

EFFECTS OF CHANGING PH UPON THE  
UPTAKE AND TRANSLOCATION OF IRON IN  
ANDROPOGON GERARDI

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## INTRODUCTION

According to Wallace and DeKock (1965), iron is an important plant element. They stated that one of iron's most important aspects is regulating the synthesis of one or more enzymes that have a key role in chlorophyll synthesis.

It has been recognized (Wallace, 1962) that different chelating agents and pH levels affect the uptake and translocation of iron in plants. Northen (1968) reported that certain cultural factors such as high pH may cause symptoms identical to those seen in iron chlorosis. Wallace et al. (1969) stated that extremely low pH may keep plants from taking up and translocating iron. However, a slightly acidic solution increases uptake and translocation of iron. Likewise, O'Connor et al (1971) have reported that chelating agents play an important part in the uptake and translocation of iron. EDDHA (Ethylenediamine Di-(O-Hydroxyphenyl Acetic)) was shown to be the best chelate for this purpose. Similarly, Wallace (1962) has stated that plants of different species change their ability to take up, translocate and reduce iron from the ferric to the ferrous state when the pH of the external solution is adjusted.

This project was undertaken to determine the effects of different pH changes on the uptake, translocation and reduction of iron in Andropogon gerardi (Big Bluestem grass). A. gerardi was selected for this study because of its importance in the tall grass prairie.

## METHODS AND MATERIALS

Seeds of Andropogon gerardi were obtained from the Plant Materials Center in Manhattan, Kansas. In order to provide different age groups, the seeds were planted at two week intervals on an alternating schedule. One hundred fifty to two hundred seeds were selected at random and planted in 16 x 20 cm plastic pots filled with potting soil. The pots were then placed in a green house for 10 days to facilitate germination and seedling growth. The pots were then transferred to a growth chamber for a more controlled environment. The growth chamber (Percival, model PT 80, No. 8336.2) was set at 28 C day - 26 C night, with an illumination intensity of 1600 f.c.. 800 cm above the plastic pots on a 14-10 hour day-night cycle in order to simulate middle June growing conditions. After the seedlings were established in the growth chamber, A. gerardi plants were transplanted into 16 x 19 cm plastic pots filled with 4:3:2 mixture of peat moss, potting soil, and sand, respectively. The A. gerardi age groups used in the uptake and translocation test were: five weeks, seven weeks, and nine weeks.

The radioactive iron (Fe-59) was supplied by New England Nuclear. A total of 4.80 mCi were used for the application. Five mCi of the Fe-59 was mixed with 10 ml KOH and 1.0 gm EDDHA (Ethylenediamine-Di(O-Hydroxyphenyl Acetic)). This solution was diluted to one liter with H<sub>2</sub>O and aerated overnight to facilitate chelation (Hoagland and Arnon, 1950). The Fe-59 EDDHA-H<sub>2</sub>O solution was then mixed with an additional

liter of water and this divided into eight 360 ml portions. The portions were adjusted to the desired pH values with a Beckman/Zeromatic pH meter. The pH of the starting Fe-59 EDDHA-H<sub>2</sub>O solution was 9.7. A 0.05% acetic acid solution was used to adjust the pH of the various solutions.

All test groups were given equal amounts of radioactive Fe-59 (0.02 mCi) at their respective pH ranges. The pH values selected for the study were 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. These values were selected to provide a low and high range that could be found in a prairie habitat. Sixty ml of the radioactive solution previously described were added to each plant set-up which consisted of three plants in 100 ml cups filled with white quartz sand. The plants for the 100 ml cups were removed from the plastic pots described previously. There were two cups per pH grouping. The three age groups of A. gerardi were subjected to the pH ranges 5.0 to 8.5.

The plants were allowed to absorb the radioactive Fe for three hours at room temperature (27 C) and normal room light (80-100 f.c.). The plants were then removed from the Fe-59 EDDHA-H<sub>2</sub>O sand mixture and washed with running tap water. The two plants from each pH and age group were used for the radioautographs and each of the other four plants were sectioned into three parts for isotope activity counts. The sections of the plant used for activity counts consisted of the tip (tip of each leaf of the four plants - two cm in length), stem and the



root (a two cm section). The sections were cut differently due to the size of the plant. The stem sections were cut as follows: five week old plants - one cm from the root stem junction; seven week old plants - five cm from the root stem junction; and nine week old plants - three cm from the root stem junction. The root sections were cut as follows: in five week old plants a two cm section was taken from the root stem junction down; in seven and nine week old plants, a two cm section was taken four cm from the root stem junction. The various sections were labelled and dried at 46 C for three days.

The dried plant sections were weighed and placed into metal planchets for counting. The radioactivity of the sections were determined by an automatic Geiger-Muller gas flow counter (Nuclear-Chicago, Planchet Sample Changer, Model 1042, and Decade Scaler, Series 8703). Background counts were repeated four times at the time of initial counting and the average obtained. Two samples of each section (tip, stem, root) were weighed and counted twice. The mean counts were obtained for each sample. Radioactivity of Fe-59 was corrected for decay (Chase and Robinowitz, 1967).

The identification of Fe reduction sites in the roots were determined under the same environmental conditions as those used in the uptake study. The sites of iron reduction can be identified by Prussian blue precipitation when a mixture of Fe EDDHA and potassium ferricyanide are added together in a growth media (Ambler et al. 1971). Therefore, a one

hundred and sixty ml mixture of the reducing solution Fe EDDHA and potassium ferricyanide (each at five ppm., etc.) was mixed in equal amounts and adjusted to pH values already mentioned in the uptake study (5.0 to 8.5).

The reducing solution (160 ml) was added to each plant set-up. The only change in the experimental design was that no sand was used. The plant roots were placed directly into the solution. This was done to facilitate Prussian blue precipitation. The cups were then placed in the growth chamber (Shere-Gillett Co., Model Cel 25-7, No. 108-0114-46) for 24 hours at 25 C; the lights (1400 f.c.) were left on during the entire time period. Sites of Fe reduction were indicated by the formation of Prussian blue precipitate which appeared in the root tissue when Fe EDDHA was reduced. The roots were then sectioned with a freezing microtome (Model 800, ser. no. 24803), observed under a Herrbrug Wild Microscope, (1.25 x 10), and photographed (Herrbrug Wild camera, 12180). The pH intervals of 5.0, 6.0, 7.0 and 8.0 were used for analysis.

#### Statistical Analysis:

A t test at  $p=0.05$  was used to determine significant differences.

## RESULTS

### Andropogon gerardi

#### (a) Five week old plants

Activity of Fe-59 in five week old plants was quite high in the tip samples compared to the root and stem samples (Fig. 1). The activity of the tip samples was highest in the pH 5.0 groups and then decreased as the pH increased to pH 5.5, however, the activity of the tip samples from pH groups 5.5 to 8.5 remained constant. At these same pH values the root sample activity increased to levels above the tip sample activity. At pH 6.5 the tip sample activity increased again and the root sample activity dropped. From pH 7.0 to 8.5 the tip sample activity decreased as the pH increased. The root sample activity again increased above the tip sample activity at pH 8.0 to 8.5. The stem activity was always well below the activity of the tip or root samples.

1. Samples of the same tissue type were compared against pH groups.
  - a. Tip samples: There was significant difference between pH groups 5.0 when compared to pH groups 5.5, 7.0, 8.0 and 8.5. There was also a significant difference between pH group 6.5 when compared to pH group 8.0.
  - b. Stem samples: There was a significant difference between pH group 5.0 when compared to pH groups 7.0, 7.5 and 8.0.
  - c. Root samples: There was a significant difference between pH group 5.0 when compared to pH groups 6.5, 7.0 and 7.5.

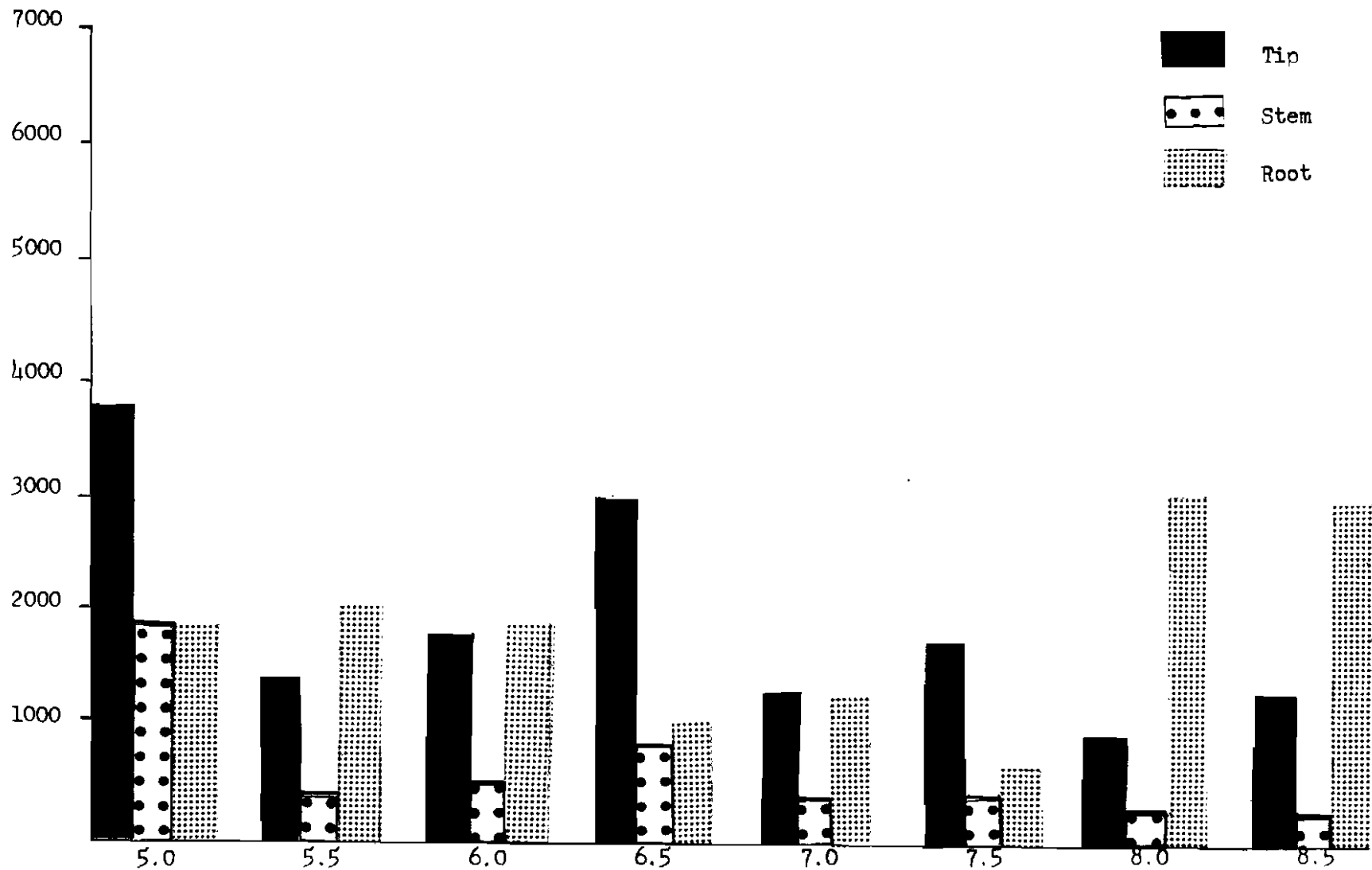


Fig. 1. Mean activity of Fe-59 in tip, stem and root portions of Andropogon gerardi plants at five week growth in pH ranges 5.0 to 8.5.

There were also significant differences between pH group 5.5 and 7.5. The pH group 6.0 was significantly different from pH groups 6.5 and 7.5. The pH group 7.5 was significantly different from pH group 8.0.

2. Samples of the tip vs stem, tip vs root and stem vs root were compared at each pH level.
  - a. At pH level 5.0 and 6.5: There was a significant difference between the tip and stem samples and also between the tip and root samples.
  - b. At pH level 7.0, 7.5, 8.0 and 8.5: There was a significant difference between the tip and stem samples and also between the stem and root samples.
  - c. At pH level 5.5 and 6.0: There was a significant difference between the stem and root samples.
3. The total samples of the tip vs stem, tip vs root and stem vs root samples were compared: There was a significant difference between the tip, stem and root samples.
4. The total samples of the plants (tip, stem and root) were compared against pH levels: There were no significant differences between pH groups.

(b) Seven week old plants

The activity of Fe-59 in the seven week old plants was similar to the five week old plants, i.e., the activity of the tip samples was

higher than the root and stem samples (Fig. 2). The highest activity of the tip samples was at pH 5.0. The activity of the tip samples activity then dropped at pH 5.5 and 6.0. At pH 6.5 it increased and continued to increase to pH 7.5. As the pH increased beyond 7.5, the activity decreased. The root activity was greater than the tip activity only at pH 8.0 and 8.5. Activity of the stem samples stayed well below that of the root and tip samples.

1. Samples of the same tissue type were compared against different pH groups.
  - a. Tip samples: There was a significant difference between pH group 5.0 when compared to pH groups 5.5, 6.0, 8.0 and 8.5. There was also a significant difference between pH groups 7.5 and 8.0. There was also a significant difference between pH groups 8.0 and 8.5.
  - b. Stem samples: There was a significant difference between pH groups 5.0 and 5.5 as compared to pH group 6.0. There was also a significant difference between pH group 6.0 and pH groups 6.5 and 7.0.
  - c. Root samples: There was a significant difference between pH groups 5.0, 5.5, 6.0 and 7.5 as compared to pH group 8.0. Also there was a significant difference between pH groups 5.0 and 7.5 as compared to pH group 8.5.
2. Samples of the tip vs stem, tip vs root and stem vs root were

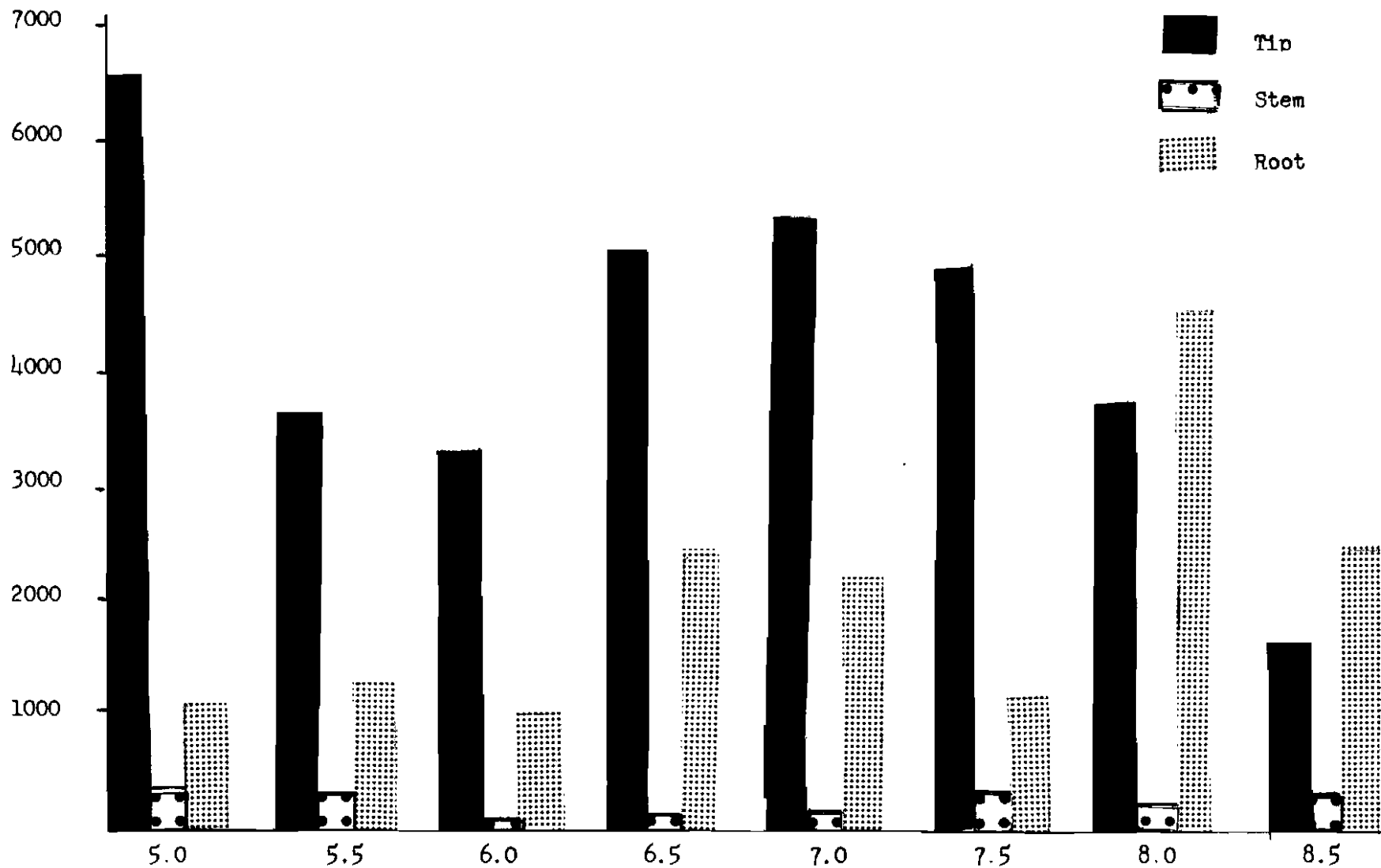


Fig. 2. Mean activity of Fe-59 in tip, stem and root portions of Andropogon gerardi plants at seven week growth in pH ranges 5.0 to 8.5.

compared at each pH level.

- a. At pH levels 5.0 and 7.5: All samples were significantly different from each other.
  - b. At pH levels 5.5, 6.0, 7.0, 8.0 and 8.5: There was a significant difference between the tip and stem samples and also between the stem and root samples.
3. The total samples of the tip vs stem, tip vs root and stem vs root were compared: There was a significant difference between the tip, stem and root samples.
  4. The total samples of the plants (tip, stem and root) were compared against pH levels: There was a significant difference between pH groups 5.0 and 5.5 as compared to all other pH levels.

(c) Nine week old plants

Activity of Fe-59 was similar to the other two age groups (Fig. 3). The highest activity was in the tip sample at pH 5.5. At pH 6.0 the activity of the tip sample dropped rapidly and stayed relatively stable from pH 6.5 to 8.5. At pH 6.0 the tip sample activity dropped to a reading almost equal to that of the root activity. The root activity stayed the same at all pH values. The trend of the tip and root sample activity stayed the same as the pH increased from 6.5 to 8.5. The stem activity was, as in the other two age groups, quite low compared to the tip and root samples.

1. Samples of the same tissue types were compared against different



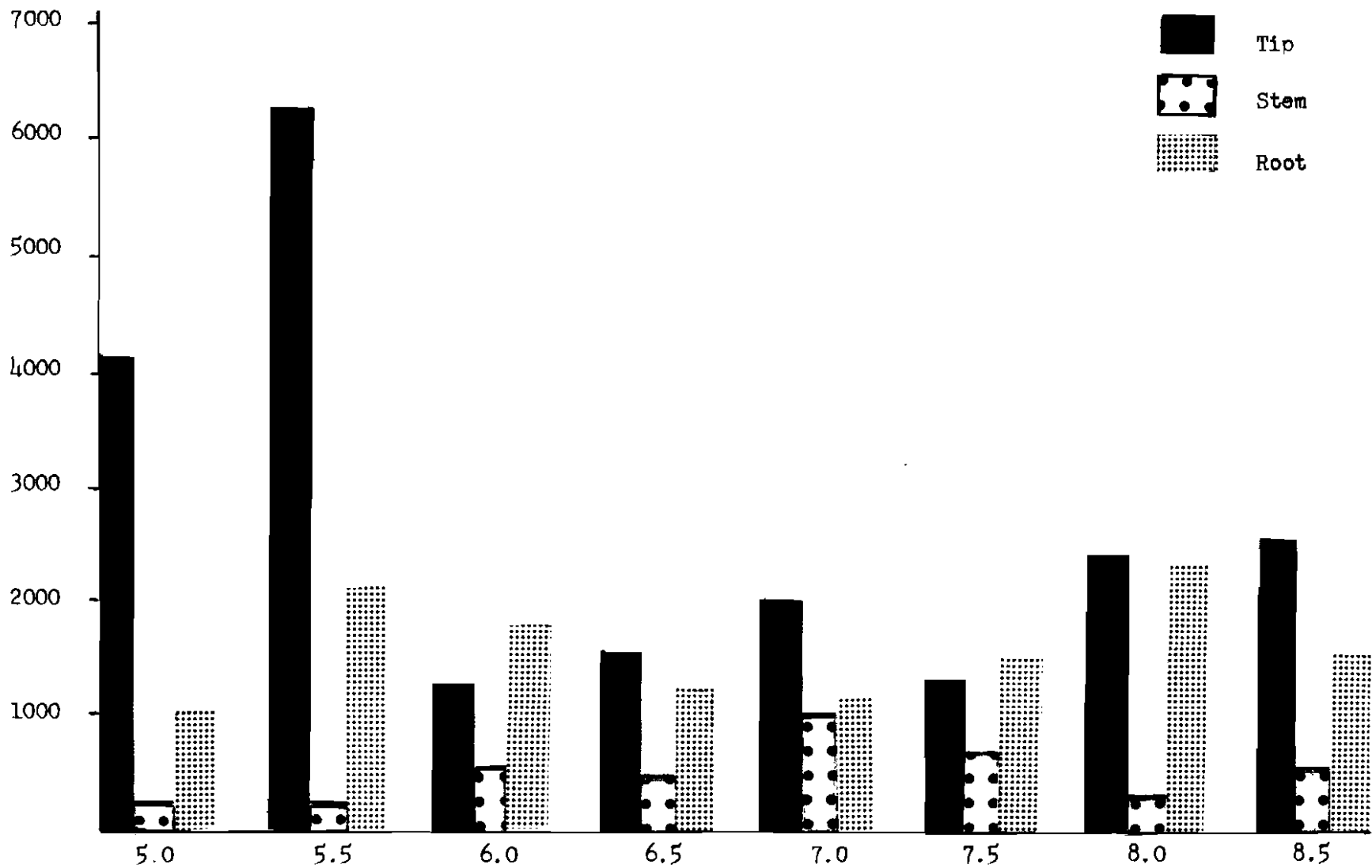


Fig. 3. Mean activity of Fe-59 in tip, stem and root portions of Andropogon gerardi plants at nine week growth in pH ranges 5.0 to 8.5.

pH groups.

- a. Tip samples: There was a significant difference between pH groups 5.0 and 5.5 as compared to pH groups 6.0, 6.5, 7.0, 7.5 and 8.5. There was also a significant difference between pH group 8.5 as compared to pH groups 6.0 and 6.5.
  - b. Stem samples: There was a significant difference between pH groups 5.0 and 5.5 as compared to pH groups 6.0, 7.5 and 8.5. Also pH group 7.5 was significantly different from pH groups 8.0 and 8.5.
  - c. Root samples: There was a significant difference between pH groups 8.0 as compared to pH groups 5.0 and 7.0.
2. Samples of the tip vs stem, tip vs root and stem vs root were compared at each pH level.
    - a. At pH levels 5.0 and 8.5: All samples were significantly different from each other.
    - b. At pH levels 5.5 6.0, 6.5, 7.0 and 8.0: There was a significant difference between the tip and stem samples and also between the stem and root samples.
  3. The total samples of the tip vs stem, tip vs root and stem vs root were compared: There was a significant difference between the tip, stem and root.
  4. The total samples of the plants (tip, stem and root) were compared against different pH levels. There was a significant

difference between pH levels 5.0 and 5.5 as compared to all other pH levels.

(d) The same tissue samples of the three age groups were compared against each other at each pH level.

1. Tip samples:

- a. At pH 5.0: There was a significant difference between five and seven week old tip and also between seven and nine week old tips.
- b. At pH 6.5: There was significant difference between seven and nine week old tips.
- c. At pH 7.5: There was a significant difference between five and seven week old tips and also between seven and nine week old tips.
- d. At pH 8.5: There was a significant difference between five and nine week old tips.

2. Stem samples:

- a. At pH 5.0: There was a significant difference between five and seven week old stems and also between five and nine week old stems.
- b. At pH 6.0: There was a significant difference between seven and nine week old stems.
- c. At pH 6.5: There was a significant difference between five and seven week old stems.

- d. At pH 7.0: There was a significant difference between five and seven week old stems.
- e. At pH 7.5: There was a significant difference between five and nine week old stems.

3. Root samples:

- a. At pH 5.0: There was a significant difference between five and seven week old roots and also between five and nine week old roots.
- b. At pH 6.5: All age groups were significantly different from each other.
- c. At pH 7.5: There was a significant difference between five and seven week old roots.

(e) The different age groups of each species were combined for purposes of statistical analysis. This was done to give an overview of the uptake and translocation of Fe-59 in the plants.

1. Samples of the same tissue type were compared against different pH groups.

- a. Tip samples: There was a significant difference between pH groups 5.0 and 5.5 as compared to pH groups 6.0 and 8.5.
- b. Stem samples: There were no significant differences between pH groups.
- c. Root samples: There was a significant difference between pH groups 5.0 and 8.0 and between pH groups 7.5 and 8.0.

2. Samples of the tip vs stem, tip vs root and stem vs root were compared:

All samples were significantly different from each other.

3. The total samples of the plants (tip, stem and root) were compared against different pH levels:

There were no significant differences between pH groups.

### General

A. gerardi tip sample portions were generally the highest in activity. The root samples were the next highest in activity and the stem portions were the lowest.

The radioautographs, in general, verified the reading of the activity counts. The concentration of Fe-59 was greater in the tip and root portions but less in the stem portions.

The observations of the Fe EDDHA reduction study showed Prussian blue precipitate was found only in the rootlets in the younger root portions. The precipitate was observed at all pH levels and in every age group.

## DISCUSSION

Activity of Fe-59 in Andropogon gerardi, in all age groups studied, is significantly greater in the tip samples than in the stem or root portions. The root samples had significantly higher activity than the stem portions. It has been reported by Brown et al. (1964) that iron is translocated particularly to the actively growing regions of the plant.

Wallace and DeKock (1965), Wallace et al. (1961), Falade (1972), Jenny (1961), and Olsen (1958) have reported that the concentration of  $H^+$  ions change the uptake and translocation of iron in some species of plants. It has also been reported by these investigators that as the pH increases, the iron uptake and translocation decreases. The uptake of Fe-59 in plants at five week of growth is significantly greater at the lower pH values than at the higher pH values. This would indicate that as the  $H^+$  ion concentration decreases the uptake of Fe-59 decreases. The translocation of Fe-59 is significantly greater at the low pH of 5.0 but then decreases at pH values of 5.5 and 6.0. However, at pH 6.5 the translocation of Fe-59 increases but decreases again at pH values 7.0 to 8.5. This would indicate that the concentration of  $H^+$  ions does affect the translocation of Fe-59 but does not affect it completely. This is shown by the high translocation of Fe-59 at pH 6.5.

The uptake of Fe-59 in plants at seven week old growth is different from that of the five week old plants. The uptake is significantly lower at the lower pH values and increases at the higher pH values. This does

not fit the trend of  $H^+$  ion concentration as stated in the literature (Brown and Jones, 1962). The translocation of Fe-59 is significantly higher at pH 5.0 but then decreases at pH values of 5.5 and 6.0. However, it increases again at pH values 6.5 to 8.0 and is not significantly different from that of the pH 5.0 level. This also does not fit the trend of  $H^+$  ion concentration as stated in the literature (Brown et al., 1962).

The uptake of Fe-59 in plants at nine weeks growth is not significantly greater at the lower pH values or the higher pH values. This would indicate that the  $H^+$  ion concentration does not significantly affect the uptake of Fe-59 in this age group. The translocation of Fe-59 is significantly greater at lower pH values of 5.0 and 5.5 and significantly lower at the higher pH values. This would indicate that the  $H^+$  ion concentration affects the translocation of Fe-59 in this age group.

The differences in the uptake and translocation of Fe-59 found in these age groups might be caused by the age factor. Also the rate of growth might have made this difference. The seven week old plants were larger than the five or nine week old plants. This size difference is caused by the fact that the plants do not grow at the same rate even under the same conditions (also the groups were planted at different times and were put in the green house the first 10 days).

The results of the uptake, translocation and reduction sites of Fe-59 in the three age groups studied were combined and statistical analysis was shown in the results. This was done to give an overview of the uptake and

translocation of Fe-59. This combined data gives a more complete and factual analysis of the uptake and translocation of Fe-59 in A. gerardi.

The total results presented in this paper indicate that as the pH increased the uptake and translocation of Fe-59 does decrease slightly, but generally, there is no significant difference between pH groups in the decrease or on the uptake and translocation of Fe-59.

The uptake of Fe-59 in A. gerardi roots does significantly increase when pH 8.0 is reached. At pH 8.5 no significant difference is found. This would indicate that the uptake of Fe-59 in the roots is not significantly affected by the concentration of  $H^+$  ion in the external solution as suggested in the literature. The translocation of Fe-59 to the tip portions of the plant is only significantly decreased at pH 6.5 and 8.5. The inability of the plant to translocate Fe-59 effectively at pH 6.0 is not understood. It could be caused by terminal oxidase enzyme systems that effect translocation of pH 6.0 (Brown, 1956), though it may also have been caused by some error in the experiment.

The inability of the plant to translocate iron at the higher pH of 8.5 might be due to the Fe-59 not being removed from the chelate caused by insufficient amount of  $H^+$  ions. The function of the chelate is to transport Fe into the root cortex. This is done by an ion removing the Fe-59 from the chelate like that of a  $H^+$  ion (Wallace, 1962).

The general activity of the total plant (tip, stem, root), at the various pH groups indicated no significant differences in uptake ability.



Also, in the rootlets of A. gerardi Prussian blue was observed in every pH group sectioned. This would indicate that A. gerardi reduces Fe EDDHA over a wide range of pH levels.

The results found in this study indicate that A. gerardi is a good reducer plant at all pH values studied, i.e., A. gerardi is able to remove about the same amount of Fe-59 from its chelate at lower pH values as it can from higher pH values. Also it is able to translocate about the same amount of Fe-59 at lower pH values as at higher pH values. Olsen (1958) found that some plants that grow in acid soil become chlorotic in a high pH solution and plants that grow in basic soil become iron intoxicated in a low pH solution. This would indicate that A. gerardi will not become iron chlorotic at a relatively high pH or become iron intoxicated at a relatively low pH.

## CONCLUSION

The results of the nine week old plants indicated that as a plant becomes older and perhaps less active the  $H^+$  ion concentration affects the uptake and translocation of Fe-59. Also, in the five week old plants the  $H^+$  ion concentration does affect the translocation of Fe-59 but not the uptake. The  $H^+$  ion concentration generally does not affect the uptake and translocation of Fe-59 in the five week old plants. The seven week old plants were essentially unaffected by the  $H^+$  ion concentration. Only at pH 8.5 does the translocation of iron decrease significantly.

The plants accumulate more iron in the active terminal growing area of the plant than is accumulated in the stem or root areas of the plant. The root accumulates a large portion of iron, but the stem retains very little iron. This would indicate that iron is absorbed into the roots and directly translocated to the active growing area, but does not accumulate in the stem. The uptake and translocation of iron in the seven week old plant study does not fit any kind of pattern of increase or decrease in  $H^+$  ion concentrations as reported in the literature for other kinds of plants.

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## LITERATURE CITED

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