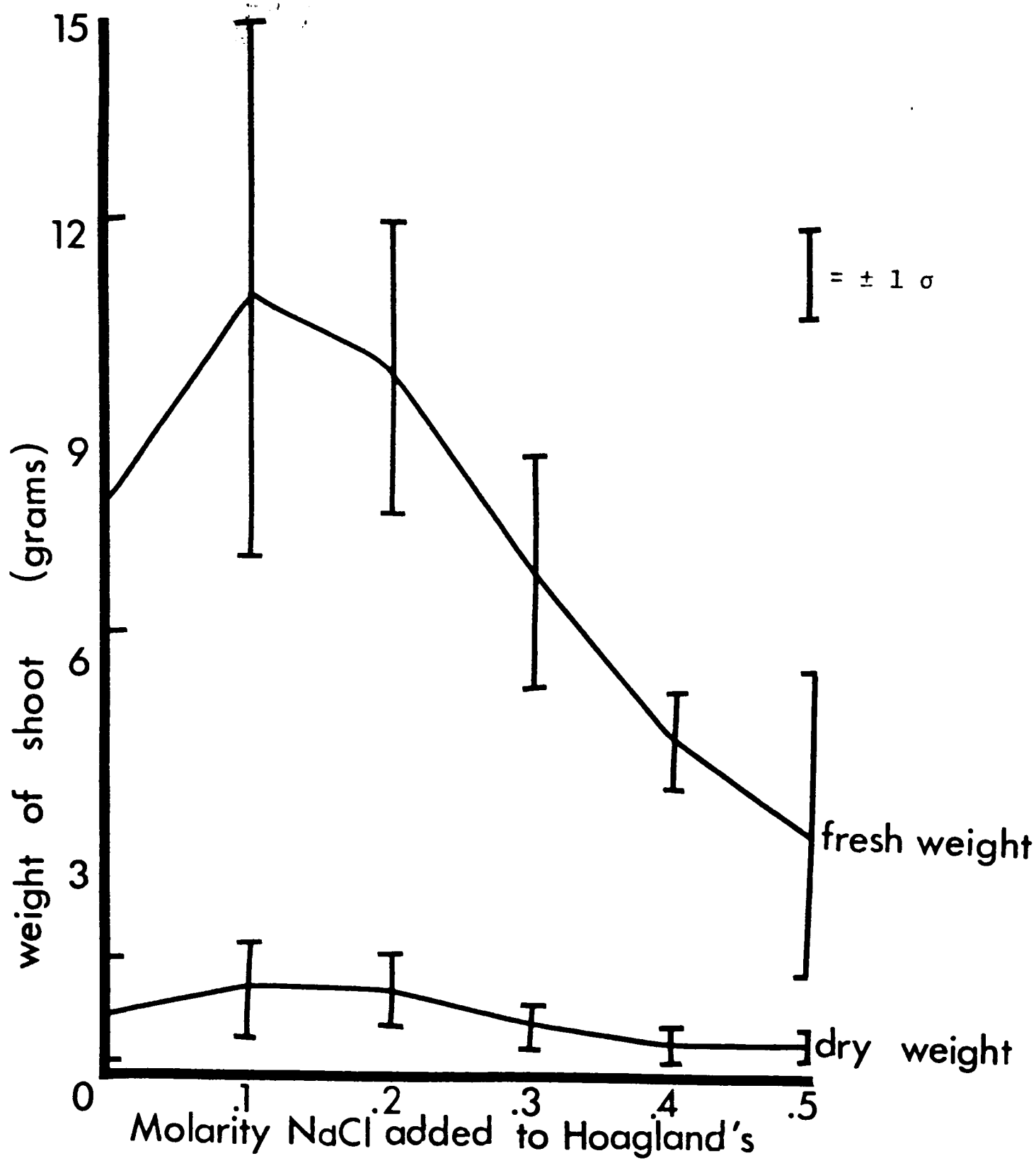

14
12
10
8
6
4
2
0
weight of plant (grams)
σ = 2.4
σ = 2.7
σ = 1.8
= ± 1 σ
σ = .67
σ = 2.5
σ = 1.2
fresh weight
σ = .66
σ = 1.67
σ = .46
σ = .23
σ = .43
σ = .26
dry weight
.1
.2
.3
.4
.5
molarity NaCl added to Hoagland's

Figure 4. Fresh and dry weights of A. hortensis shoots.
Mean of 3-6 shoots for each treatment (f.w.); 6-9 shoots for each treatment (d.w.).


15
12
9
6
3
0
weight of shoot (grams)
= ± 1 σ
fresh weight
dry weight
.1
.2
.3
.4
.5
Molarity NaCl added to Hoagland's

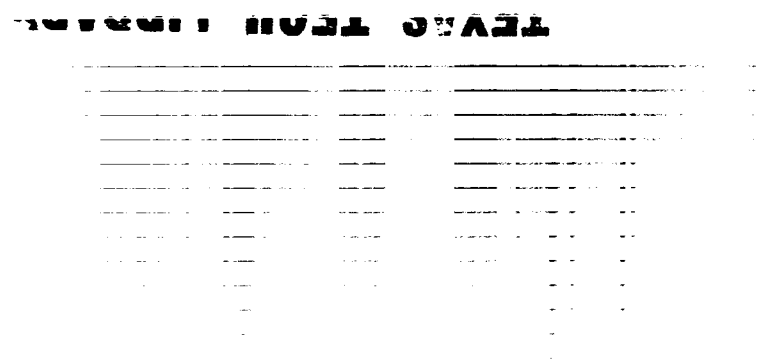
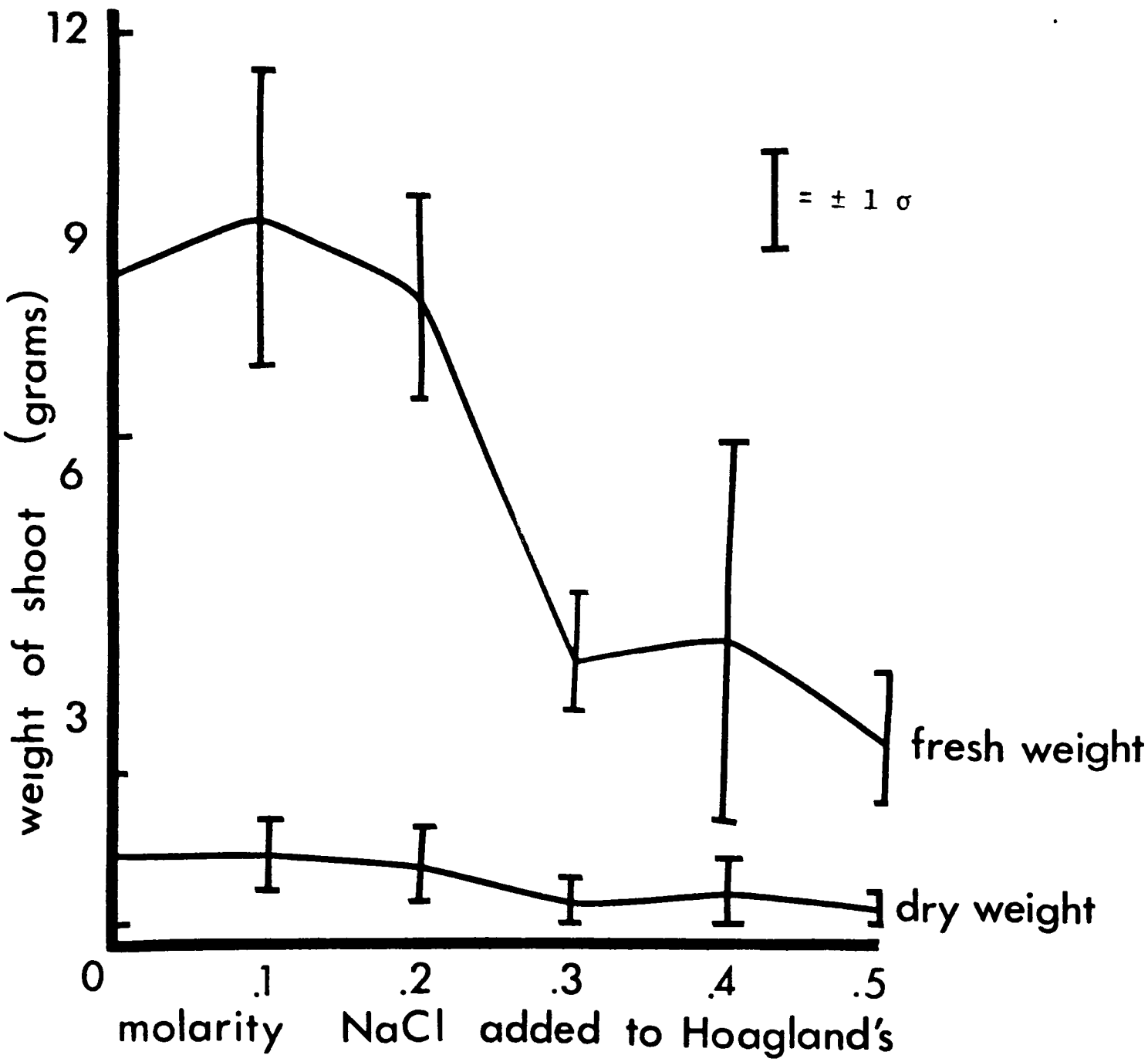


Figure 5. Fresh and dry weights of A. halimus shoots. Mean of 3-5 shoots for each treatment (f.w.); 5-8 shoots for each treatment (d.w.).


12
9
6
3
0
weight of shoot (grams)
= ± 1 σ
fresh weight
dry weight
.1
.2
.3
.4
.5
molarity NaCl added to Hoagland's

it was difficult to dry the roots completely for fresh weight determinations without dehydrating them, dry weights for roots are probably more accurate than fresh weights. The roots of both species decreased in weight as the concentration of sodium chloride increased (Figures 6 and 7).

A decrease in the top;root ratio is a common effect of an increase in salinity (Bernstein and Hayward, 1958). Ashby and Beadle (1957) found that the top:root ratio of tomatoes decreased as salinity increased, but that the top:root ratios for salt-tolerant Atriplex species increased for those plants receiving added salt. In these experiments, variability of shoot-root ratios was so great that no correlation can be seen between shoot-root ratio and salt concentration (Figure 8; Appendix A).

Increase in leaf succulence may often occur as a result of salinity. With Atriplex species, contradictory results have been reported. Gates (1972) reported an increase in succulence in Atriplex nummularia. Chatterton and McKell (1969) found no correlation between succulence and salt concentration in A. polycarpa. Ashby and Beadle (1957) obtained an increase in succulence in salt-treated A. inflata but only with very high concentrations of sodium chloride (0.4 and 0.6 M). With A. halimus, Gale and Poljakoff-Mayber (1970) found increased succulence, but Mozafar (1969) did not. In my experiments, no correlation was obtained between leaf succulence and salinity in either A. halimus or A. hortensis (Figure 9), although leaves of A. hortensis grown in high concentrations of NaCl visually appeared thicker than leaves of A. hortensis controls.

Shakhov had reported in 1952 (cited by Nieman, 1962) that salt tolerance is associated with a high rate of photosynthesis and a low

Figure 6. Root growth in A. hortensis: fresh and dry weights. Mean of 3-5 roots for each treatment (f.w.); 6-9 roots for each treatment (d.w.).

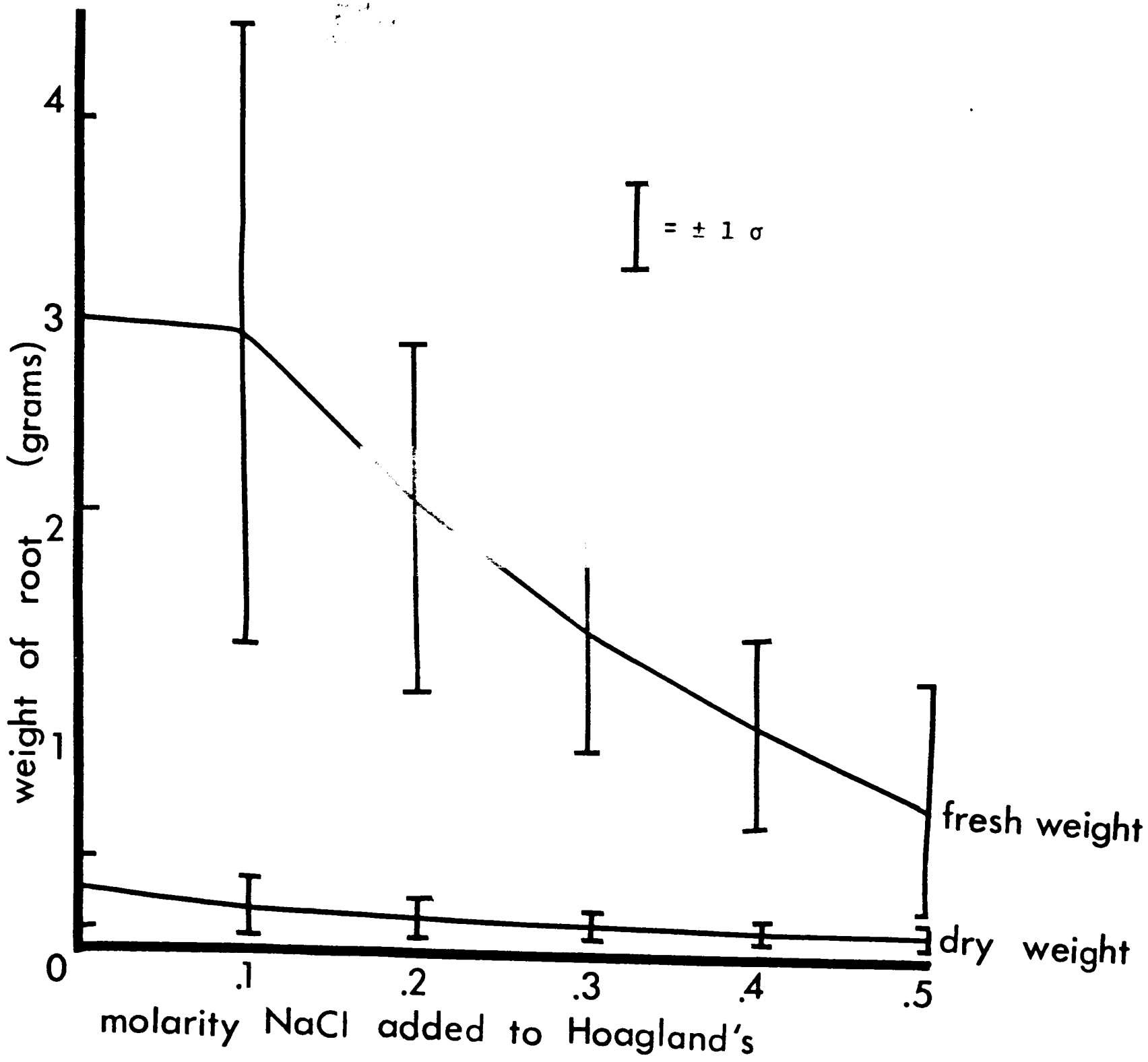

4
3
2
1
0
weight of root (grams)
= ± 1 σ
fresh weight
dry weight
.1
.2
.3
.4
.5
molarity NaCl added to Hoagland's

Figure 7. Root growth in A. halimus: fresh and dry weights. Mean of 3-5 roots for each treatment (f.w.); 5-8 roots for each treatment (d.w.).

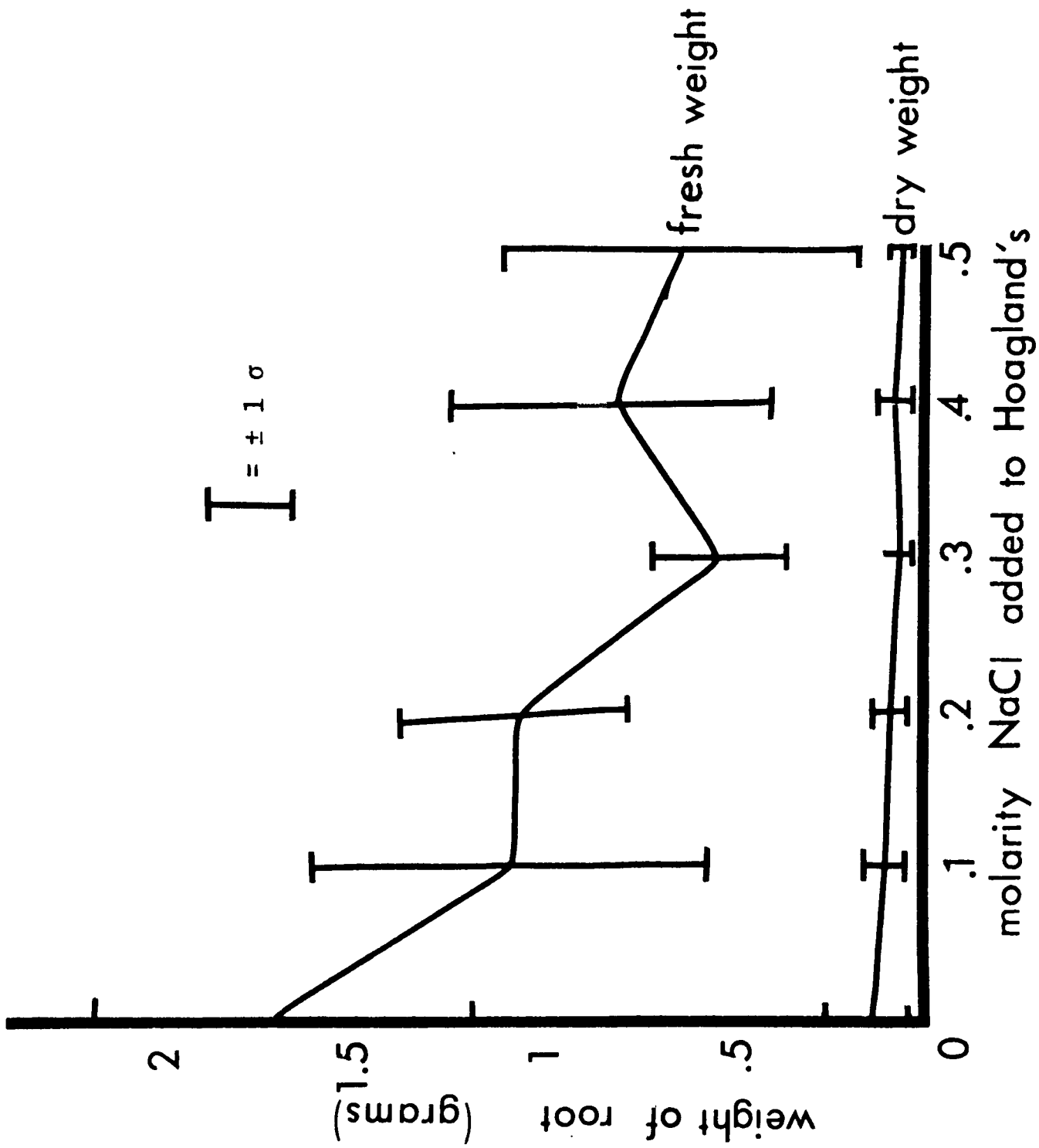

= ± 1 σ
fresh weight
dry weight
weight of root (grams)
2
1.5
1
.5
0
.1
.2
.3
.4
.5
molarity NaCl added to Hoagland's

Figure 8. Shoot: Root ratios of A. halimus and A. hortensis (fresh and dry weights). Mean of 6-9 plants for each treatment.

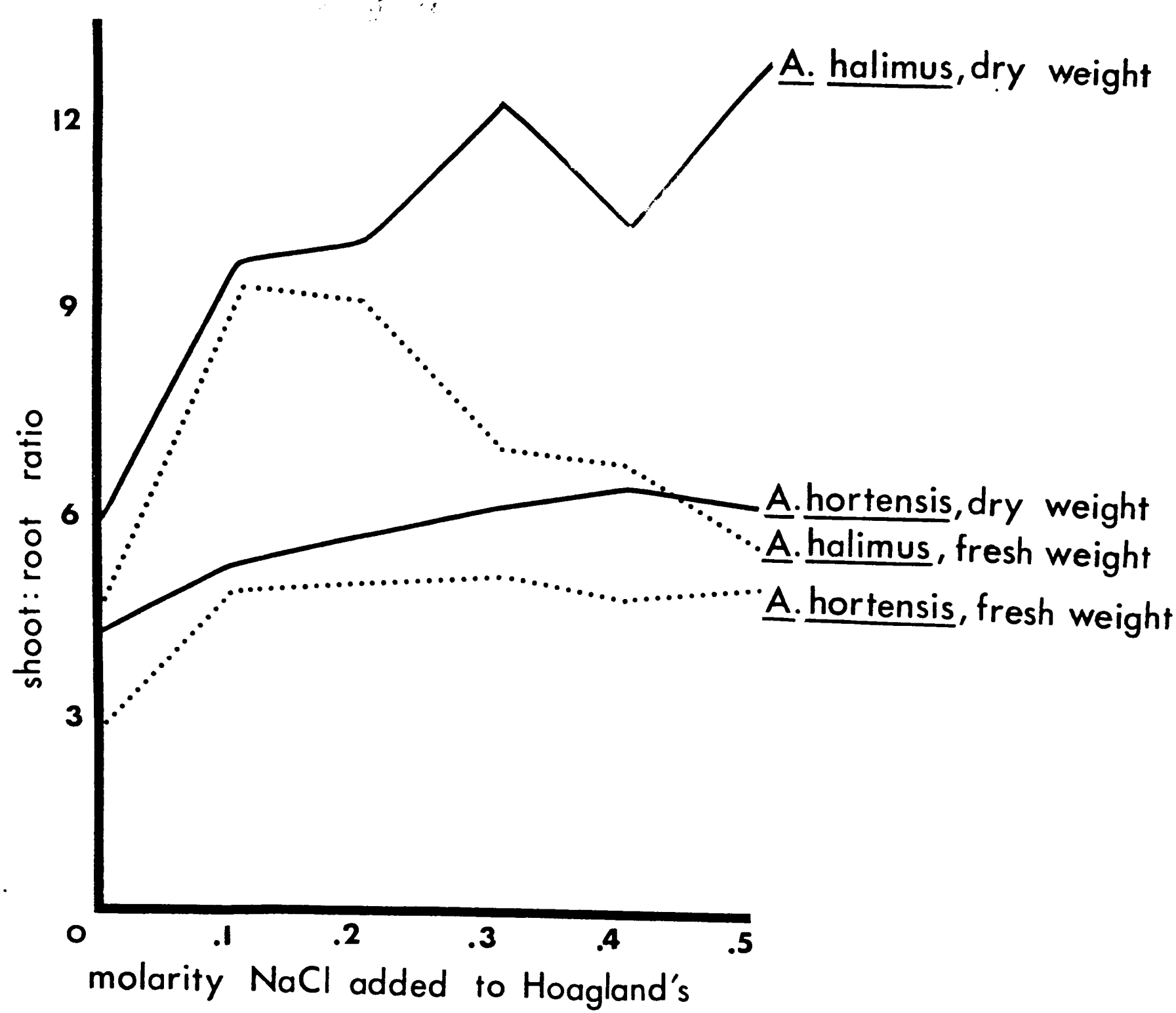
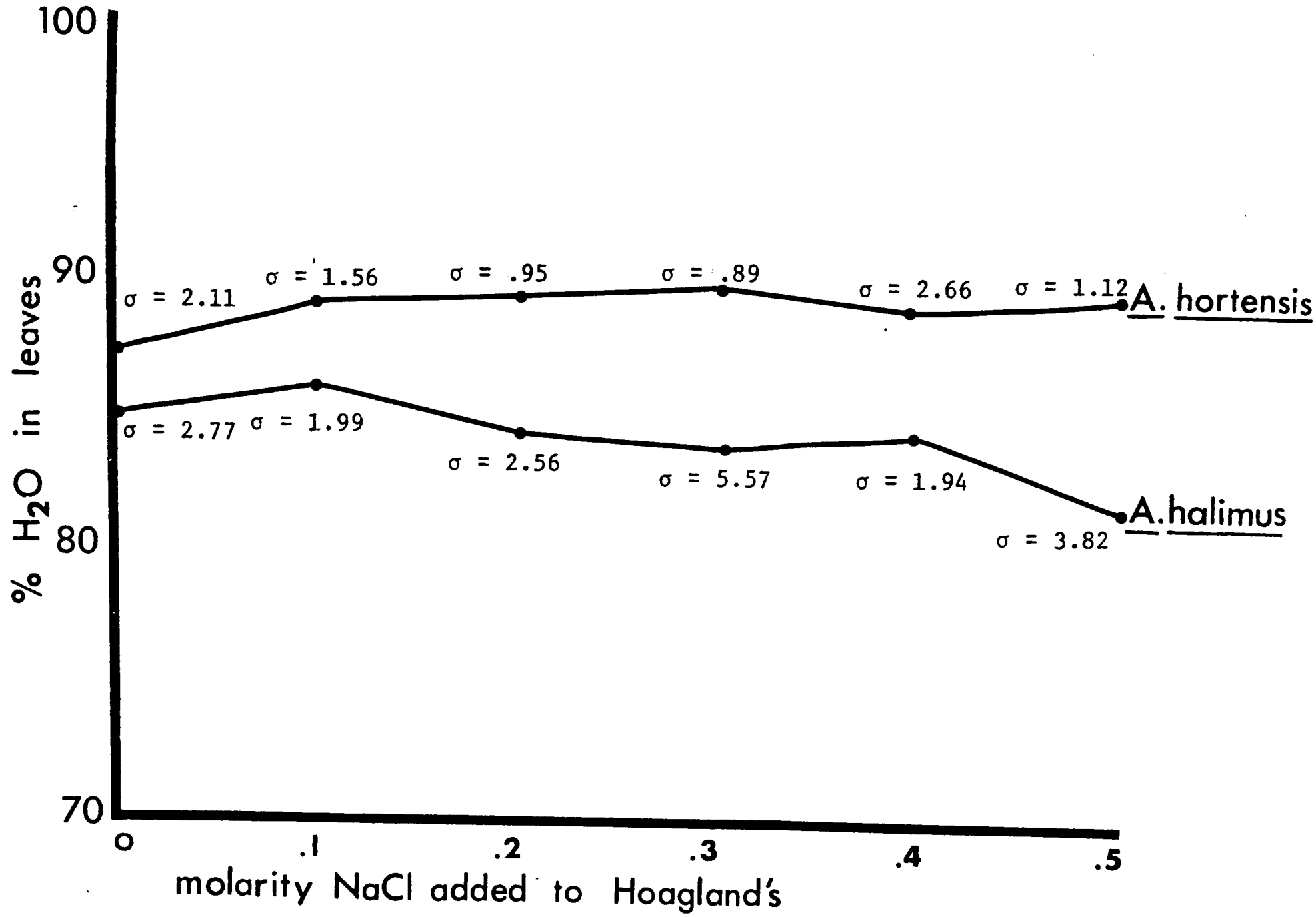

A. halimus, dry weight
A. hortensis, dry weight
A. halimus, fresh weight
A. hortensis, fresh weight
shoot: root ratio
12
9
6
3
0
.1
.2
.3
.4
.5
molarity NaCl added to Hoagland's

Figure 9. Leaf succulence (% H_2O in leaves) in A. halimus and A. hortensis.


100
90
80
70
% H_2O in leaves
σ = 2.11
σ = 1.56
σ = .95
σ = .89
σ = 2.66
σ = 1.12
A. hortensis
σ = 2.77
σ = 1.99
σ = 2.56
σ = 5.57
σ = 1.94
σ = 3.82
A. halimus
0
.1
.2
.3
.4
.5
molarity NaCl added to Hoagland's

rate of respiration, which seemed to predict that a C_4 plant would be more salt tolerant than a C_3 plant. One might therefore expect A. halimus to be more salt tolerant than A. hortensis. This was not found to be true. A. hortensis, a C_3 plant, even flowered and produced seed at 0.5 M NaCl.

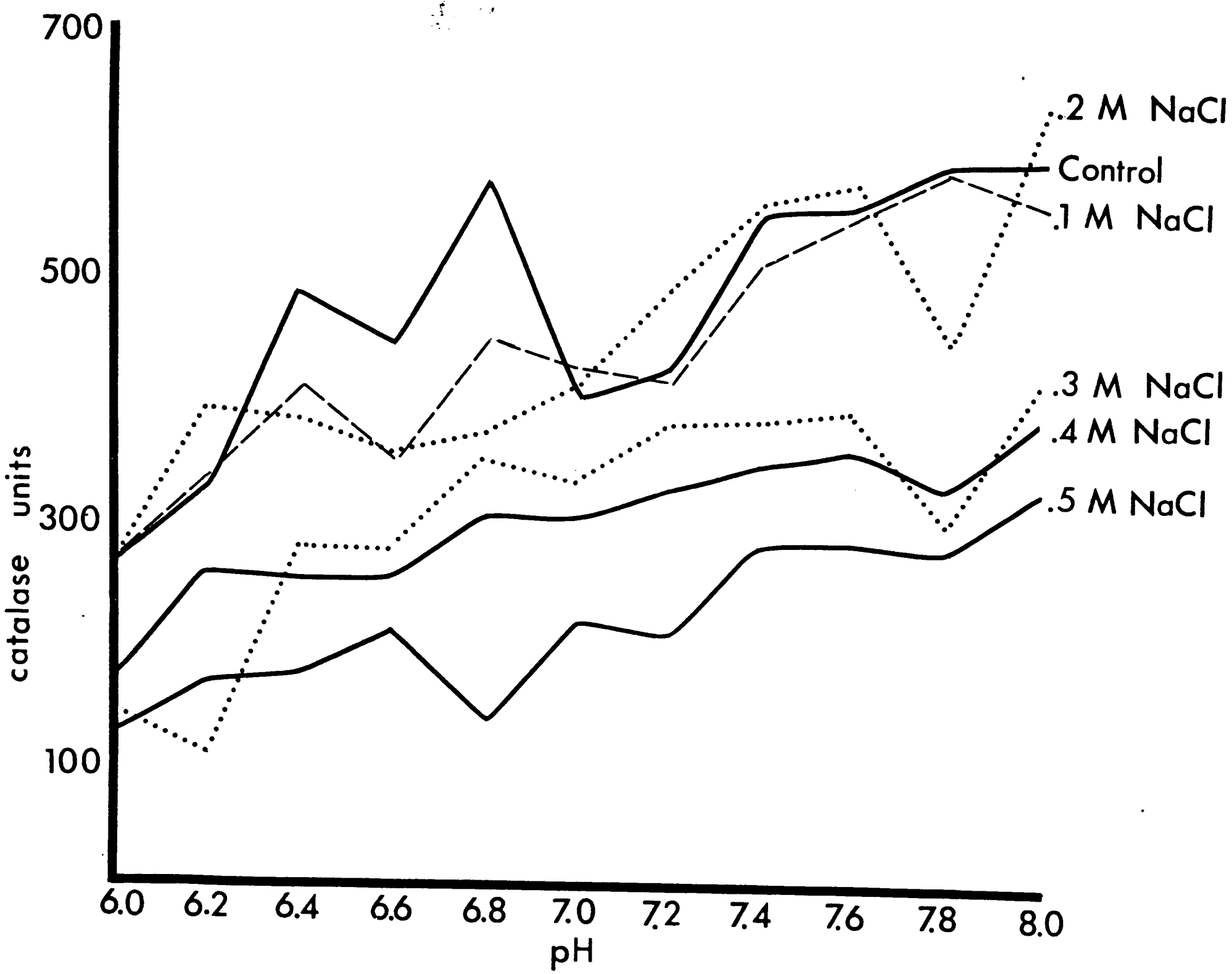
Catalase Activity

The spectrophotometric method of determining catalase activity was found to be a more accurate method than measurement of catalase activity by measuring the volume of O_2 produced. When a single determination was repeated by the latter method, results varied by as much as 100%, partially because the volume of air in the rubber tubing varied with each determination, because it was difficult to shake the tube in the same way each time, and because heat transmitted to the tube during shaking was probably not constant. When a determination was repeated with the spectrophotometric method, the results were consistent. Only the results from the spectrophotometric method will be discussed here.

Response of enzymes to ions is often associated with a shift in pH optima which may be mistaken for an overall increase or decrease in enzyme activity (Greenway and Osmond, 1972). For this reason, catalase activity was measured over a pH range from 6.0 to 8.0. The mean value of catalase activity for each treatment was used to give a value for overall catalase activity in the control.

In A. halimus, catalase activity was highest in the control, and activity decreased as sodium concentration increased (Figure 10, Table 1). In A. hortensis, catalase activity was highest in the control, but did

Figure 10. Catalase activity in A. halimus over a pH range of 6 to 8.


700
500
300
100
catalase units
.2 M NaCl
Control
.1 M NaCl
.3 M NaCl
.4 M NaCl
.5 M NaCl
6.0
6.2
6.4
6.6
6.8
7.0
7.2
7.4
7.6
7.8
8.0
pH

not decrease steadily as salt concentration increased (Figure 11; Table 1). No clearcut shift in pH optima was seen with NaCl treatments. Catalase activity was about three times as great in A. hortensis as in A. halimus. This is in agreement with the findings of Frederick and Newcomb (1969, 1971) that more catalase-containing peroxisomes are found in plants with high photorespiration than in plants with low photorespiration.

Table 1. Overall Activity (Catalase Units) in A. halimus and A. hortensis.

Species	Catalase Units Treatment (Molarity of NaCl)					
	Control	0.1	0.2	0.3	0.4	0.5
A. halimus	469	437	430	309	303	222
A. hortensis	1600	926	834	1203	1313	1014

Mozafar (1969) reported an increase in catalase activity of NaCl-treated plants when the homogenates were left standing in an ice bath for 2 hours. He hypothesized that catalase is bound to some subcellular particle from which it migrates as the homogenates stand to produce an increase in activity. A consistent increase in activity was not found with these plants (Figure 12). When A. halimus homogenates were left standing in an ice bath for 4 hours, overall catalase activity decreased in the control, 0.1, 0.2, and 0.4 M NaCl treatments; increased slightly in the 0.3 M NaCl treatment, and increased by 26% in the 0.5 M NaCl treatment. In A. hortensis homogenates, catalase activity after 4 nours decreased in the controls, 0.2 M NaCl, and 0.4 M NaCl treatments; and increased in the 0.1, 0.3, and 0.5 M NaCl treatments.

Figure 11. Catalase activity in Atriplex hortensis over a pH range of 6 to 8.

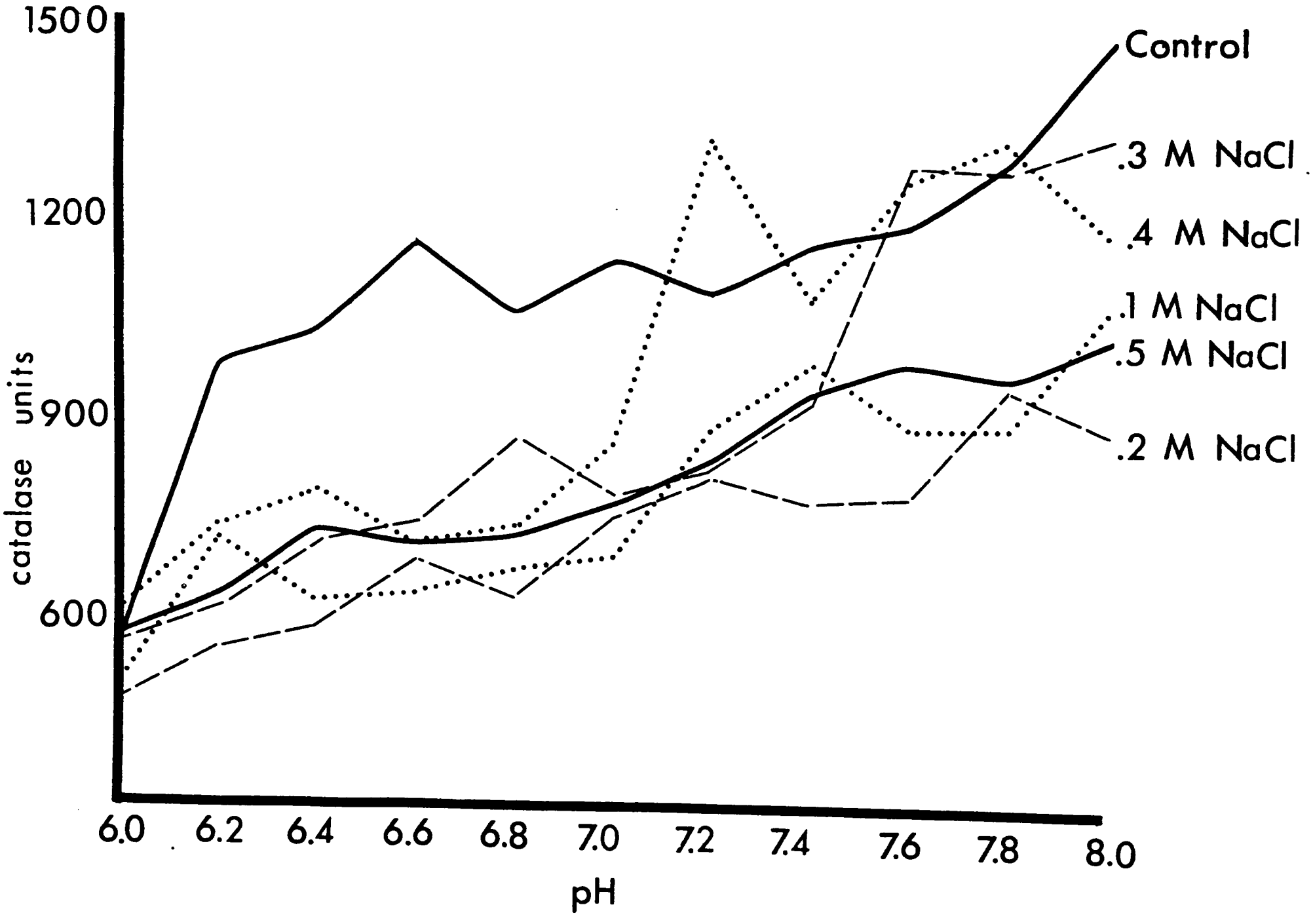
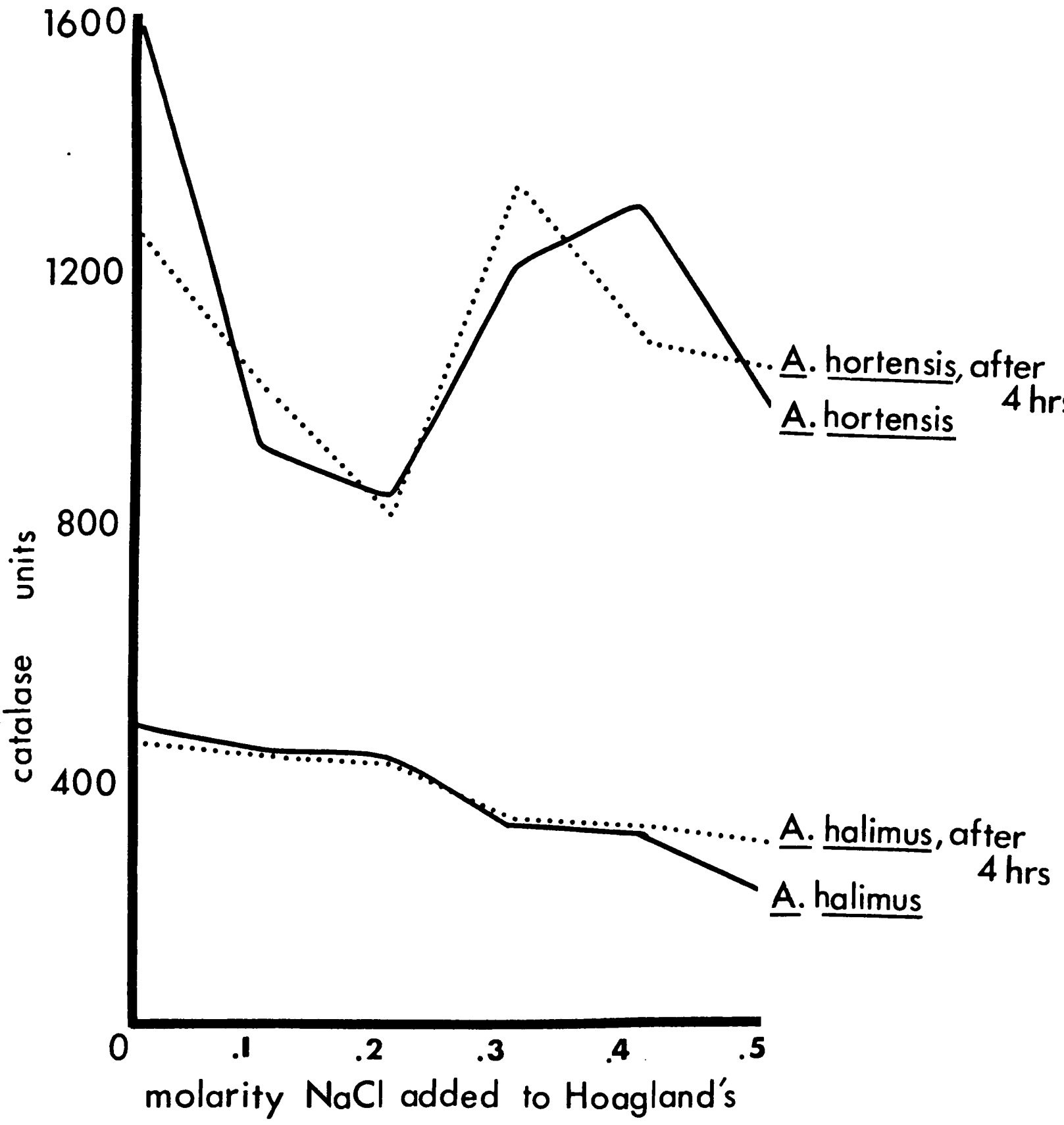

1500
1200
900
600
catalase units
Control
.3 M NaCl
.4 M NaCl
.1 M NaCl
.5 M NaCl
.2 M NaCl
6.0
6.2
6.4
6.6
6.8
7.0
7.2
7.4
7.6
7.8
8.0
pH

Figure 12. Change in catalase activity (0 and 4 hours after homogenation).


1600
1200
800
400
0
.1
.2
.3
.4
.5
catalase units
molarity NaCl added to Hoagland's
A. hortensis, after 4 hrs
A. hortensis
A. halimus, after 4 hrs
A. halimus

In another part of the experiment, salt was added to the H_2O_2-buffer solutions to see if catalase from plants grown in salinized media was more tolerant to NaCl than catalase from control plants grown in pure Hoagland's solution. If this were true, it would perhaps indicate that salt tolerant isozymes of catalase were produced by the salinized plants. In both A. halimus and A. hortensis, a greater decrease in activity was seen in enzyme extracts from plants grown in saline media (Figures 13 and 14; Table 2). It is possible that most of the catalase is located in the peroxisomes of these plants (Frederick and Newcomb, 1969a,b, 1971; Tolbert et al., 1969a,b), and thus the catalase is protected from high concentrations of ions. It is also possible that the constant removal of ions to vesiculated hairs prevents high concentrations of ions in the cell, even in plants grown in high concentrations of salt (Greenway and Osmond, 1972; Mozafar and Goodin, 1970).

Table 2. Decrease in Catalase Activity with Salt Added to Enzyme Extracts (Overall Values in Enzyme Units).

Treatment	Buffer Only	0.1 M NaCl Added to Buffer	% Decrease
A. halimis, Control	504	437	13%
A. halimus, 0.2 M NaCl	338	193	43%
A. hortensis, Control	1563	1381	12%
A. hortensis, 0.2 M NaCl	560	375	33%

Figure 13. Change in catalase activity with salt added to enzyme extract of A. halimus.

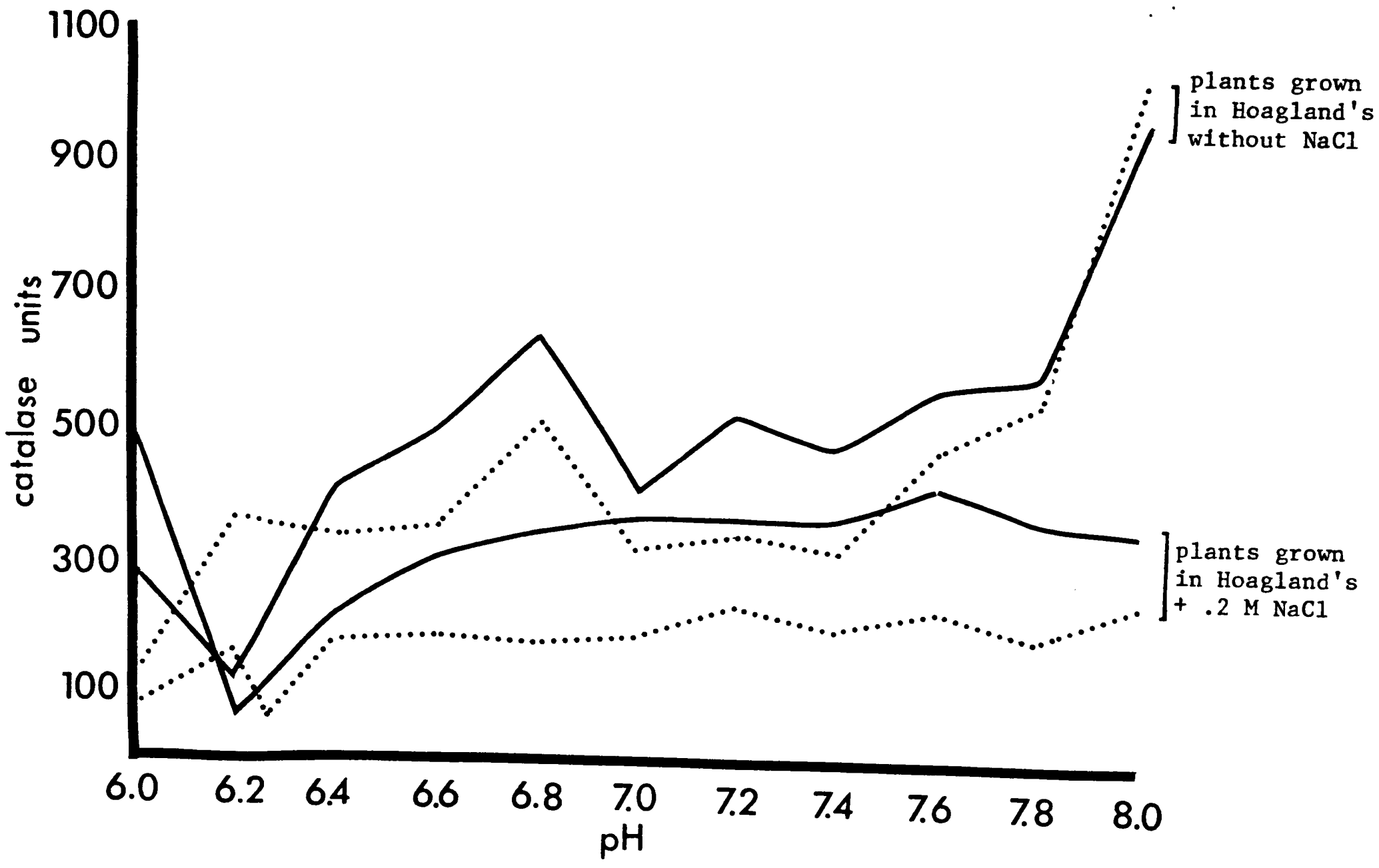
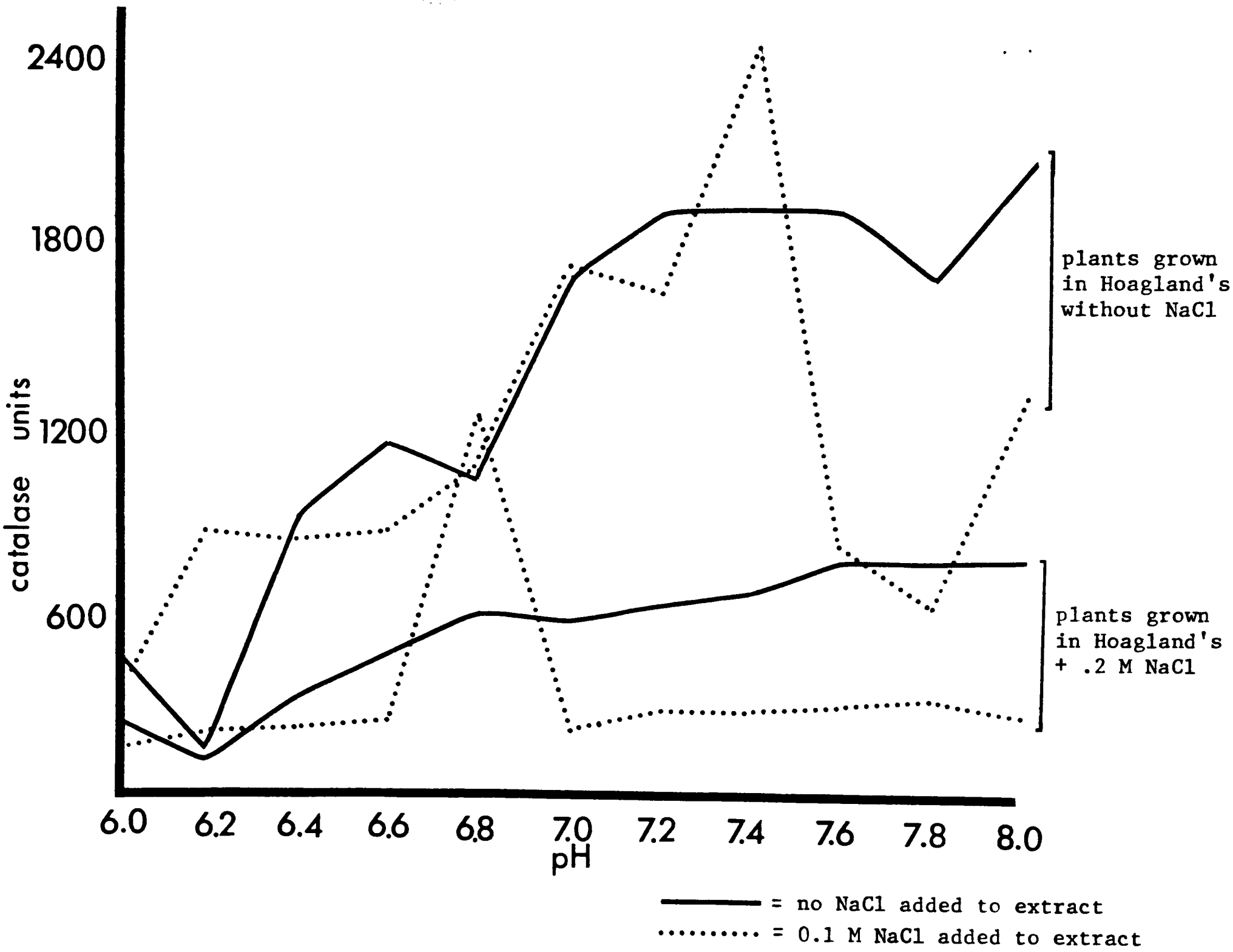

1100
900
700
500
300
100
catalase units
6.0
6.2
6.4
6.6
6.8
7.0
7.2
7.4
7.6
7.8
8.0
pH
plants grown in Hoagland's without NaCl
plants grown in Hoagland's + .2 M NaCl
= no NaCl added to extract
= 0.1 M NaCl added to extract

Figure 14. Change in catalase activity with salt added to enzyme extract of A. hortensis.


2400
1800
1200
600
catalase units
plants grown in Hoagland's without NaCl
plants grown in Hoagland's + .2 M NaCl
6.0
6.2
6.4
6.6
6.8
7.0
7.2
7.4
7.6
7.8
8.0
pH
= no NaCl added to extract
..... = 0.1 M NaCl added to extract

Oxalate Content

Oxalate levels were similar in A. halimus and A. hortensis. When expressed in meq per 25 leaf discs or as mg/g dry weight, oxalate content in both species did not change significantly with increased salt (Figures 15 and 16; Appendix B). An analysis of variance showed no significant differences between salt treatment and oxalate content (F=.5004 for A. hortensis, F=.9855 A. halimus).

Mozafar (1969) reported that in A. halimus oxalate content does not increase as salinity increases, although the highest NaCl concentration used in his study was 0.2 M NaCl. Since catalase is involved in oxalic acid production, we had hypothesized that catalase activity and oxalate production might be correlated. This does not appear to be true. Levels of catalase activity are high in 0.1 and 0.2 M NaCl and decrease in higher concentrations of NaCl. Oxalate levels do not change significantly with higher salt concentrations in these plants. Catalase activity seems to be a fairly good indicator of overall growth of plants in a given treatment. Probably catalase is used to break down H_2O_2 produced in many reactions so that activity of catalase is a better indicator of overall metabolic activity than an indicator of production of an organic acid.

Summary

Atriplex halimus and A. hortensis are salt tolerant plants that have potential for use as forage plants in dry and saline areas. Both species show a positive growth response to levels of sodium chloride as high as 0.2 M. Since levels of oxalic acid apparently do not

Figure 15. Oxalate content (meq in 25 leaf discs) of A. halimus and A. hortensis (Mean of 4-6 determinations per treatment).

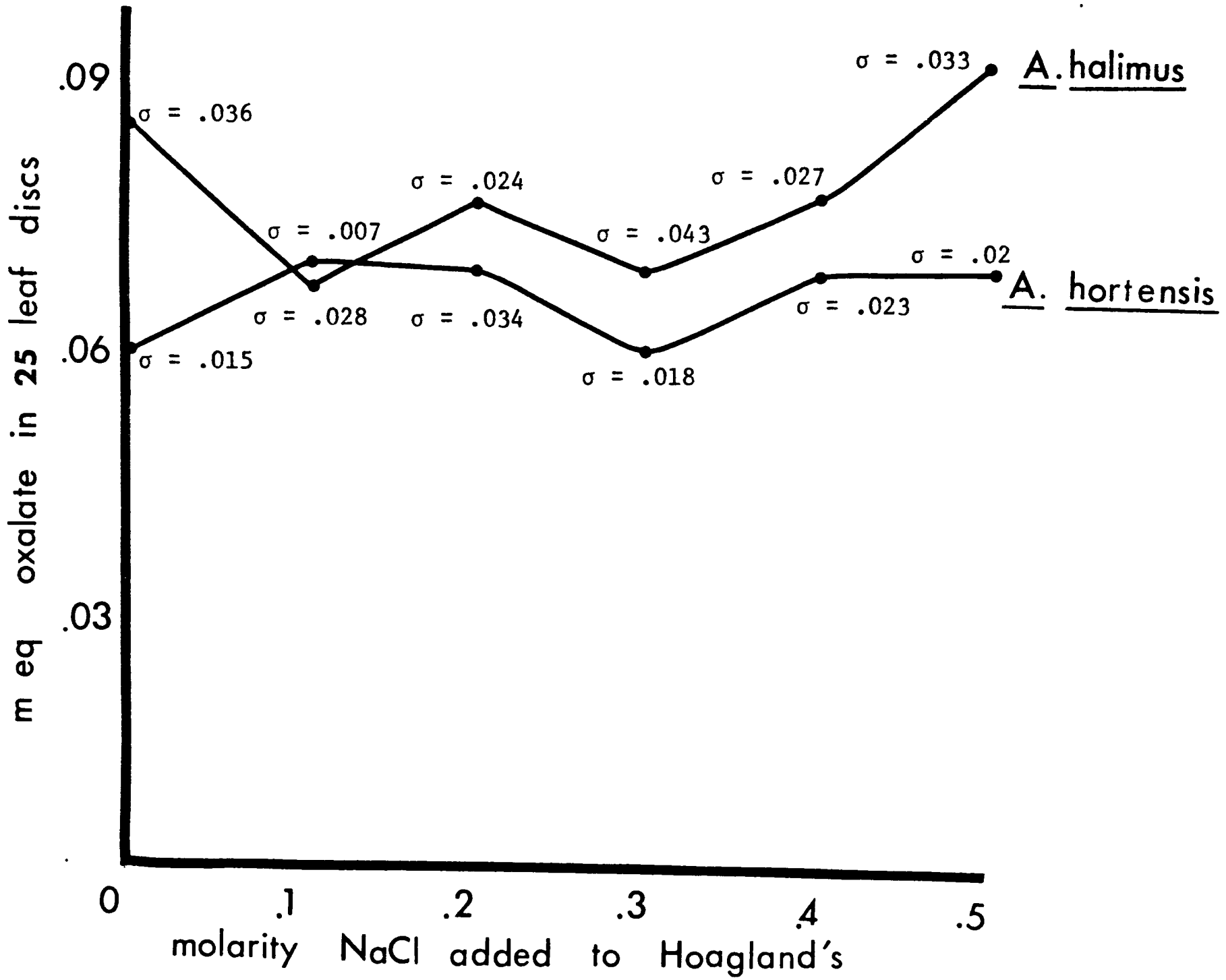
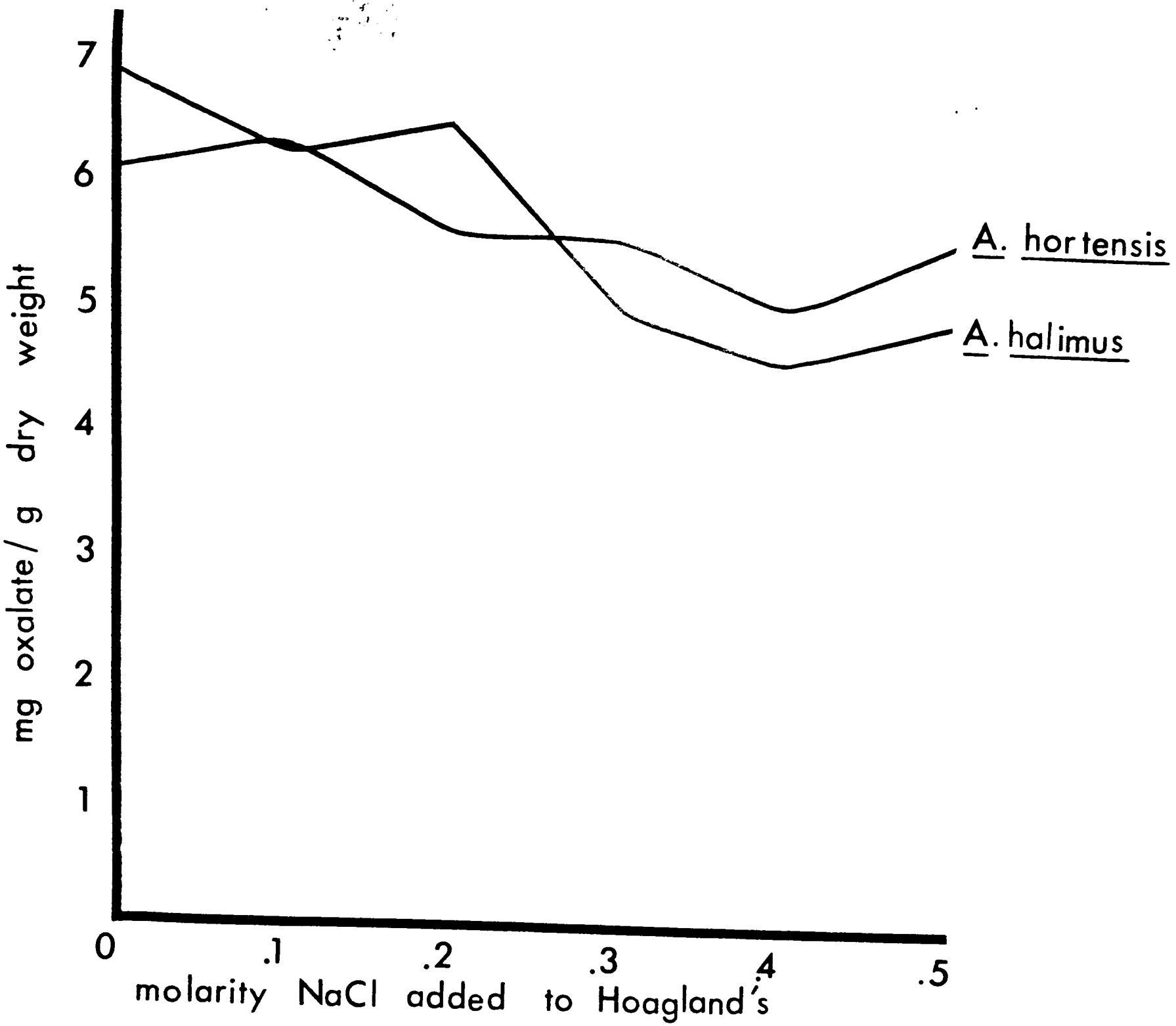

.09
.06
.03
m eq oxalate in 25 leaf discs
σ = .036
σ = .028
σ = .007
σ = .024
σ = .034
σ = .015
σ = .018
σ = .043
σ = .027
σ = .033
A. halimus
σ = .02
σ = .023
A. hortensis
0
.1
.2
.3
.4
.5
molarity NaCl added to Hoagland's

Figure 16. Oxalate content (mg/g) of A. halimus and A. hortensis (Mean of 4-6 determinations per treatment).


7
6
5
4
3
2
1
0
.1
.2
.3
.4
.5
mg oxalate/ g dry weight
molarity NaCl added to Hoagland's
A. hortensis
A. halimus

increase with increased salinity in these plants, they could perhaps be used as forage plants on highly salinized soils where other species of Atriplex would be dangerous to animals.

Although catalase is involved in the production of oxalic acid, total catalase activity of the plant is not indicative of the amount of oxalic acid produced. Catalase activity in both control and salt-treated plants is reduced when sodium chloride is added to the enzyme extract. This indicates that the enzyme itself does not adapt to NaCl when the plant is grown in saline media, but that it is somehow protected from the harmful effects of the NaCl, either by compartmentalization in organelles (peroxisomes) or by the active extrusion of the salt to vesiculated hairs.

As the problem of salinity increases, so does the need to understand mechanisms of salt tolerance in halophytes. One way to approach the study of salinity is to study the effects of salt on metabolism and enzyme activity in halophytes. No complete explanation of the increase in size in salt-treated Atriplex has yet been made. It might be interesting to compare changes in the top:root ratio in Atriplex and in non-halophytes when treated with salt. A more complete understanding of plant response to salt could lead to an improvement of these plants as forage crops and might offer the possibility of adapting non-salt tolerant plants for growth on saline soils.

REFERENCES

Arnon, D. I. and P. R. Stout. 1939. The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant Physiol. 14:371-375.

Ashby, W. C. and N. C. W. Beadle. 1957. Studies in halophytes. III. Salinity factors in the growth of Australian saltbushes. Ecology 38:344-352.

Baker, C. J. L. 1952. The determination of oxalates in fresh plant material. Analyst 77:340-344.

Beadle, N. C. W. 1952. Studies in halophytes. I. The germination of the seed and establishment of the seedlings of 5 species of Atriplex in Australia. Ecology 33:49-62.

Beers, R. F. and I. W. Sizer. 1952. A spectrophotometric method for measuring the breakdown of H_2O_2 by catalase. J. of Biol. Chem. 195:133-139.

Berg, C. van den. 1952. The influence of absorbed salts on growth and yield of agricultural crops of salty soils. Versl. Landb. Ned. 58:1-118. Cited by Slatyer, 1967.

Bernstein, L. and H. E. Hayward. 1958. Physiology of salt tolerance. Ann. Rev. of Plant Physiol. 9:25-46.

Black, R. F. 1954. The leaf anatomy of Australian members of the genus Atriplex. I. A. vesicaria Heward and A. nummularia Lindl. Aust. J. Bot. 2:269-286.

Black, R. F. 1960. Effect of NaCl on the ion uptake and growth of A. vesicaria Heward. Aust. J. Biol. Sci. 13:249-266.

Blumenthal-Goldschmidt, S. and A. Poljakoff-Mayber. 1968. Effect of substrate salinity on growth and on submicroscopic structure of leaf cells of A. halimus L. Aust. J. Bot. 16:469-478.

Brownell, P. F. 1965. Sodium as an essential micronutrient element for a higher plant (Atriplex vesicaria). Plant Physiol. 40:460-468.

Brownell, P. F. 1968. Sodium as an essential micronutrient element for some higher plants. Plant and Soil 28:161-164.

Brownell, P. F. and J. G. Wood. 1957. Sodium as an essential micronutrient element for A. vesicaria, Heward. Nature 179:635-636.

Card, K. A., B. Mahall, and J. H. Troughton. 1974. Salinity and carbon isotope ratios in C_3 and C_4 plants. Ann. Rep. of the Dir. Carnegie Institution. 784-785.

Chatterton, N. J., J. R. Goodin, and C. Duncan. 1971. Nitrogen metabolism in Atriplex polycarpa as affected by substrate nitrogen and NaCl salinity. Agron. J. 63:271-273.

Chatterton, N. J. and C. M. McKell. 1969. Atriplex polycarpa. I. Germination and growth as affected by NaCl in water cultures. Agron. J. 61:448-450.

Eaton, F. M. 1942. Toxicity and accumulation of Cl^- and SO_4^{-2} salts in plants. J. Agr. Res. 64:357-399.

Flowers, T. J. 1972a. Salt tolerance in Suaeda maritima (L.). The effect of sodium chloride on growth, respiration, and soluble enzymes in a comparative study with Pisum sativum L. J. Exp. Bot. 23:310-321.

Flowers, T. J. 1972b. The effect of NaCl on enzyme activities from four halophyte species of Chenopodiaceae. Phytochemistry 11: 1881-1886.

Franclet, A. and H. Le Houérou. 1971. Les Atriplex en Tunisie et en Afrique du Nord. Institut de Reboisement, Tunisia. Rapport technique 7. FAO, Rome. 249 pp.

Frederick, S. E. and E. H. Newcomb. 1969a. Cytochemical localization of catalase in leaf microbodies (peroxisomes). J. Cell Biol. 43:343-356.

Frederick, S. E. and E. H. Newcomb. 1969b. Microbody-like organelles in leaf cells. Science 163:1353-1355.

Frederick, S. E. and E. H. Newcomb. 1971. Ultrastructure and distribution of microbodies in leaves of grasses with and without CO_2-photorespiration. Planta 96:152-174.

Gale, J., R. Naaman, and A. Poljakoff-Mayber. 1970. Growth of Atriplex halimus L. in NaCl salinated culture solutions as affected by the relative humidity of the air. Aust. J. Biol. Sci. 23:947-952.

Gale, J. and A. Poljakoff-Mayber. 1970. Interrelations between growth and photosynthesis of salt bush (Atriplex halimus L.) grown in saline media. Aust. J. Biol. Sci. 23:937-945.

Gates, C. T. 1972. Ecological response of the Australian native species Acacia harpophylla and Atriplex nummularia to soil salinity: Effects on water content, leaf area, and transpiration rate. Aust. J. Bot. 20:261-272.

Greenway, H. 1968. Growth stimulation by high chloride concentrations in halophytes. Israel J. Bot. 17:169-177.

Greenway, H. and C. B. Osmond. 1972. Salt responses of enzymes from species differing in salt tolerance. Plant Physiol. 49:256-259.

Grillot, G. 1954. The biological and agricultural problems presented by plants tolerant of saline or brakish water and the employment of such water for irrigation. Arid Zone Programme. IV. Reviews of Research on the Problems of Utilization of Saline Water. UNESCO, Paris. pp. 9-35.

Hayward, H. E. and L. Bernstein. 1958. Plant-growth relationships on salt-affected soils. Bot. Rev. 24:584-635.

Hayward, H. E. and C. H. Wadleigh. 1949. Plant growth on saline and alkali soils. Advances Agron. 1:1-38.

Hoagland, D. R. and D. I. Arnon. 1950. The water culture method for growing plants without soil. Univ. Calif. Agr. Exp. Sta. Circ. 347. Berkeley.

Jaffa, M. E. 1899. Composition and food value of the saltbushes. Univ. of Calif. Agr. Exp. Sta. Bull. 125. Berkeley. 30 pp.

Jennings, D. H. 1968. Halophytes, succulence, and sodium in plants - A unified theory. New Phytol. 67:899-911.

Johnson, H. S. and M. D. Hatch. 1968. Distribution of the C_4-dicarboxylic acid pathway of photosynthesis and its occurrence in dicotyledonous plants. Phytochemistry 7:375-380.

Jones, R. (ed.) 1970. The biology of Atriplex. Div. of Plant Ind., CSIRO, Canberra, Australia. 128 pp.

Kalir, A. and A. Poljakoff-Mayber. 1975. Malic dehydrogenase from Tamarix roots. Plant Physiol. 55:155-162.

Kaplan, A. and J. Gale. 1972. Effect of NaCl salinity on the water balance of Atriplex halimus. Aust. J. Biol. Sci. 25:895-903.

Keilin, D. and E. F. Hartree. 1945. Properties of catalase. Catalysis of coupled oxidations of alcohols. Biochem. J. 39:293-301.

Kelley, D. B. 1974. Salinity effects on growth and fine structure of Atriplex halimus L. M.S. Thesis. Texas Tech Univ. 52 pp.

Kenten, R. H. and P. J. G. Mann. 1952. Hydrogen peroxide formation in oxidations catalyzed by plant -hydroxyacid oxidase. Biochem. J. 52:130-134.

Kisaki, T. and N. E. Tolbert. 1969. Glycolate and glyoxylate metabolism by isolated peroxisomes or chloroplasts. Plant Physiol. 44:242-250.

LaHaye, P. A. and E. Epstein. 1969. Salt toleration by plants: Enhancement with Ca^{++}. Science 166:395-396.

Lehninger, A. L. 1970. Biochemistry. Worth Publ. Co., New York.

Livne, A. and N. Levin. 1967. Tissue respiration and mitochondrial oxidative phosphorylation of NaCl-treated pea seedlings. Plant Physiol. 42:407-414.

Lück, H. 1965. Measurement of enzyme activity, pp. 886-888. In H. U. Bergmeyer (ed.) Methods of enzymatic analysis. Academic Press, New York.

Lüttge, U. 1971. Structure and function of plant glands. Ann. Rev. of Plant Physiol. 22:23-44.

Machlis, L. and J. G. Torrey. 1956. Plants in action: A laboratory manual of plant physiology. W. H. Freeman and Co., San Francisco, pp. 113-114.

Magistad, O. C. et al. 1943. Effect of salt concentration, kind of salt, and climate on plant growth in sand cultures. Plant Physiol. 18:151-166.

Milthorpe, F. L. 1970. Atriplex--Atlantean or Atrabilarian?, pp. 123-125. In R. Jones (ed.) The biology of Atriplex. Div. of Plant Ind., CSIRO, Canberra, Australia.

Mooney, H., O. Björkman, and J. Troughton. 1974. Seasonal changes in the leaf characteristics of the desert shrub Atriplex hymenelytra, Ann. Rep. Dir., Carnegie Institution. p. 846.

Mozafar, A. 1969. Physiology of salt tolerance in Atriplex halimus L.: Ion uptake and distribution, oxalic acid content, and catalase activity. Ph. D. Dissertation. Univ. of Calif., Riverside. 154 pp.

Mozafar, A. and J. R. Goodin, 1970. Vesiculated hairs: A mechanism for salt tolerance in Atriplex halimus L. Plant Physiol. 45:62-65.

Nieman, R. H. 1962. Some effects of sodium chloride on growth, photosynthesis, and respiration of 12 crop plants. Bot. Gaz. 123:279-285.

Nieman, R. H. and L. L. Poulsen. 1967. Interactive effects of salinity and atmospheric humidity on the growth of bean and cotton plants. Bot. Gaz. 128:69-73.

Nobs, M. A. and W. G. Hagar. 1974. Analysis of germination and flowering rates of dimorphic seeds from Atriplex hortensis. Ann. Rep. Dir., Carnegie Institution, pp. 859-864.

O'Leary, J. W. 1971. Physiological basis for plant growth inhibition due to salinity, pp. 331-336. In W. McGinnies, B. J. Goldman, and P. Paylore (eds.) Food, fiber, and the arid lands. Univ. of Ariz. Press, Tucson, Ariz.

Osmond, C. B. 1963. Oxalates and ionic equilibria in Australian saltbushes (Atriplex). Nature 198:503-504.

Osmond, C. B. 1966. Divalent cation absorption and interaction in Atriplex. Aust. J. Biol. Sci. 19:37-48.

Osmond, C. B. 1967. Acid metabolism in Atriplex. I. Regulation of oxalate synthesis by the apparent excess cation absorption in leaf tissue. Aust. J. Biol. Sci. 20:575-587.

Osmond, C. B. and P. N. Avadhani. 1968. Acid metabolism in Atriplex. II. Oxalate synthesis during acid metabolism in the dark. Aust. J. Biol. Sci. 21:917-927.

Osmond, C. B., J. H. Troughton and D. J. Goodchild. 1969. Physiological, biochemical, and structural studies of photosynthesis and photorespiration in two species of Atriplex. Zeitschr. Pflanzenphysiol. 61:218-237.

Osmond, C. B. et al. 1969. Ion absorption in Atriplex leaf tissue. II. Secretion of ions to epidermal bladders. Aust. J. Biol. Sci. 22:797-814.

Pallaghy, C. K. 1970. Salt relations of Atriplex leaves, pp. 57-62. In R. Jones (ed.) The biology of Atriplex. Div. of Plant Ind., CSIRO, Canberra, Australia.

Palmer, J. K. 1955. Chemical investigation of the tobacco plant. X. Determinations of organic acids by ion exchange chromatography. Conn. Agr. Exp. Sta. Bull. 589. New Haven.

Pokrovskaia, E. I. 1957. Certain data on oxidation-reduction processes in halophytes. Pamiati Akad. N. A. Maksimova. sobornik statei. Moskva, Akad, Nauk., USSR, pp. 268-274. In Russian. Cited by Mozafar, 1969.

Porath, E. and A. Poljakoff-Mayber. 1964. Effect of salinity on metabolic pathways in pea root tips. Israel J. Bot. 13:115-121.

Saunders, B. C. et al. 1964. Peroxidase. Buttwerworths and Co., Washington.

Scandalios, J. G. 1965. Subunit dissociation and recombination of catalase isozymes. Proc. Nat. Acad. Sci. 53:1035-1040.

Scandalios, J. G. 1969. Genetic control of multiple molecular forms of enzymes in plants: A review. Biochem. Genetics 3:37-79.

Siew, D. and S. Klein. 1968. The effect of sodium chloride on some metabolic and fine structural changes during the greening of etiolated leaves. J. Cell Biol. 37:590-596.

Shakhov, A. A. 1956. Salt resistance of plants. Acad. Sci., USSR, Moscow. Cited by Nieman, 1962.

Slatyer, R. O. 1967. Plant-water relationships. Academic Press, London. 366 pp.

Tolbert, N. E. et al. 1968. Peroxisome from spinach leaves containing enzyme related to glycolate metabolism. J. Biol. Chem. 243: 5179-5184.

Tolbert, N. E. et al. 1969. A survey of plants for leaf peroxisomes. Plant Physiol. 44:135-147.

Waisel, Y. 1972. Biology of halophytes. Academic Press, New York. 395 pp.

Weimberg, Ralph. 1970. Enzyme levels in pea seedlings grown on highly salinized media. Plant Physiol. 46:466-470.

Welkie, G. W. and H. Caldwell. 1970. Leaf anatomy of species in some dicotyledon families as related to the C_3 and C_4 pathways of carbon fixation. Canad. J. Bot. 48:2135-2146.

Williams, M. C. 1960. Effect of sodium and potassium salts on growth and oxalate content of Halogeton. Plant Physiol. 35:500-505.

Williams, O. B. 1960. The selection and establishment of pasture species in a semi-arid environment -- An ecological assessment of the problem. J. Aust. Inst. Agr. Sci. 26:258. Cited by Mozafar, 1969.

Appendix A. Standard Deviations for Data Given in Figure 8, p. 35.

Treatment	Standard Deviation	
	fresh weight	dry weight
A. halimus, Control	1.01	3.35
A. halimus, 0.1 M NaCl	3.82	7.07
A. halimus, 0.2 M NaCl	2.39	5.09
A. halimus, 0.3 M NaCl	2.83	11.36
A. halimus, 0.4 M NaCl	2.54	2.08
A. halimus, 0.5 M NaCl	2.03	12.83
A. hortensis, Control	0.32	1.59
A. hortensis, 0.1 M NaCl	2.14	3.29
A. hortensis, 0.2 M NaCl	1.35	2.33
A. hortensis, 0.3 M NaCl	2.10	1.60
A. hortensis, 0.4 M NaCl	1.33	4.88
A. hortensis, 0.5 M NaCl	1.96	3.15

Appendix B. Standard Deviations for Data Given in Figure 16, p. 55.

Treatment	Standard Deviation
A. halimus, Control	3.11
A. halimus, 0.1 M NaCl	2.73
A. halimus, 0.2 M NaCl	0.80
A. halimus, 0.3 M NaCl	3.29
A. halimus, 0.4 M NaCl	1.44
A. halimus, 0.5 M NaCl	1.51
A. hortensis, Control	2.07
A. hortensis, 0.1 M NaCl	0.93
A. hortensis, 0.2 M NaCl	1.70
A. hortensis, 0.3 M NaCl	1.20
A. hortensis, 0.4 M NaCl	1.35
A. hortensis, 0.5 M NaCl	1.81