# THE INFLUENCE OF THIAMIN ADDITIONS ON THE GERMINATION AND GROWTH OF CERTAIN GRASSES AND OF WHITE CLOVER

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## INTRODUCTION

The physiological reactions in animals as related to thiamin (vitamin $B_1$) nutrition are fairly well established and understood today. The etiology of beriberi and its experimental cure are attributed to thiamin. Its relation to many other physiological disturbances, particularly those of the nervous system, of body metabolism, and glandular function, are being clarified and are of practical significance to modern medical science.

Within the past several years, the utilization of thiamin in the growth of certain of the higher plants has, according to some investigators, resulted in marked increases in total dry matter produced by these plants. Further investigations have proven that thiamin is produced in the leaves of all green plants. From the leaves it is translocated to the roots where it exerts a marked stimulatory effect on root elongation. Our knowledge concerning its specific function in plant responses is negligible at present. Undoubtedly the important economic plants selected by man have survived because of their inherent ability to produce abundant quantities of thiamin and thereby develop large and extensive root systems. Thus its presence in the higher plants is not by mere chance, but rather to secure certain physiological responses which insure the survival of a species under continual natural competition.

The quantity of thiamin produced in the higher plants varies with the species, with the stage of development, and probably with the amount supplied by the soil. The vitamin content adsorbed by the clay complex of the soil may be supplemented by plant residues and organic fertilizers. Recent investigations indicate that the slow growing perennial species respond most markedly to additions of thiamin. The important fast-growing annual crop plants such as corn, wheat, and tomatoes do not seem to respond at all to added thiamin.

It is the purpose of these experiments to demonstrate the effect of thiamin supplements to perennial forage and turf species. Some of the species studied are rapid in their growth and sod forming action whereas others require several years to produce a mature, healthy turf. It is further proposed to clarify our knowledge regarding the influence which applications of thiamin and of natural and artificial fertilizers have on the thiamin content of these turf and forage species.

## REVIEW OF LITERATURE

The first evidence that certain accessory growth substances are necessary for normal plant development was presented by Bottomley (8) (9). In his classical researches (8) (9) (10) (11) (12) (13) lasting from 1912 to 1920 he succeeded in isolating a substance or substances from bacterized peat by means of a phosphotungstic acid precipitate, which on being added to Knop's and Detmer's nutrient solutions greatly stimulated growth and reproduction in the two water plants Lemna major and Lemna minor. Cooper and Funk (16) had previously shown that the curative substance for beri-beri could be precipitated by phosphotungstic acid from an aqueous solution of an alcoholic extract of rice polishings. Later Funk (17) fractionated the phosphotungstic precipitate of anti-beri-beri substance, and succeeded in isolating a substance which he called "vitamine", and which cured polyneuritis in pigeons. In 1912 Hopkins (19) found that the active growth substances in his experiments with rats could be obtained from the phosphotungstic acid precipitate of proteid-free milk. Funk considered the substance which he had isolated from rice polishings to be of the nature of a pyrimidine base. To these accessory growth substances Bottomley gave the name "auximones" and suggested that they were similar to the vitamins necessary for the growth of animals. His work was substantiated by Mockeridge (21) who also attempted to isolate the accessory growth promoting substances from peat and manurial composts. She succeeded in finding nucleic acid, a dinucleotide, and free purine and pyrimidine bases which occurred in various proportions in the organic composts, but she was unable to determine which if any of these substances acted as auximones.

Auximones were once sold on a commercial scale in England to greenhouse operators and garden fanciers. Bottomley secured several patents for the manufacture of auximones from bacterized peat including a patent for this process from the Canadian government (13). The substance which Bottomley precipitated from the filtrate of bacterized peat by means of phosphotungstic acid seems to be related to vitamin $B_1$ as it is known today.

In 1923 Clark and Roller (15) conclusively demonstrated that auximones were not necessary for normal healthy growth of Lemna major as maintained by Bottomley. They pointed out that suitable mineral salt concentrations in the nutrient medium was the significant growth determinant. The stimulating properties of manure to plant growth was described by Breazeale (14) in 1927 to rest primarily on the black, water soluble, organic matter and not on the plant foods, nitrogen, phosphorous or potassium that manures contain.

The structural formula for thiamin was worked out by Williams (28) in 1936 and the synthesis of this vitamin in pure crystalline form was begun shortly thereafter. In 1938 Bonner and Greene (5), working with the pure vitamin, reported data indicating that this vitamin had a stimulating effect on top and root growth of several of the higher plants when supplied in a standard nutrient medium. They also reported that thiamin was produced in the green leaves of plants in the presence of light and translocated to the roots where it stimulated root elongation. In a second paper published a year later Bonner and Greene (6) presented additional data to substantiate their work and further reported that certain plant species are capable of synthesizing, in the presence of light, sufficient thiamin in their leaves for maximum growth, whereas, in other plant species the amount of thiamin produced in the leaves is relatively small and acts as a limiting factor in plant growth and development. The most favorable concentration of thiamin in nutrient medium for maximum plant response was found to be 0.01 mg. per liter of solution. Similar results were found with plants grown in soil. Bonner and Greene also found that when thiamin was supplied to the roots of plants grown in sand cultures, the vitamin content of the leaves was increased over that of control plants not supplied with the vitamin. Since the completion of the experimental work with green plants herein described, Arnon (2) and Hamner (18) have reported only negative responses by several plant species to additions of thiamin supplied in nutrient media. Among the species which they studied were Cosmos, Mustard, and Cocklebur, previously reported by Bonner and Greene to give a stimulatory response.

The hormone action of thiamin in stimulating root elongation has been experimentally demonstrated on excised pea (1) and tomato roots (22). During seed formation large quantities of this vitamin are stored in the aleurone layer as in barley or wheat and in the cotyledons as in the pea (6). The presense of thiamin is readily demonstrated in such forms of organic matter as manure, peat, composts, and decomposing plant constituents, and therefore is probably present in soils which contain any of these materials.

Several chemical tests and biological assays have been utilized in determining the thiamin content of plant tissues and plant products. Of these several tests, the Phycomyces assay appears to be the most simple and results in a high degree of accuracy. This test was developed by Schopfer (25) who showed a quantitative relationship between the thiamin present in the test medium and the dry weight produced by Phycomyces blakesleeanus. Asparagine and glucose are necessary in the medium in excess and the test holds only for micro quantities of vitamin. In a later paper (26) Schopfer found that plant extracts had the same effect on Phycomyces growth as did traces of added thiamin. Bonner and Buckman (7) and Bonner and Greene (6) have further clarified the Phycomyces test as applied to plant tissue and have shown that dried and ground leaves can readily be tested for thiamin by this means.

The influence of fertilizer treatments as affecting the thiamin content of plants and plant products is a comparatively new field of research. Scheunert and Wagner (23) found that additions of NP and NPK to soil cultures had no effect on the thiamin content found in the seeds of rye and barley. In a further investigation (24) with potatoes, fertilization with manure, NP, and NPK failed to give any differences in thiamin content of the tubers.

## EFFECT OF THIAMIN ON SEED GERMINATION AND GROWTH OF SEEDLINGS

### Materials and Methods

Laboratory studies were conducted on four forage species, namely, Kentucky bluegrass, timothy, perennial ryegrass, and white clover. The effects of additions of dilute concentrations of thiamin on seed germination and seedling development were studied.

Open-pollinated commercial seed was secured for each species listed above. Lots of 50 seeds each of Kentucky bluegrass, timothy, and white clover, and lots of 25 seeds of perennial ryegrass were placed in sterile petri dishes on ash free filter paper. The white clover seed was scarified with sand paper before testing. Six treatments consisting of a control and 0.01, 0.1, 0.4, 0.8, and 1.6 mg. of thiamin per liter of solution were studied. Three complete germination tests were run for each species and in each case 3 replications were used per treatment. The rules specified for seed germination tests (29) were adhered to excepting for the additions of thiamin. At the prescribed time for final germination counts each species was harvested and the results with the grasses, excepting Kentucky bluegrass, were determined by separating the roots, tops and attached caryopses of the seedlings and securing oven dry weights. The root growth of Kentucky bluegrass seedlings was almost negligible and therefore only the tops were separated from the caryopses. Because of the difficulty encountered with white clover seedlings in separating the seed coat and the root and top growth, only the total oven dry weights were recorded.

Table 1 indicates that additions of thiamin to seed of Kentucky bluegrass, timothy, perennial ryegrass,

Table 1. Effect of Varying Concentrations of Thiamin on Percentage Seed Germination of Several Forage Species

| Treatment | Distilled Water | Concentrations of Thiamin, Mg. Per Liter of Solution | | | | |
|---|---|---|---|---|---|---|
| | | 0.01 | 0.1 | 0.4 | 0.8 | 1.6 |
| Kentucky bluegrass* | 87 | 88 | 87 | 86 | 87 | 84 |
| Timothy* | 83 | 85 | 86 | 84 | 86 | 87 |
| Perennial ryegrass* | 98 | 96 | 96 | 97 | 98 | 97 |
| White clover* | 88 | 85 | 87 | 85 | 87 | 86 |
| Mean for all species | 89.0 | 88.5 | 89.0 | 88.0 | 89.5 | 88.5 |

* Each figure is the average of 9 replicates.

and white clover had no apparent effect on the germination of these seeds. The mean values are consistently uniform with a range of only 88.0 to 89.5 per cent. In table 2 are presented the results on the rate of dry matter accumulation by seedling growth as effected by thiamin concentrations. The comparative results given in this table also show a high degree of similarity indicating only negative response to thiamin additions.

## EFFECT OF THIAMIN ON GROWTH OF GREEN PLANTS SAND CULTURE STUDIES IN THE GREENHOUSE

### Materials and Methods

A standard culture solution (20) with reduced concentration was added to seeds of commercial *Poa pratensis* germinated on filter paper in sterile petri dishes. When the seedlings were about 2.5 cms. high they were transplanted to screened acid- and alkali-washed sand in 3-gallon glazed crocks with good drainage, twelve uniform seedlings being placed in each of 12 cultures. These cultures were in turn divided into two groups so that the calcium nitrate supplied in nutrient solution to 6 cultures had a partial volume molecular concentration of 0.0045 and the calcium nitrate supplied in nutrient solution to the other 6 cultures had a partial volume molecular concentration of 0.00018. These two groups were randomized and

Table 2. Average Weights in Mgs. for 100 Seedlings for Each of Four Forage Species

As Affected by Varying Concentrations of Thiamin

| Treatment | Distilled Water | Thiamin Concentrations in Mg. Per Liter of Solution | | | | | Distilled Water | Thiamin Concentrations in Mg. Per Liter of Solution | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.01 | 0.1 | 0.4 | 0.8 | 1.6 | | 0.01 | 0.1 | 0.4 | 0.8 | 1.6 |
| Species | Perennial Ryegrass | | | | | | Timothy | | | | | |
| Dry Wt. Tops | 25.3 | 25.8 | 25.4 | 25.8 | 25.7 | 24.5 | 12.6 | 12.4 | 12.6 | 11.9 | 12.3 | 12.6 |
| Dry Wt. Roots | 17.1 | 16.9 | 17.3 | 17.9 | 16.3 | 17.1 | 4.0 | 3.8 | 4.2 | 4.3 | 4.0 | 4.6 |
| Dry Wt. Caryopses | 28.2 | 29.4 | 30.0 | 27.6 | 28.9 | 27.2 | 8.6 | 7.9 | 7.2 | 7.4 | 7.0 | 7.4 |
| Dry Wt. Roots and Tops | 42.4 | 42.7 | 42.7 | 43.7 | 42.0 | 41.6 | 16.6 | 16.2 | 17.8 | 16.2 | 16.3 | 17.2 |
| Species | Kentucky Bluegrass | | | | | | White Clover | | | | | |
| Dry Wt. Tops | 8.5 | 8.2 | 9.0 | 8.4 | 8.5 | 8.3 | | | | | | |
| Dry Wt. Roots and Caryopses | 10.1 | 9.3 | 9.9 | 9.8 | 9.9 | 10.5 | | | | | | |
| Dry Wt. Tops, Roots and Caryopses | | | | | | | 74.5 | 76.2 | 72.6 | 76.2 | 73.8 | 74.9 |

sub-divided so that three cultures in each group received thiamin at the rate of 0.01 mg. per liter of solution and the other three served as controls. Thus a total of 36 Kentucky bluegrass plants was under trial for each of four treatments. The medium and low concentrations of nitrate nitrogen were used with Kentucky bluegrass in order to determine whether the synthesis of thiamin might be related to nitrogen metabolism and vegetative vigor as indicated by growth rates.

Homogenic cuttings (undoubtedly containing minute traces of thiamin) were taken from one plant each of _Poa trivialis_ and _Agrostis tenuis_ and first placed in acid- and alkali-washed sand. A standard nutrient solution of reduced atmospheric concentration was supplied to these cuttings and within one week the cuttings had become well rooted and they were transplanted to the experimental crocks. Six crocks each containing eight uniform cuttings were allotted to each species. Finally three cultures (24 cuttings) from each species were supplied with thiamin at the rate of 0.01 mg. per liter of culture solution and the remaining cultures served as the controls. In this experiment all of the cultures were supplied with a solution containing calcium nitrate at a partial volume molecular concentration of 0.0045. The cultures were randomized in two rows along a single supporting bench in the greenhouse.

The culture medium used as a basis of operation was Shive's 3-salt solution modified to meet the requirements of the experiment. The modifications are given in table 3.

Table 3. Composition of Nutrient Solutions

| Solution Type | Nutrient Salts* — Partial Vol. Mol. Concentration of Salts | | | |
|---|---|---|---|---|
| | $KH_2PO_4$ | $Ca(NO_3)_2$ | $MgSO_4$ | $CaCl_2$ |
| 1 | 0.0023 | 0.00018 | 0.0023 | 0.00432 |
| 2 | 0.0023 | 0.0045 | 0.0023 | |

* Boron, iron, manganese, and zinc were added at the rate of 0.25, 0.1, 0.1, and 0.1 p.p.m. respectively.

The amount of calcium chloride added to the solution was enough to replace any calcium nitrate that was withheld. Such a replacement permits varying the nitrogen content while maintaining the calcium at a uniform concentration. Approximately one liter of solution was supplied to each crock daily by the continuous flow method of Shive and Stahl (27). One liter of fresh solution was flushed through each of the cultures daily, thus obviating the accumulation of salts through the loss of water by evaporation and transpiration. A fresh stock solution of thiamin was made up at weekly intervals and kept in a refrigerator at low temperatures to insure its preservation.

Germination of seed of Kentucky bluegrass was begun on February 8 and the young seedlings were transplanted to the experimental crocks on February 15. The first harvest of top growth was made on May 6. It was intended to take a second clipping but this growth was injured in spraying for aphids and had to be discarded. The roots were harvested immediately after injury to the tops on May 23. Vegetative cuttings of rough stalked meadow bluegrass and colonial bent grass were made on June 16 and they were transplanted to the experimental cultures on June 24. The first top cutting was made on August 8 and the second clipping and roots were harvested on September 28.

## Experimental Results

During the entire course of these experiments it was impossible to note any real differences between comparative replications. The results are given in table 4.

The dry weights of the tops for the thiamin-treated medium nitrate cultures of Kentucky bluegrass averaged 18.34 gms. as compared to 17.58 gms. for the control cultures. The average dry weights of the roots in this series was 7.9 gms. for the thiamin-treated cultures

Table 4. Dry Matter Yield of Top and Root Growth of Three Grass Species Grown in Sand Cultures and Supplied Nutrient Solutions With and Without Additions of Thiamin

| Species | Dry Weight of Top Growth | | Statistical Estimate of Difference Necessary for Significance | Dry Weight of Root Growth | | Statistical Estimate of Difference Necessary for Significance |
|---|---|---|---|---|---|---|
| | Control* | Thiamin* | | Control* | Thiamin* | |
| | gms. | gms. | gms. | gms. | gms. | gms. |
| Kentucky Bluegrass | | | | | | |
| Med. N Series | 18.34 | 17.58 | 0.88 | 7.9 | 7.6 | 0.48 |
| Low N Series | 3.43 | 3.33 | 0.22 | 0.6 | 0.6 | 0.05 |
| Colonial Bent Grass | | | | | | |
| 1st Clipping | 8.49 | 8.77 | 0.96 | | | |
| 2nd Clipping | 4.78 | 4.59 | 0.40 | 4.24 | 4.28 | 0.08 |
| Rough Stalked Meadow Bluegrass | | | | | | |
| 1st Clipping | 9.78 | 9.28 | 0.80 | | | |
| 2nd Clipping | 6.42 | 6.35 | 0.44 | 5.18 | 5.20 | 0.24 |

*Each figure is average of three replicates.

and 7.6 gms. for the control cultures. Similar results can be noted for the thiamin and the control cultures of Kentucky bluegrass in the low nitrate series. The small differences between corresponding average yields do not show any statistical significance. These results show that Kentucky bluegrass did not respond to additions of thiamin in nutrient solutions of normal and low nitrate concentrations when the vitamin was supplied at the rate of 0.01 mg. per liter of solution.

The results from the first top growth clipping of colonial bent grass and rough stalked meadow bluegrass, shown in table 4, also indicate negative results to thiamin additions. No differences could be noted at any time during the course of this experiment between the cultures supplied with thiamin and those serving as controls. Figure 1 page 17 shows representative cultures of colonial bent grass, and figure 2 page 18 shows representative cultures of rough stalked meadow bluegrass. The thiamin-treated cultures of colonial bent grass produced an average of 8.49 gms. of dry weight as compared to 8.77 gms. per culture for the controls. The rough stalked meadow bluegrass cultures averaged 9.78 gms. dry weight for those treated with thiamin and 9.28 gms. for the controls. These data show that thiamin had no significant influence on the yield of tops in this experiment as is indicated by the


Figure 1. The top and root growth of these two cultures of colonial bent grass were essentially the same. Thiamin failed to give any stimulating response.


Figure 2. No evidence of growth stimulation can be noted in these cultures of rough stalked meadow bluegrass. The control plants grew just as well as did the thiamin-treated plants.

statistical analysis.

The results for the second clipping of tops and the root harvest of colonial bent grass present further negative evidence. At no time could any effect pertaining to color of foliage or height of second growth from the thiamin treatment be detected. The dry weight of the second clipping of the thiamin-treated colonial bent grass cultures averaged 4.78 gms. as compared to 4.59 gms. for the controls. The average dry weight of root yield for the thiamin-treated cultures was 4.24 gms. compared to 4.28 gms. for the controls. Similar results can be noted for rough stalked meadow bluegrass. No significant differences between the thiamin-treated and the control cultures can be noted for either the second clipping or the root yields of colonial bent grass or of rough stalked meadow bluegrass.

It is interesting to note the differences in the percentage moisture found in the top growth for the several species and treatments. When a solution containing a normal concentration of nitrogen was supplied, Kentucky bluegrass averaged 78.5 per cent. moisture whereas the percentage moisture of Kentucky bluegrass supplied with a solution having a low concentration of nitrogen was 73.6 per cent., representing a difference of 5 per cent. Rough stalked meadow bluegrass and colonial bent grass both received the same solution and the same treatment, but the rough stalked meadow bluegrass averaged 85.0 per cent. moisture whereas the colonial bent grass averaged 79.9 per cent. or again a difference of 5 per cent. This last difference may possibly present an interesting ecological adaptation both from the standpoint of plant associations and plant morphology.

## Soil Amendment Studies in the Greenhouse

### Materials and Methods

A Sassafras loam soil having the following chemical analysis of readily available nutrients was used in this study: Ca, low; P, very low; K, low; Mg, high; Mn, low; Al, medium; $NO_3$, low. The pH of this soil was found to be 5.10. It is evident that the nutrient supply and the pH of this soil were much below optimum for normal growth of the grasses and the white clover used in this experiment. This soil was mixed with an equal amount of fine sand and the mixture was air dried. Into 1-gallon glazed pots which had been tared to a uniform weight, was placed 8 pounds of the soil mixture per pot.

The soil amendments used in this study on the basis of rate per acre consisted of 20 tons of peat; 20 tons of barnyard manure; 1 ton of ground limestone; and 1 ton of ground limestone plus 1,000 pounds of a 5-10-5 fertilizer mixture. The 5-10-5 fertilizer was made up of nitrate of soda, superphosphate, bone meal, and muriate of potash. These fertilizers were applied by thoroughly mixing the soil in each pot with its particular amendment.

Open-pollinated commercial seed of the several species was sown at the following acre rates: Kentucky bluegrass, 8 pounds; timothy, 8 pounds; perennial ryegrass, 20 pounds; white clover, 2 pounds. In the case of colonial bent grass and rough stalked meadow bluegrass, 20 uniform seedlings of each were placed in the respective pots.

Water and thiamin solution were added regularly to keep the soil at about 50 per cent. of its water holding capacity. The thiamin solution was made up in tap water at a concentration of 0.1 mg. per liter. The moisture content of the pots was normally maintained by adding tap water at fairly regular intervals; however, at least once every week the tap water solution of thiamin was added. In this manner several hundred cc. of thiamin solution were added weekly.

The ryegrass was seeded December 9, 1939 and harvested February 21, 1940. Kentucky bluegrass was seeded December 9, 1939 and harvested February 26, 1940. Timothy and white clover were planted on March 1, 1940 and harvested on April 27 and May 2, 1940, respectively. A second clipping and the roots of these two species were harvested on May 22 and May 23, 1940. The colonial bent grass and rough stalked meadow bluegrass were seeded in acid and alkali-washed sand on June 8, 1940 and transplanted to the experimental pots on June 20, 1940. These species were harvested on November 1 and 2, 1940.

## Experimental Results

The rate of accumulation of dry matter of the roots and tops did not appear to be influenced by the thiamin additions under any of the soil fertility conditions. No exhibition of greater general vigor over the control plants as indicated by taller, healthier plants, was found. Neither was any negative inhibiting effect demonstrated indicating that the thiamin added apparently was passive as effecting dry matter accumulation of roots or tops for these six species. These conclusions are borne out by the data given in tables 5 and 6.

## Field Studies on Fine Turf

### Materials and Methods

Six well established bent grass strains growing on a fertile soil were selected for thiamin studies in the field. These bent grasses consisted of two strains of Agrostis tenuis, namely Colonial and German mixed; one strain of Agrostis canina, or Highland velvet; and three strains of Agrostis stolonifera, namely Washington creeping, Seaside, and Metropolitan creeping. Each bent grass

Table 5. Effect of Added Thiamin on the Accumulation of Dry Weights of Tops for Six Turf and Forage Species Grown with Various Fertilizer Treatments

| Treatment | Check | | Lime | | 5-10-5 Fertilizer | | Peat | | Manure | | Statistical Estimate of Difference Necessary for Significance |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Thiamin | Control | Thiamin | Control | Thiamin | Control | Thiamin | Control | Thiamin | |
| | gms. | gms. | gms. | gms. | gms. | gms. | gms. | gms. | gms. | gms. | gms. |
| Perennial Ryegrass* | 0.8 | 0.6 | 0.9 | 0.9 | 4.7 | 4.8 | 3.7 | 3.6 | 7.8 | 7.4 | 1.48 |
| Kentucky Bluegrass* | 1.0 | 0.9 | 1.1 | 1.1 | 3.3 | 3.4 | 3.3 | 3.1 | 3.7 | 4.1 | 0.72 |
| Timothy* | | | | | | | | | | | |
| 1st Clipping | 1.3 | 1.1 | 1.2 | 1.2 | 6.0 | 5.7 | 3.8 | 3.4 | 10.3 | 10.8 | 2.02 |
| 2nd Clipping | 0.2 | 0.3 | 0.3 | 0.3 | 0.8 | 1.0 | 0.6 | 0.6 | 2.6 | 2.8 | 0.52 |
| White Clover* | | | | | | | | | | | |
| 1st Clipping | 2.6 | 2.5 | 2.7 | 2.9 | 3.9 | 4.0 | 3.6 | 3.8 | 8.2 | 7.8 | 1.16 |
| 2nd Clipping | 1.4 | 1.2 | 1.5 | 1.5 | 1.5 | 1.4 | 1.5 | 1.2 | 4.8 | 4.6 | 0.72 |
| Colonial Bent Grass* | 0.9 | 0.7 | 1.0 | 0.9 | 4.8 | 4.9 | 6.8 | 6.6 | 7.4 | 7.5 | 1.64 |
| Rough Stalked Meadow Bluegrass* | 1.5 | 1.4 | 1.5 | 1.7 | 6.8 | 7.1 | 7.5 | 7.6 | 7.6 | 7.2 | 1.76 |
| Average | 1.2 | 1.1 | 1.3 | 1.3 | 4.0 | 4.0 | 3.9 | 3.7 | 6.6 | 6.5 | |

*Each figure is average of five replicates.

Table 6. Effect of Added Thiamin on the Accumulation of Dry Weights of Roots for Six Turf and Forage Species Grown with Various Fertilizer Treatments

| Treatment | Check | | Lime | | 5-10-5 Fertilizer | | Peat | | Manure | | Statistical Estimate of Difference Necessary for Significance |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Thiamin | Control | Thiamin | Control | Thiamin | Control | Thiamin | Control | Thiamin | |
| | gms. | gms. | gms. | gms. | gms. | gms. | gms. | gms. | gms. | gms. | gms. |
| Perennial Ryegrass* | 3.3 | 2.8 | 3.5 | 3.4 | 5.5 | 5.6 | 5.9 | 6.7 | 6.3 | 6.0 | 0.84 |
| Kentucky Bluegrass* | 1.2 | 1.1 | 1.3 | 1.1 | 1.6 | 1.6 | 1.6 | 1.6 | 1.4 | 1.5 | 0.20 |
| Timothy* | 2.0 | 1.8 | 2.2 | 1.8 | 5.0 | 5.3 | 4.2 | 4.1 | 6.2 | 6.9 | 2.04 |
| White Clover* | 0.7 | 0.6 | 0.8 | 1.1 | 2.0 | 1.9 | 2.0 | 1.7 | 3.8 | 3.2 | 0.60 |
| Colonial Bent Grass* | 1.7 | 2.0 | 2.9 | 2.8 | 4.8 | 5.0 | 6.1 | 6.3 | 7.6 | 7.3 | 1.20 |
| Rough Stalked Meadow Bluegrass* | 3.2 | 3.2 | 3.3 | 3.6 | 5.1 | 5.5 | 5.9 | 6.0 | 6.2 | 6.0 | 0.80 |
| Average | 2.0 | 1.9 | 2.3 | 2.3 | 4.0 | 4.2 | 4.3 | 4.4 | 5.3 | 5.2 | |

*Each figure is average of five replicates.

plot was 20 by 30 feet in size. These were divided into 3 equal units of 10 by 20 feet, thus affording 3 replications per bent grass strain and giving a total of 18 plots.

Three treatments consisting of tap water, 0.01 p.p.m., and 0.001 p.p.m. of thiamin were applied to the respective plots once every two weeks with a 5-gallon sprinkling can. The thiamin concentrations added were determined on the basis of soil weight to a 6 inch depth. The plots receiving 0.01 p.p.m. of thiamin thus were given 0.036 gms. of thiamin every two weeks, and those receiving 0.001 p.p.m. of thiamin were given 0.0036 gms. of thiamin every two weeks. Immediately following the thiamin treatment, tap water was sprinkled from a pressure hose for 1 or 2 minutes per plot to wash the thiamin into the soil and prevent its loss by evaporation. Treatment with thiamin began April 15, 1940. At this early spring period the grass roots and rootlets are being rapidly formed, and good opportunity to effect root stimulation is presented.

The results of this experiment were measured by taking clippings from each plot at weekly intervals for dry weight comparisons, and further by securing dry weights of roots and root depth penetrations according to Blaser's (3) soil prism method. The root samples were taken in triplicate for each strain and treatment on August 1, 1940. No distinction is made between roots and rhizomes.

## Experimental Results

At no time during the course of this experiment were there visible differences between the thiamin-treated and the control plots of bent grasses. The results for the accumulation of dry matter of the tops and roots and for root penetrations are given in tables 7 and 8.

No significant differences were found between treatments for accumulation of dry matter for the tops and roots nor was there any significant response to thiamin by greater root penetration for any of the six strains of bent grass.

Table 7. Effect of Added Thiamin on the Accumulation of Dry Weights of Tops for Six Strains of Bent Grass

| Strain of Bent Grass | Yields in Grams of Dry Matter Produced | | |
|---|---|---|---|
| | Control | Thiamin 0.01 p.p.m. | Thiamin 0.001 p.p.m. |
| Seaside | 594.7 | 454.5 | 520.3 |
| German | 542.7 | 578.2 | 459.4 |
| Colonial | 453.9 | 543.0 | 608.6 |
| Highland | 472.8 | 501.8 | 593.3 |
| Metropolitan | 556.7 | 485.3 | 605.3 |
| Washington | 468.8 | 390.8 | 422.2 |
| Average* | 514.9 | 492.3 | 534.8 |

*Statistical estimate of differences between averages necessary for significance = 63.1 gms.

Table 8. Effect of Added Thiamin on Accumulation of Dry Matter of the Roots and on Root Penetration for Six Strains of Bent Grass

| Strain of Bent Grass | Yield in Grams of Dry Matter Produced* | | | Penetration of Roots in Inches* | | |
|---|---|---|---|---|---|---|
| | Control | Thiamin 0.01 p.p.m. | Thiamin 0.001 p.p.m. | Control | Thiamin 0.01 p.p.m. | Thiamin 0.001 p.p.m. |
| Seaside | 0.940 | 1.105 | 1.176 | 8.8 | 10.2 | 9.5 |
| German | 1.435 | 1.256 | 1.082 | 10.3 | 9.5 | 9.7 |
| Colonial | 0.998 | 1.258 | 1.421 | 10.3 | 9.2 | 9.5 |
| Highland | 1.001 | 1.153 | 1.055 | 10.3 | 9.5 | 10.1 |
| Metropolitan | 0.938 | 1.019 | 0.919 | 9.3 | 9.0 | 8.5 |
| Washington | 0.960 | 0.964 | 0.893 | 8.3 | 8.7 | 8.3 |
| Average | 1.045 | 1.126 | 1.091 | 9.6 | 9.4 | 9.3 |

* Each figure is the average of three replicates.

Statistical estimate of differences necessary for significance:

1. Between averages for gms. of dry matter produced = 0.43 gms.
2. Between averages for root penetration = 0.68 inches.

## ASSAY OF PLANT TISSUES FOR THIAMIN BY MEANS OF THE PHYCOMYCES TEST

### Materials and Methods

The materials assayed for thiamin consisted of the dried top growth of six forage species grown under five soil fertility treatments and of the top growth of three forage species grown in sand culture experiments as heretofore reported. These tissues were first dried at 70 degrees Centigrade, ground to a fineness suitable for mineral analysis, and then re-dried at 60 degrees Centigrade.

The basic nutrient medium used in the following experiments is that of Schopfer (25) and has the composition shown in table 9. The experimental cultures were made with 25 cc. of this nutrient medium in 125 cc. Pyrex Erlenmeyer flasks. Test samples each consisting of 10 mg. of dried and ground plant tissue were added to this medium, and the culture flasks were then autoclaved for 15 minutes at a pressure of 15 pounds.

Table 9. Composition of the Basic Medium for the Growth of Phycomyces blakesleeanus

| | |
|---|---|
| $MgSO_4 \cdot 7H_2O$ | 0.5 gms. |
| $KH_2PO_4$ | 1.5 " |
| Asparagin | 4.0 " |
| Dextrose | 100.0 " |
| Distilled Water | 1000.0 ml. |

Stock cultures of Phycomyces blakesleeanus were kept growing on a natural medium of 2 per cent. malt and 2 per cent. agar. The cultural vessels were inoculated with 1 cc. of a uniform spore suspension made from these stock cultures. After inoculation the cultures were incubated for 10 days at 25 degrees Centigrade, at which time the mycelial growth was filtered off, washed, dried at 60 degrees Centigrade for 24 hours, and weighed. Each test was replicated four times. Since asparagin is known to contain minute quantities of thiamin, control cultures were inoculated with Phycomyces for each experiment, and the weight of the growth produced in these control cultures was subtracted from the test cultures.

## Experimental Results

### Standard Growth Curve

In order to interpret the assays for the various turf and forage samples, it was necessary to make a standard growth curve by measuring the mycelial growth produced in cultures to which known concentrations of thiamin were added. The results are graphically presented in figure 3. This graph shows that, under the conditions of this experiment, there is a direct increase in dry weight accumulation with an increase in thiamin concentration up to 0.80 of a microgram. A definite vertical decline occurs from

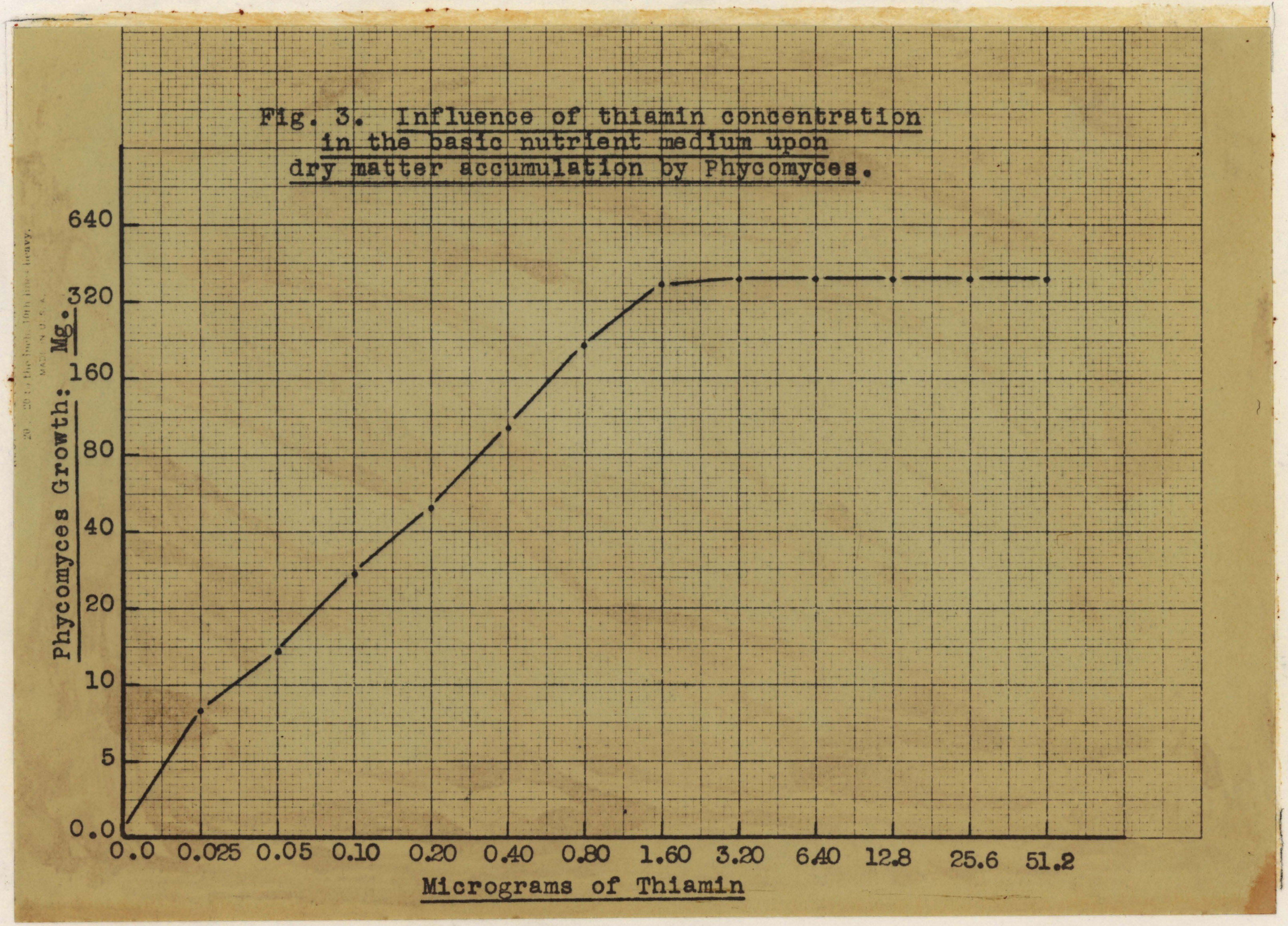


Fig. 3. Influence of thiamin concentration in the basic nutrient medium upon dry matter accumulation by Phycomyces.

0.80 to 3.20 micrograms, and at still greater concentrations no further growth was secured, indicating that, at these high concentrations of thiamin, some substance (probably asparagin) was limiting the growth response of Phycomyces. The essential part of this graph from the standpoint of interpreting the experimental results that follow, lies between the points representing 0.025 and 0.20 micrograms of thiamin. Since this range of concentration gives a straight line growth response for Phycomyces, no difficulty was experienced in interpreting mycelial growth in terms of micrograms of thiamin present in a sample containing a higherto unknown quantity.

## Concentration of Thiamin in Grass Leaves as Effected by Additions of Thiamin in Nutrient Solutions

The Phycomyces assays for the three grass species grown with nutrient cultures in the greenhouse are presented in tables 10 and 11.

The statistical analysis strongly indicates a significant increase in thiamin concentration in the leaves of those plants which were supplied thiamin in the nutrient medium as compared to the control plants. While the assay on the second clipping of colonial bent grass failed to show a thiamin increase in the leaf tissue of the plants supplied with vitamin, the cumulative data of the other five tests strongly suggest that thiamin can be absorbed

Table 10. Effect of Thiamin Additions in Nutrient Medium to Thiamin Content of Several Grasses, as Measured by Dry Matter Accumulation by Phycomyces

| Species | Weight of Mycelial Growth | | Statistical Estimate of Difference Necessary for Significance |
|---|---|---|---|
| | Control | Thiamin | |
| | mgs. | mgs. | |
| Kentucky Bluegrass* | | | |
| Low Nitrate | 10 | 39 | 5.92 |
| Medium Nitrate | 9 | 20 | 2.96 |
| Colonial Bent Grass* | | | |
| 1st Clipping | 15 | 22 | 3.56 |
| 2nd Clipping | 16 | 15 | 1.16 |
| Rough Stalked Meadow Bluegrass* | | | |
| 1st Clipping | 19 | 25 | 4.72 |
| 2nd Clipping | 14 | 22 | 1.48 |
| Mean for all species | 14 | 24 | |

* Each figure is average of three replicates.

Table 11. Effect of Thiamin Additions in Nutrient Medium on the Thiamin Concentrations Found in the Leaves of Three Grasses

| Species | Mgs. of Thiamin per kg. of Dry Matter | | Statistical Estimate of Difference Necessary for Significance |
|---|---|---|---|
| | Control | Thiamin | |
| Kentucky Bluegrass | | | |
| Low Nitrate | 3.8 | 15.8 | 2.40 |
| Medium Nitrate | 3.4 | 7.5 | 1.11 |
| Colonial Bent Grass | | | |
| 1st Clipping | 4.9 | 8.0 | 1.30 |
| 2nd Clipping | 5.3 | 4.9 | 0.38 |
| Rough Stalked Meadow Bluegrass | | | |
| 1st Clipping | 7.0 | 8.7 | 0.31 |
| 2nd Clipping | 4.7 | 8.0 | 0.15 |
| Mean for all species | 4.9 | 8.8 | |

by plants and can accumulate at least temporarily in the green leaves.

The data in these tables also suggest that greater quantities of thiamin per unit weight of dry matter accumulated in the leaves of the Kentucky bluegrass which received a low concentration of nitrate nitrogen, than the Kentucky bluegrass which was supplied with a normal nitrate nitrogen treatment. This result appears feasible since equal quantities of thiamin were supplied the low and normal nitrogen cultures of Kentucky bluegrass. Since a reduction of growth stimulation was found in the low nitrate grass cultures there was a smaller total plant area over which to distribute the absorbed thiamin, and therefore greater quantities would be found per unit of dry weight of plant tissue.

The data further show that at least some of the absorbed thiamin is first translocated to the leaves. If this absorbed thiamin is used by the green plant for meristematic activity of root tips it must be returned to the roots. No indication of the length of time that it will remain in the leaves nor whether or not it is translocated again can be secured from these data.

Upon the basis of the average values obtained in the control series of plants it appears that the leaves of rough stalked meadow bluegrass and colonial bent grass were about equal in thiamin production, whereas Kentucky bluegrass produced a considerably smaller quantity. However, Kentucky bluegrass was not grown at the same time in the greenhouse as the other two grasses, and environmental differences may have had some influence here. The concentration of nitrate nitrogen in the nutrient medium had no effect on the thiamin concentration found in Kentucky bluegrass.

## Concentration of Thiamin in the Top Growth of Forage and Turf Plants Grown in Soil Cultures as Effected by Thiamin Additions and by Fertility Amendments

The results of the Phycomyces assay of the six forage species grown under various soil fertility treatments are given in tables 12 and 13. Since interest centers particularly on the thiamin concentrations, the discussion will be limited to this latter table.

The statistical estimates for significant differences between comparable means, as shown in table 13, do not give as clear cut results as might be desired. Nevertheless, with a majority of the comparable mean values there is an increase in thiamin concentration as found in the top growth of those cultures given thiamin additions. The general mean values for this table show consistent increases. While this increase is not great, it must be

Table 12. Effect of Thiamin Additions and Soil Fertility Treatments on the Thiamin Concentrations in Several Grass and Legume Species as Measured by Dry Matter Accumulation by Phycomyces

| Species | Check | | Lime | | 5-10-5 Fertilizer | | Peat | | Manure | | Statistical Estimate of Difference Necessary for Significance |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Thiamin | Control | Thiamin | Control | Thiamin | Control | Thiamin | Control | Thiamin | |
| | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | mgs. | |
| Perennial Ryegrass* | 18 | 19 | 18 | 19 | 19 | 20 | 19 | 20 | 18 | 20 | 1.04 |
| Kentucky Bluegrass* | 12 | 13 | 12 | 13 | 14 | 14 | 14 | 15 | 14 | 15 | 1.44 |
| Timothy* | 4 | 6 | 5 | 7 | 7 | 7 | 4 | 9 | 12 | 12 | 2.12 |
| White Clover* | 16 | 17 | 16 | 18 | 15 | 17 | 19 | 18 | 15 | 18 | 1.28 |
| Colonial Bent Grass* | 15 | 16 | 15 | 15 | 16 | 16 | 16 | 16 | 16 | 16 | 0.88 |
| Rough Stalked Meadow Bluegrass* | 17 | 18 | 17 | 19 | 17 | 18 | 18 | 18 | 20 | 19 | 1.28 |
| Mean for all species | 14 | 15 | 14 | 15 | 15 | 15 | 15 | 16 | 16 | 17 | |

* Each figure is the average of four replicates.

Table 13. Effect of Thiamin Additions and Soil Fertility Treatments on the Thiamin Concentrations in the Top Growth of Several Grasses and Legumes

| Species | Mgs. of Thiamin Per Kg. of Dry Matter | | | | | | | | | | Statistical Estimate of Difference Necessary for Significance |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Check | | Lime | | 5-10-5 Fertilizer | | Peat | | Manure | | |
| | Control | Thiamin | Control | Thiamin | Control | Thiamin | Control | Thiamin | Control | Thiamin | |
| Perennial Ryegrass | 6.4 | 7.0 | 6.4 | 7.0 | 7.0 | 7.5 | 7.0 | 7.5 | 6.4 | 7.5 | 0.39 |
| Kentucky Bluegrass | 4.3 | 4.5 | 4.3 | 4.5 | 4.7 | 4.7 | 4.7 | 4.9 | 4.7 | 4.9 | 0.47 |
| Timothy | 1.4 | 2.2 | 1.8 | 2.6 | 2.6 | 2.6 | 1.4 | 3.4 | 4.3 | 4.3 | 0.76 |
| White Clover | 5.3 | 5.8 | 5.3 | 6.4 | 4.9 | 5.8 | 7.0 | 6.4 | 4.9 | 6.4 | 0.46 |
| Colonial Bent Grass | 4.9 | 5.3 | 4.9 | 4.9 | 5.3 | 5.3 | 5.3 | 5.3 | 5.3 | 5.3 | 0.29 |
| Rough Stalked Meadow Bluegrass | 5.8 | 6.4 | 5.8 | 7.0 | 5.8 | 6.4 | 6.4 | 6.4 | 7.5 | 7.0 | 0.47 |
| Mean for all species | 4.7 | 5.2 | 4.8 | 5.4 | 5.1 | 5.4 | 5.3 | 5.7 | 5.5 | 5.9 | |

remembered that the soil cultures received only several hundred cc. of dilute thiamin solution weekly as compared to the continuous drip solution used for the sand culture studies previously described, which received several thousand cc. of dilute thiamin solution each week. Since these results are fairly regular and since a majority of the comparable mean values indicate significant differences, it seems justifiable to conclude that added thiamin can be taken up from soil by plant roots and can be detected in the top growth.

To determine the effect of fertilizer treatment on the production of thiamin by these species, the mean values for those plants not supplied thiamin may be compared. Most of these comparable mean values are not significantly different. However, the timothy species seems to give positive responses in thiamin concentration, depending on the fertilizer treatments. Since timothy is the only stemmy species in these tests it appears possible that under the higher fertility levels there is an increase in the relative percentage of leafiness, which in turn may be responsible for the increase in thiamin content as measured by the total plant assay. If the values for timothy are eliminated from these considerations the comparable mean values for Phycomyces growth shown in table 12 for all species are as follows: check = 16; lime = 16; 5-10-5 fertilizer = 16; peat = 17; and manure = 17. The proximity of these mean values to each other strongly indicates that soil fertility did not tend to increase the thiamin content of these forage species to any significant concentration. It appears therefore that physiological and genetic factors are primarily responsible for the thiamin production in green plants, rather than cultural conditions.

It is difficult to compare directly the concentration of thiamin in these several turf and forage species, under the conditions of these experiments. The species were not grown at the same time in the greenhouse under comparable environmental conditions. Furthermore, in the case of white clover the stolons and petioles were composited with the leaves, whereas most of the grasses, excepting timothy, consist almost entirely of leaves. Considering a thiamin analysis representing the entire top growth for these species, and further allowing for some variability due to environmental differences and differences in the age of these plants, they might roughly be ranked in the following order as to thiamin content: perennial ryegrass, rough stalked meadow bluegrass, colonial bent grass, white clover, Kentucky bluegrass and timothy. Perennial ryegrass was found to have the highest concentration of thiamin, and timothy the lowest.

## DISCUSSION

Investigations on the effects of thiamin relative to seed germination, seedling growth and rate of accumulation of dry matter for a number of grass and legume species have given only negative results. The discrepancies between the experiments herein reported and those of Bonner and Greene (5) (6), cannot be explained at present.

Open-pollinated seed of commercial origin of Kentucky bluegrass, timothy, perennial ryegrass, and white clover were placed in sterile petri dishes and treated with distilled water and five concentrations of thiamin, namely; 0.01, 0.1, 0.4, 0.8, and 1.6 mg. per liter of distilled water. These concentrations of thiamin neither increased nor retarded the seed germination of these four species. The mean germination values for the control and all thiamin concentrations were consistently uniform, ranging from 88.0 to 89.5 per cent. Similarly the dry weights for the seedlings of these four species, grown with a control and five concentrations of thiamin, showed no increase in dry matter accumulation of tops or roots, nor were any differences in height or leaf color detectable at any time during the course of these experiments.

Kentucky bluegrass seedlings from open-pollinated commercial seed were grown in sand cultures with two nutrient solutions; one contained a low nitrate nitrogen concentration, and the other had a medium nitrate nitrogen supply. Thiamin was added to one half of these cultures at a concentration of 0.01 p.p.m., which, according to Bonner and Greene (6), is the most favorable concentration for maximum plant response. The control cultures of the medium nitrate series produced, in terms of dry matter, 17.58 gms. of top and 7.6 gms. of root growth, as compared to the thiamin treated medium nitrate series which produced 18.34 gms. of top and 7.9 gms. of root growth. Simmilar results were found for the low nitrate series, excepting that the total yields were smaller. Additions of thiamin in no way effected these results.

Homogenetic cuttings of colonial bent grass and of rough stalked meadow bluegrass were grown in sand cultures with a standard nutrient solution. No significant differences were found between the control and the thiamin-treated plants. Bonner and Greene (6) point out that in two months their cultures of rough stalked meadow bluegrass which received thiamin produced a total of more than 13 gms. dry weight of clippings per crock, whereas the control crocks averaged only 2.05 gms. In the present experiment the average total dry weight of grass per control crock at the end of three months was 16.20 gms., and 15.63 gms.

for those crocks to which thiamin was added in nutrient medium. The yield of dry matter from the control crocks of rough stalked meadow bluegrass reported in this experiment, was many times greater than in the experiments reported by Bonner and Greens.

The addition of thiamin to plants growing in a Sassafras loam soil with fertility amendments of lime, 5-10-5 fertilizer, peat, and manure, failed to give any increase in dry matter production of either tops or roots when compared to suitable controls. Perennial ryegrass, Kentucky bluegrass, timothy, white clover, rough stalked meadow bluegrass, and colonial bent grass were grown for this study. Thiamin was not a factor in plant growth stimulation under conditions of either low or high fertility, nor with additions of mineral or organic fertilizers. Statistical differences which could be attributed to additions of thiamin as compared with suitable controls were in no cases significant.

Experiments with six mature, well established bent grasses used for fine turf were conducted in the field. Thiamin was added weekly to replicated and randomized plots in concentrations of 0.001 and 0.01 mg. on the basis of soil weight per plot to a 6 inch depth. Studies of top and root yields of dry matter failed to give any significant responses over the yields of the control plots.

A soil prism study of root penetration indicated that the added thiamin did not increase root depth for these six bent grasses. The thiamin additions to these plots were started in April at the time when new roots are being formed by grasses.

From the standpoint of dry matter accumulation or growth increase, it appears that thiamin has played only a passive role with the species herein reported. The results secured with the various strains and species under laboratory, sand culture, soil culture, and field conditions, and under varying conditions of soil fertility, were markedly different in many cases, but the addition of thiamin in no way changed these relationships.

The Phycomyces assay for thiamin was used to determine the concentration of this vitamin in the top growth of the species grown under sand and soil culture conditions in the greenhouse. Additions of thiamin in nutrient solution and at a concentration of 0.01 p.p.m. to cultures of Kentucky bluegrass, rough stalked meadow bluegrass, and colonial bent grass increased the concentration of thiamin as found in the leaves of these plants, as compared to the controls. The general mean values for the controls was 4.9, and for the thiamin-treated plants 8.8 mgs. of thiamin per kilogram of dry matter. A low concentration of nitrate nitrogen in the nutrient solution compared to a medium concentration did not effect the amount of thiamin found in the leaves of Kentucky bluegrass. Six grass and legume species growing under several soil fertility levels were analyzed for thiamin. In general the mean values show that thiamin additions in tap water at weekly intervals tended to give a small increase in the thiamin content of the top growth, indicating that plants absorb thiamin from the soil even though this absorbed thiamin apparently does not stimulate plant growth.

The fertility treatments consisting of lime, 5-10-5 fertilizer, peat, and manure, did not materially effect the concentration of thiamin as found in the leaves of these six grass and legume species. Omitting the mean values for timothy which are not consistent with the other mean values, the general means in mgs. of thiamin for the fertilizer treatments are as follows: check = 5.3; lime = 5.3; 5-10-5 fertilizer = 5.6; peat = 6.1; and manure = 5.8. Comparable mean values for the control and thiamin-treated plants grown under these several fertilizer treatments show only small increases in thiamin concentrations in those plants given 5-10-5 fertilizer, peat, and manure, as compared to the check and lime series. In general it is concluded that fertilizer treatment did not greatly effect the thiamin production of the six grass and legume species as herein reported. Strong, healthy, vigorous plants apparently do not produce significantly higher quantities of thiamin per unit weight as compared to less vigorous plants. The small increases found in the tests reported here are probably attributable to the thiamin absorbed by the plants from the organic fertilizers. Since the quantity of thiamin in the soil or in the organic fertilizers is relatively low, only very small increases in thiamin content of top growth could be expected.

## SUMMARY AND CONCLUSIONS

1. Thiamin concentrations of 0.01, 0.1, 0.4, 0.8, and 1.6 mg. per liter of distilled water were added to seed of Kentucky bluegrass, timothy, perennial ryegrass, and white clover germinating in sterile petri dishes. These thiamin concentrations neither stimulated nor retarded the percentage germination of these seeds when compared to a control series supplied distilled water only.

2. Thiamin concentraions of 0.01, 0.1, 0.4, 0.8, and 1.6 mg. per liter of distilled water were added to seedlings of Kentucky bluegrass, timothy, perennial ryegrass, and white clover growing in sterile petri dishes. No visible differences of top or root growth could be noted for any of these species. No significant differences were found in the accumulation of dry matter.

3. Thiamin additions had no effect upon the accumulation of dry matter of Kentucky bluegrass growing

in sand cultures and supplied with nutrient solutions containing medium and low concentrations of nitrate nitrogen. Rough stalked meadow bluegrass and colonial bent grass supplied with a standard nutrient solution, also failed to give any significant differences which could be ascribed to thiamin. The thiamin was supplied at the rate of 0.01 mg. per liter of solution.

4. Thiamin was added at weekly intervals to soil cultures of Kentucky bluegrass, perennial ryegrass, timothy, white clover, rough stalked meadow bluegrass, and colonial bent grass. Five levels of soil fertility were examined, namely; check, lime, 5-10-5 fertilizer, peat, and manure series. No visible differences were detected between the control plants and those receiving thiamin. No significant differences were obtained in the accumulation of dry matter.

5. Six established plots of bent grass were studied in the field. Thiamin was added at the rate of 0.001 and 0.01 p.p.m. on the basis of soil weight of the plots to a six inch depth. No growth stimulation or effects from thiamin additions of any kind were found in top growth, root growth, or root penetration.

6. The Phycomyces assay for thiamin showed that thiamin added in nutrient cultures to plants growing in sand will, at least temporarily, increase the concentration

of this vitamin in the top growth. A low concentration of nitrate nitrogen did not effect the amount of thiamin found in the leaves of Kentucky bluegrass, as compared to a medium concentration of nitrate nitrogen. Thiamin added to plants growing in soil was also detectable in the top growth of these plants.

7. Fertility treatments consisting of lime, 5-10-5 fertilizer, peat, and manure did not materially effect the thiamin content of the different plants growing in soil culture. Healthier plants resulting from proper nutritional procedure did not seem to increase the thiamin content as measured on a unit weight basis.

8. Thiamin concentration in the species studied did not prove to be a limiting factor in growth as measured by dry matter accumulation of tops and roots.

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