THE INFLUENCE OF TEMPERATURE, CALCIUM AND ARSENIOUS ACID ON SEEDLINGS OF KENTUCKY BLUEGRASS

AUBREY W. NAYLOR *

The natural range of Kentucky bluegrass over the northern half of the United States and southern Canada indicates that it is adapted to cool conditions. In addition, it has been observed that bluegrass grows slowly in hot weather and, when well established, most vigorously at temperatures ranging between 60° and 75° F. Also it is known that bluegrass will flourish and grow well on calcareous soils, either because of their lime content or because of other associated nutritive conditions.

During recent years there have been reports that certain chemicals, other than those usually considered as nutrients, will stimulate growth. Thus arsenic salts have been found in some cases to cause a distinct increase in growth of well established bluegrass. These observations were made, as have been most other investigations, on mature plants.

Some preliminary work, however, has indicated that Kentucky bluegrass, once it has become established, will grow well under conditions which are unfavorable for the germination of its seed and for early growth. In the experiments reported here, attention is directed primarily to the effect of temperature, calcium content of soil, and arsenic on germination of seeds and on the growth of seedlings.

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* Agent in the Bureau of Plant Industry cooperating with the University of Chicago.

Materials and Methods

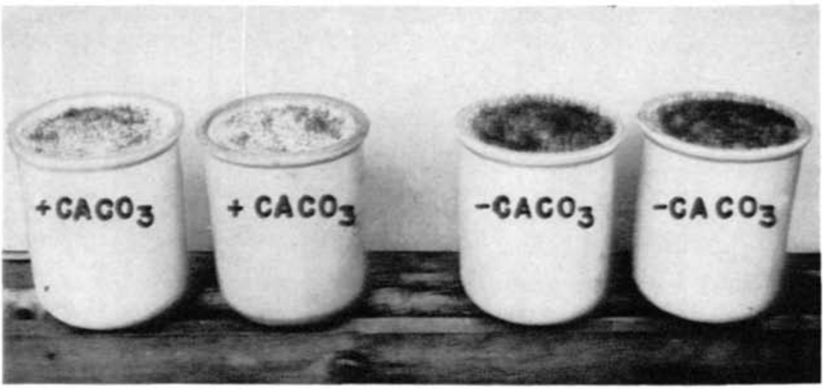
Kentucky bluegrass seed of high purity was planted in washed quartz sand contained in 2-gallon glazed crocks. Three grams of seed were used in each crock, except in the calcium carbonate series set up March 20, 1938, in which two grams were used. The seed was evenly distributed, then lightly covered with sand and watered daily with distilled water until germination began. Each crock then received biweekly applications of a nutrient solution containing nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, and traces of zinc, boron, manganese, copper, and iron.

While making trial runs it was found that fine quartz sand was unsuitable for winter work, perhaps because damping-off fungi thrived under the prevailing warm, moist, cloudy conditions. The most successful practice was to use coarse quartz sand and to spray the seed at the time of planting and at frequent intervals thereafter with a dilute solution of a mercury fungicide.

Ninety days after the experiment was started, half of the pots in each series were sampled to determine the relative number of plants which survived. For this purpose, plants from 1 centimeter squares (about 1/6 of a square inch) along two diameters of each crock were counted. At the termination of the experiments dealing with calcium carbonate, fresh and dry weights of the grass were taken.

Experiments with Calcium Carbonate

In the calcium carbonate series started March 20, 1938, 33 crocks were filled with sand which had approximately one-half pound of calcium carbonate per crock mixed with it. This was equivalent to working limestone into the top 6½ inches of soil at the rate of 37,000 pounds to the acre. The solution which dripped from the crocks after watering had a reaction of pH 8.0. The crocks were watered at intervals with water



Effect of lime on germination and early growth of Kentucky bluegrass seedlings. The two crocks on the left marked "+ $CaCO_3$" each contained quartz sand mixed with one-half pound of calcium carbonate. The two crocks marked "—$CaCO_3$" contained only pure quartz sand. All of the crocks received equal amounts of a complete nutrient solution. Thirteen days after planting, when this picture was taken, there were noticeably fewer and smaller seedlings in the +$CaCO_3$ than in the —$CaCO_3$ crocks.

heavily charged with calcium carbonate to avoid any chance of a decrease in pH during the course of the experiment. Twenty-eight crocks were set up in the same manner except that no calcium carbonate was added. The reaction of the solution which dripped from these latter crocks was fairly constant at pH 5.6.

The effect of high calcium carbonate content of the sand on germination of the seed was noted and the effect on early growth of the grass seedlings was followed for 35 days after germination, at the end of which time the plants in 21 of the crocks containing calcium carbonate and 8 without calcium carbonate were harvested. During these early stages of growth the temperature of the sand was not controlled.

The remaining crocks were placed in tanks which kept soil temperatures constant to within 1° C. Half of these were maintained at a soil temperature of 15° C. (59° F.); the other half were kept at 25° C. (77° F.). Fifty-five days after making the temperature adjustments, photographs were taken and harvests made.

Effect of Calcium Carbonate on Germination

In the initial trials of this experiment it was very difficult to get an even stand with the various treatments. In the final one, germination began 6 days after planting (March 26) in the crocks lacking calcium carbonate. There were no signs of germination in the other crocks. This same condition had been observed, in a less pronounced form, in a previous experiment.

Germination was delayed approximately a week in the crocks containing calcium carbonate. After germinating, some of the grass grew for only a few days and then withered, leaving isolated clumps. If moist chambers were placed over individual crocks further germination ensued and fairly good growth followed, probably because of the high humidity produced. Even with this treatment, however, some grass died. It is not likely that death can be attributed to damping-off fungi because that disease generally produces different symptoms.

Shortly after germination in the calcium carbonate series the leaves appeared to be vigorous but the root systems of the seedlings were poorly developed. This may be contrasted with the grass grown without calcium carbonate, which germinated in about 6 days, produced leaves, and developed root systems rapidly.

Slow root development of the germinating seedlings in the calcium carbonate series may possibly have been caused by the low availability of phosphorus at high pH. Phosphorus is considered necessary for development of roots, and some work

Effect of Presence of Calcium Carbonate on Growth of Bluegrass Seedlings During the First 35 Days After Planting.

Treatment	Number of crocks	Portion of plant	Average length in inches	Average dry weight per crock in grams	Top/root ratio
High calcium carbonate		Tops	2.6	1.15	
(pH 8.0)	21	Roots	3.3	2.21	0.52
No calcium carbonate		Tops	2.0	2.1	
(pH 5.6)	8	Roots	1.9	6.05	0.35

with wheat seedlings has indicated that its uptake is greatest in the early stages of development. It is possible that in this experiment it was a limiting factor in the early stages. Later, absorption of phosphorus was sufficient for more extensive growth.

Thirty-five days after planting, 21 crocks treated with calcium carbonate and 8 not so treated were harvested. Average dry weights and lengths of tops and roots were taken. From the table on this page it can be seen that, on the basis of weight of both tops and roots, the stand of grass grown in crocks at pH 8.0 was much less than that grown at pH 5.6. This was to be expected since fewer plants were growing in those crocks containing calcium carbonate in which germination had been inhibited and many more young seedlings had died, than in the calcium carbonate-free crocks.

Once growth had started in the crocks containing calcium carbonate, the plants grew more rapidly than in those not having calcium carbonate. This may have been associated with the reduced competition due to the smaller number of plants in the crocks containing calcium carbonate. At this time, also, the growth was apparently equally as uniform in the crocks containing lime as in those without it. The average length of roots in the series high in calcium carbonate was almost twice as great as in the minus calcium carbonate series. Also the tops were about 0.6 inch longer in the former series than in the latter.

The root systems of the seedlings grown at pH 8.0 in the presence of calcium carbonate were characteristically long, thick, and white with few or no branches. Where root branches were present, they were short except near the growing point of the main root. In contrast with this, seedlings grown at pH 5.6 in the absence of calcium carbonate had shorter, thin, primary roots and a better development of root branches.

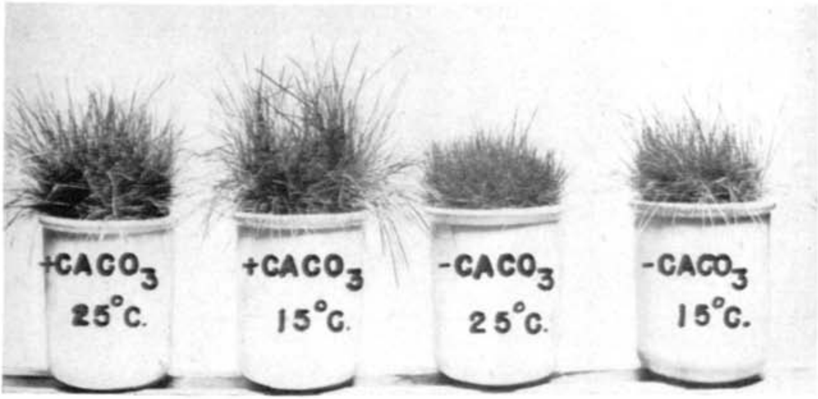
Effects of Temperature and Calcium Carbonate Content of Sand on Growth of Seedlings

After the harvest at the end of 35 days, the remaining crocks were used in soil temperature experiments as previously described. At the end of 55 days of growth, photographs were taken and harvests made.

The effect of calcium on the growth of the grass seedlings is difficult to judge because of the much larger number of seedlings present in the crocks without lime than in the ones with lime. Due to the effect of lime on germination and the very young seedlings, there were only approximately one-third as many seedlings in the calcium carbonate crocks as in those which had not received calcium carbonate, as is shown in the table on page 38. This means that in the calcium-free crocks, competition was three times as great as in those with lime. However, this early effect of lime was approximately the same at 59° as at 77°. It is possible, therefore, to estimate the effect of the temperature of the sand on the growth of the grass in the calcium-free crocks and in the crocks containing calcium.

Once the bluegrass seedlings were established in sand having a high calcium carbonate content they had longer and darker green leaves than those grown on sand lacking lime. Furthermore, as shown in the table on page 36, the average dry weight of tops produced by each crock was approximately one-third greater in the calcium carbonate series than in the series which did not receive calcium carbonate although the crocks in the calcium carbonate series contained only about one-third the number of plants present in the calcium-free crocks. Thus it becomes apparent that the individual seedlings increased many more times in dry weight when calcium carbonate was present than when absent. This may probably be explained, at least in part, by the decrease in competition between the plants for food and light and the consequent increase in rates of metabolism and growth of the plants in the crocks containing lime.

In considering the effect of the temperature of the sand on the growth of the seedlings it has been generally true that 59° was a more favorable soil temperature than 77°. From the figures in the table on page 38 it has been calculated that in the presence of calcium the dry weight of 100 seedlings was 27 percent greater at 59° than at 77° whereas in the absence of calcium it was 69 percent greater at 59° than at 77°.



Effect of soil temperature and lime on the growth of Kentucky bluegrass seedlings. On the left there are two crocks marked "$+CaCO_3$" which contained one-half pound of calcium carbonate mixed with the quartz sand in which the grass was growing. The two crocks on the right marked "$-CaCO_3$" contained pure quartz sand. All four crocks had been watered throughout the 90 days of the experiment with equal quantities of a complete nutrient solution. The soil in one of each pair of crocks had been kept at 25° C. (77° F.) and that of the other at 15° C. (59° F.), for 55 days prior to the time the picture was taken. As a result of the effect of the lime on the germination and early growth of the seedlings there were only about one-third as many plants in the $+CaCO_3$ as in the $-CaCO_3$ crocks. Note more extensive growth in the crocks in which the soil was kept at 15° C. than in comparable crocks kept at 25° C.

In general, top growth was greatest at the lower temperature. The plants tended to be longer and to have more succulent blades and darker green leaves than grass grown at 77°. However, as shown in the table on page 36 the dry weights of tops of the plants of the two series show very little difference at the two temperatures.

The densest, most finely branched, and longest root systems were produced by plants grown at 59° in the presence of lime. The least root growth was produced by those plants grown at the higher temperature and in the absence of calcium. The plants grown under these conditions produced very little lateral growth and the primary roots failed to penetrate to the bottom of the crocks. The figures in the table on this page indicate that the lower temperature has been of much more importance

RELATIVE EFFECTS OF CALCIUM CARBONATE AND TEMPERATURE ON DEVELOPMENT OF KENTUCKY BLUEGRASS SEEDLINGS.

Soil treatment	Soil temperature (° F.)	Number of crocks	Average dry weight in grams of roots per crock	Average dry weight in grams of tops per crock	Top/root ratio
High calcium carbonate	59°	5	18.44	15.32	0.83
(pH 8.0)	77°	5	10.32	15.26	1.48
No calcium carbonate	59°	9	13.48	12.01	0.89
(pH 5.6)	77°	9	7.05	10.85	1.53

in the development of abundant root growth than has the calcium carbonate content of the sand. From the figures in the column of "Average dry weight in grams of roots per crock" it has been calculated that in the presence of calcium the root weight was 78.7 percent greater at 59° than at 77° and in the absence of calcium it was 91 percent greater at 59° than at 77°. It was found that, in spite of much less competition, the low temperature root growth was only 36.8 percent greater in the presence of calcium than in its absence. At the higher temperature, the root growth in the presence of calcium was only 46.4 percent greater than in its absence.

The top-root ratio was also predominantly influenced by temperature because those plants grown at 77° had much poorer root development than those grown at 59°. As may be seen in the table on page 38, this difference cannot be attributed to a difference in the degree of competition, since there were relatively equal numbers of plants per crock in the temperature series. On the basis of the figures in the table on page 36 the calcium carbonate content of the sand and the resultant decrease in competition among the grass plants played a negligible role in altering the top-root ratio.

Rhizome development, which was just beginning at the time of harvest, was most noticeable in the crocks with open and sparse growth.

The general conclusion from this experiment, therefore, is that calcium in large quantities has been detrimental to the germination of seed and the growth of young seedlings of Kentucky bluegrass. The effect of the calcium on the growth of the older seedlings was difficult to determine from these experiments because of the much smaller number of plants with the resulting decrease in competition in the crocks containing calcium carbonate. Whether as a result of the sparser distribution of the plants or as a direct effect of the calcium, the grass plants in the crocks containing calcium showed an increase in fine roots near the surface and an increase in length of the primary roots.

It should be remembered in considering these data, however, that the rate of application of lime was much heavier than the heaviest rates ever used in the field. Therefore, although the results are interesting they cannot necessarily be expected to follow the use of lime as applied at the customary rates of 1 or even 2 tons to the acre.

From the temperature series it was found that the grass seedlings grown at 59° had greener color, longer leaves, greater green and dry weights, and a lower top-root ratio than similar seedlings grown at a soil temperature of 77°. These results confirm the wisdom of the recommended practice of planting grass seed in the early autumn during periods of decreasing

RELATIVE NUMBERS AND WEIGHTS OF SEEDLINGS AS CORRELATED WITH TEMPERATURE AND CALCIUM CONTENT OF SEED.

Soil treatment	Soil temperature	Approximate number of seedlings per crock	Dry weight in grams of 100 seedlings
High calcium carbonate	59°	1,700	9.4
(pH 8.0)	77°	1,725	7.4
No calcium carbonate	59°	4,275	5.3
(pH 5.6)	77°	5,125	3.1

temperatures. Since an extensive root system is necessary for the survival of grass through the dry periods of summer, the superior root development obtained at the low temperatures promotes the establishment of turf from seed planted in the early autumn.

EXPERIMENTS WITH ARSENIOUS ACID

During the months of January to March, a series of experiments designed to show the effects of arsenious acid on germination and on seedling growth was set up in a greenhouse maintained at a temperature of approximately 60° F. In the first experiment, five crocks constituted a unit. Each unit was sprayed with a liter (1.06 quarts) of one of the following materials: distilled water, and 0.01, 0.025, 0.05, and 0.1 percent solutions of arsenious acid. The seeds were then sprinkled lightly with fine sand and moistened with distilled water.

In a second experiment two crocks composed a unit. The grass was grown until 1½ months old and the same treatments described above were given. In a third experiment the grass was 3½ months old before similar treatment was given. All three experiments were continued for 3 months following the initial treatment.

The application of arsenious acid at these rates was equivalent to applying it at the rates of approximately 0.6, 1.5, 3, and 6 pounds to 1,000 square feet. Arsenious acid applied at these rates contained arsenic in amounts equivalent to sodium arsenite applied at the rates of approximately 1, 2.5, 5 and 10 pounds to 1,000 square feet, respectively. Arsenious acid is much less soluble and does not have the burning effect on vegetation that sodium arsenite has and therefore could be applied at these heavier rates. These are much heavier rates than those ordinarily used on turf. Moreover, it should be remembered that the grass in these experiments was growing in crocks in quartz sand rather than in the field in the more complicated environment which the soil presents.

Results with Arsenious Acid on Seed

Sixteen days after planting and spraying the seeds, there was sporadic germination of those sprayed with distilled water, and some germination of those sprayed with arsenious acid at the lowest rate of 0.6 pound to 1,000 square feet. Two days later germination began in the crocks sprayed with arsenious acid at the rate of 1.5 pounds to 1,000 square feet. Thereafter, germination occurred in decreasing amounts in the crocks treated with arsenious acid at the rate of 3 and 6 pounds to 1,000 square feet. By the end of 27 days, it was apparent that the grass seed treated with arsenious acid at the 0.6-pound rate was germinating much more completely than the water-treated controls, while that treated at the 1.5-pound rate was germinating about as well as the controls. There was definite retardation in the crocks treated at the 3-pound rate and very marked toxicity to seed in the crocks treated at the 6-pound rate. The stimulating effects continued to be apparent after 2 months in the case of the seed treated at the 1.5 and 0.6-pound rates. As discussed in the January, 1939, issue of TURF CULTURE, such stimulating effects of arsenic on Kentucky bluegrass have been observed frequently in the United States Golf Association Green Section's experiments at Arlington and by others throughout the country who have been using arsenicals in weed control.

The slowness of germination of all the grass in these arsenic experiments may be partially attributed to the low temperature of the greenhouse. An essentially constant temperature of about 65° was maintained throughout the day. Constant low temperatures are not most conducive to the germination of Kentucky bluegrass seed.

RESULTS WITH ARSENIOUS ACID ON SEEDLING GRASS

In the experiments with the 1½- and 3½-month old grass it was observed 5 days after spraying that there was injury from the treatment at the 6-pound rate, the leaves appearing somewhat dried. A week after spraying, injurious effects became apparent as a result of the treatments at the 1.5, 3, and 6-pound rates, the most severe being brought about by the heaviest rate. In general the oldest grass was slowest to respond. Within 2 weeks it was apparent that the grass treated at the

Effect of arsenious acid on germination and early growth of Kentucky bluegrass. The crock on the left received no acid. The four remaining crocks received arsenious acid at the rates of 6, 3, 1.5 and .6 pounds to 1,000 square feet, reading from left to right. The crocks are marked 0.1, .05, .025 and .01, respectively, indicating the percentage of solution used immediately after the seed was sown, after which the seed were sprinkled lightly with fine sand and moistened with distilled water. The picture was taken 27 days later and shows a better stand of grass in the crocks marked .01 and .025 which received arsenious acid at the .6- and 1.5-pound rates respectively than in the untreated crock marked C., and a retardation in the crocks treated at the 3- and 6-pound rates. These effects remained evident 2 months after treatment.

6-pound rate would die, while with the remaining treatments, although there was injury, the severity decreased with decreased rates.

Slightly over a month after treatment with the very heavy arsenious acid sprays, the older grass treated at the 3- and 6-pound rates was almost completely killed. Thus an upper tolerance of Kentucky bluegrass seedlings for arsenious acid, when used as a spray, was found to be 3 pounds to 1,000 square feet. This is equivalent in arsenic content to approximately 5 pounds of sodium arsenite to 1,000 square feet. It should be remembered that these results were obtained on grass seedlings grown in crocks in quartz sand and that, therefore, the results cannot be expected to apply to grass of the same age grown in the field. The complications introduced by the colloidal nature of the soil solution in clay soils, the microflora of soils, and other factors may be expected to alter the results considerably. It has been shown by other investigators that the toxicity of arsenic is affected by the texture of the soil in which the plants are growing. Arsenic is much more toxic to plants grown in sand than in soil, and this toxicity is reduced materially even by the coarser soils.

After a few weeks the older grass, which had been injured severely, began to recover from the initial effects of arsenic "burning." The grass treated with arsenious acid at the 0.6-pound rate, however, showed no adverse effects from spraying. There seemed, on the contrary, to be a slight benefit.

In the crocks of grass which were sprayed, some seed germinated which had previously remained dormant. Practically all the seed germinating in the crocks treated with arsenious acid at the 6-pound rate were those of weeds present in the seed sample, but no weed seedlings were observed in the crocks sprayed at the lower rates. Apparently arsenious acid at the higher rates stimulated germination of weed seeds.

Another effect noted was that grass sprayed with the lower concentrations of arsenic was more drought resistant than the controls.

In the experiments reported here, seeds appear to be differentially affected by arsenious acid, a treatment at the 6-pound rate killing nearly all bluegrass seeds or seedlings, while seeds of weeds, which were present as impurities, were apparently stimulated to germinate and grow.

Summary and Discussion

Kentucky bluegrass was seeded in washed quartz sand contained in 2-gallon crocks and grown in the greenhouse. After germination each crock received bi-weekly applications of a complete nutrient solution.

In one series one-half pound of calcium carbonate was mixed in the sand in each of some of the crocks. This series was later grown at two different temperatures of 59° and 77° F.

Additional series of crocks were set up, some of which were watered with solutions of arsenious acid equivalent to applying it at the rates of .6, 1.5, 3, and 6 pounds to 1,000 square feet. These solutions were sprinkled on some of the crocks before the seed germinated and on others when the plants were at various stages of growth.

Germination of seed in crocks having a high calcium carbonate content (pH 8.0) began about a week after that in crocks having no calcium carbonate (pH 5.6).

Thirty-five days after planting, the roots of the plants growing in crocks containing calcium carbonate were long and had few if any branches, while those growing in the absence of calcium carbonate were shorter and more branched.

The average length of roots at the end of 35 days was almost twice as great when calcium carbonate was present as when it was absent.

At both 59° and 77° the average number of seedlings surviving after 90 days in the crocks containing calcium was about one-third of the number in the calcium-free crocks.

Ninety days after planting, the average top growth of grass grown on sand having a high calcium carbonate content was, on the basis of dry weight, approximately 33 percent greater than when calcium carbonate was absent. This may probably have been associated with the decreased competition in the calcium carbonate crocks.

The dry weight of seedlings was consistently greater at 59° than at 77°.

During the period of this experiment, root growth was definitely greater at 59° than at 77°. The data indicate that soil temperature is a more important factor in the growth of an extensive root system than is the calcium carbonate content of the soil.

The grass grown at a soil temperature of 59° had greener color, longer leaves, greater green and dry weights, and a larger top-root ratio than did grass grown at a soil temperature of 77°.

When arsenious acid was applied to Kentucky bluegrass seed at the 0.6 and 1.5-pound rates, germination and growth were greater than in the control crocks to which water alone had been applied. The treatment at the 0.6-pound rate was equivalent in arsenic content to the application of sodium arsenite at the rate of 1 pound to 1,000 square feet and produced a thicker and taller stand of grass in a shorter time than the controls. This advance was maintained throughout the 3 months of the experiment.

The harmful effects of arsenious acid progressively increased with increasing rates. There was a definite retardation of germination with the use of the 3-pound rate and a decidedly marked toxicity to the seed evident when the arsenious acid was applied at the 6-pound rate.

The upper tolerance of Kentucky bluegrass for arsenious acid in these sand cultures was found to be the 3-pound rate, a single application at the 6-pound rate being sufficient to kill seed or seedlings of grass.

Considerably higher concentrations of arsenious acid are required to kill the growing point of the stem than to kill the leaves of Kentucky bluegrass.

Arsenious acid is more toxic to Kentucky bluegrass seed than to seed of certain common weeds. In some cases the latter appeared to be stimulated to germination by the heavy applications of arsenious acid.

THE FEDERAL SEED ACT

The new Federal Seed Act which became operative on February 5, 1940, should be of interest to all turf culturists for this new act is another step toward providing the intelligent buyer with the facts concerning his purchases.

Heretofore seeds of turf grasses have received little attention from the various legislatures. The Seed Importation Act of 1912 covered only the importation of certain agricultural seeds and, except for the ryegrasses, seeds of interest to turf growers were not included. Most of the states now have legislation covering the labeling and quality of the seed sold in the state but these laws are not uniform and buyers of grass seed in many states have had little or no protection.

The Federal Seed Act promises to bridge many of the gaps in the existing seed laws since it regulates both the importation