

THE EFFECT OF A LIMITED SUPPLY OF PHOSPHORUS  
ON THE NITRATE-REDUCING ABILITY OF  
KENTUCKY BLUE GRASS

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According to Pozzi-Escot<sup>3</sup> the reducing enzymes were discovered by Jade, Ray, and Pailhode in 1886. Since then many experiments have been made on the nitrate-reducing ability of different kinds of plants. Apparently very few experiments have been carried on to determine the reducase activity in grasses.

The particular phase of this investigation was to determine the effect of a limited supply of phosphorus on the nitrate-reducing ability of Kentucky blue grass, and to study some of the factors influencing such activity.

Kentucky blue grass was sown in gallon crocks in pure washed sand. Ten of the crocks were given a complete nutrient solution and were considered the control pots. Twelve crocks were given a nutrient solution lacking the phosphorus. One pint of solution was applied to each pot every morning until the crop was harvested. One month after the seed was sown a trace of phosphorus was added to the minus-phosphorus plants to keep them alive until the end of the experiment.

The method of procedure was that used by Eckerson<sup>2</sup>. The plants were harvested at eight o'clock in the morning, and at four in the afternoon, to find out whether the time of day was an important factor influencing reducase activity. Separate tests were made of the leaves, leaf-bases, and rhizomes to determine in what part of the plants nitrate-reduction takes place. The test was made by the sulphanilic acid method as described by Yoe<sup>4</sup> with one modification. The standard nitrite solution was prepared in distilled water instead of using the concentrated sulphuric acid. The acid solution was not stable enough.

The results of the experiment are:

1. The leaves harvested in the morning showed an increase in reducase activity over those harvested in the afternoon. There was an increase of 15.3 per cent for the phosphorus-deficient plants and an increase of 6.9 per cent for the phosphorus plants. On the other hand the leaf bases and rhizomes harvested in the afternoon showed an increase over

those harvested in the morning: a 30.4 per cent increase for the leaf bases, and 25.5 per cent for the rhizomes of the minus-phosphorus plants, and an increase of 11.1 per cent for the leaf bases, and 9.1 per cent for the rhizomes of the plus-phosphorus plants.

There is in Kentucky blue grass a decrease in the nitrate reducing ability of the photosynthetic tissue during the day, but the deficiency is not permanent. It is restored again over night. On the other hand, the nitrate-reducing ability of the underground parts is increased in the day time and decreased during the night. It may be that in the day time the synthesis of reductase is retarded by the activity of photosynthesis; or else the reductase may be transported to the rhizomes and root during the day thereby increasing its activity in those parts.

2. The seat of reductase activity in Kentucky blue grass is mostly in the underground parts of the plant. In the phosphorus plants the rhizomes reduced 77.7 per cent more nitrates than the leaves, and the leaf-bases 58.1 per cent more than the leaves. The rhizomes of the minus-phosphorus plants reduced 53.6 per cent more than the leaves, and the leaf-bases 46.8 per cent more than the leaves. Although nitrate-reduction in Kentucky blue grass takes place mostly in the underground parts of the plants, it seems to be influenced by the carbohydrate synthesis of the leaves as is indicated by the fact that the nitrate-reducing ability increases during the day.

3. The juices of the phosphorus deficient plants of Kentucky blue grass have the ability to reduce nitrates to nitrites to a greater degree than the juices of the plus-phosphorus plants. In the minus-phosphorus plants there was an increase over the plus-phosphorus plants of 28.8 per cent in the leaves, 19.4 per cent in the leaf-bases, and 10.6 per cent in the rhizomes. Although the nitrate-reducing ability of minus-phosphorus plants is higher than that of plus-phosphorus plants, the total amount of growth made is considerably less under extreme phosphorus deficiency. This would indicate that the check in growth in phosphorus deficient plants is due to a lack of phosphorus or some other factor, and not to a shortage of reductase. There seems to be an interference with amino acid production. Reduced nitrate compounds must be present in the juice, but the plants are unable to use them. Or else, reductase is unable to change nitrates efficiently in the absence of phosphorus because of the interference of some other factor. There was no appre-

ciable difference in the acidity between the plus-phosphorus and the minus-phosphorus plants.

One of the factors which must not be overlooked in connection with the study is the high temperature under which the tests were made. During the two weeks of testing the daily temperature ranged between 81 degrees F and 103 degrees F, and it never dropped below 60 degrees F at night. High temperature seems to have been beneficial to the minus-phosphorus plants in Biddulph's<sup>1</sup> experiment with crested wheat grass. Under high temperature the phosphorus-deficient plants made better growth than the plants grown in a complete nutrient solution. In this experiment the phosphorus deficient plants changed more nitrates to nitrites than the plants grown in complete nutrients. Under high temperature grasses seem to be able to get along quite well with a limited supply of phosphorus.

#### Bibliography

<sup>1</sup> Biddulph, Orlin. Unpublished Manuscript in Department of Botany, University of S. Dak., Vermillion, S. Dak.

<sup>2</sup> Eckerson, Sophia H. "Protein Synthesis by Plants. Nitrate Reduction" *Botanical Gazette*, XXVII (June 1924), 370-390.

<sup>3</sup> Pozzi-Escot, M. Emm. "The Reducing Enzymes" *American Chemical Journal*, XXIX (1903), 517-563.

<sup>4</sup> Yoe, John H. *Photometric Chemical Analysis*, Vol. 1, John Wiley and Sons, Inc., N. Y., (1928), 308.