Effects of Environment on the Metabolism and Germination of Crested Wheatgrass Seeds[1]

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Highlight

Seeds of crested wheatgrass (*Agropyron desertorum* [Fisch. ex Link] Schult.), planted at a depth of 1 inch, germinated well because of relatively constant and favorable moisture conditions. These seeds rapidly synthesized hexose phosphate, uridine diphosphate hexose, nicotinamide adenine dinucleotide, adenosine diphosphate, adenosine triphosphate, and other phosphate esters. Synthesis began 2 to 4 weeks before germination was observed. In contrast, seeds on the soil surface failed to germinate because of generally unfavorable and rapidly fluctuating moisture conditions. Adenosine triphosphate, the principal phosphate ester formed in these seeds during brief periods of precipitation, was broken down during periods of drought. Although these measurements include only a few of the biochemical reactions that occur in seeds, they contribute to an understanding of the environmental conditions that promote or retard germination processes and help explain the reasons for success or failure of seedings on semiarid rangelands.

Severe environments often determine the success or failure of range seedings. Unfortunately, there is often little information available to determine why certain seedings result in failures. A seeding operation that is successful one year may not be successful the next even though conditions appear to be similar. Thus, there is a need to understand more precisely the conditions that are essential for success. Information is needed to determine (1) how intensively to prepare a seedbed, (2) what sites to seed, (3) what time of year to seed, and (4) what method to use in seeding. Furthermore, an understanding of critical environmental variables is needed so that plant breeders can select for physiological and morphological adaptations that will better enable seeds and seedlings to germinate and emerge under marginal conditions.

Measurements of both environment and seed responses to environment are needed. In the past, effects of environment have been evaluated in terms of the number of seedlings that emerge. But when many different environments have occurred during the weeks or months that elapse from time of planting to emergence, it has been difficult to determine how or when environment promoted or retarded the germination of seeds.

Since the germination of seeds is a complex process, one is faced with the problem of determining what measurements will meaningfully reflect the effects of environment on seeds. One of the important processes that occurs in early stages of germination is the synthesis of phosphate esters, such as phosphorylated sugars and nucleotides (Cherry and Hageman, 1961; West, 1962; Wilson and Harris, 1966). These phosphate esters are intermediates in the synthesis of respiratory substrates and of many new cell materials required for germination and seedling growth. Adenosine triphosphate and certain other phosphate esters conserve energy made available during respiration and use this energy in biosynthetic reactions (Krebs and Kornberg, 1957). In this study, measurements of synthesis and breakdown of phosphate esters were made to evaluate the effects of environment on seeds and to help explain success or failure of experimentally drilled and broadcast seedings (Nelson et al., 1970).

Materials and Methods

The experimental site, on the Snake River breaks in southeastern Washington, is representative of large areas in western United States that are too steep or rocky to seed by plowing and drilling. The soil is a shallow silt-loam over fragmented basalt. Average annual precipitation is 13 inches.

Eight grams dry weight of Nordan crested wheatgrass seeds were treated with 100 μcuries of ^{32}P-labeled Na_2HPO_4 to distinguish newly synthesized compounds from those already present in mature seeds. Seed samples were treated with 40 mg of thiram (Tetramethylthiuram disulfide) to inhibit microbiological growth, and were enclosed in flat 12- by 18-inch nylon screen bags.

The soil was rototilled prior to planting in late October, 1967, and early March, 1968. One set of samples was placed at a depth of 1 inch to simulate drilling of seeds, and another set of samples was placed on the soil surface to simulate broadcasting of seeds. Additional seed samples were moistened; killed by autoclaving; treated with ^{32}P and thiram; and placed at the 1-inch depth in late October and early March to determine how much ^{32}P microorganisms associated with seeds might incorporate in phosphate esters. The experiment was replicated twice.

After 2, 8, 16, and 32 days, seeds were taken from the soil and quickly frozen in dry ice to stop metabolic processes. Enzymes were denatured by homogenizing seeds in 10% w/v trichloroacetic acid in

Table 1. Loss (%) of ^{32}P from seeds as a result of precipitation.[1]

Sampling day	1-inch depth	Soil surface
Fall seeding:		
8 days	45	92
16 days	47	95
32 days	72	95
Spring seeding:		
8 days	64	75
16 days	72	88
32 days	—	92

[1] Estimated by radioassay of ^{32}P in orthophosphate fractions.

FIG. 1. Daily maximum and minimum temperatures, during experimental periods beginning in late October, 1967, and early March, 1968.

diethyl ether, with the homogenizer assembly held in a dry ice-acetone bath (Wilson and Harris, 1966). Phosphate esters were extracted from the residue by homogenizing three times in 50 ml of 0.6N aqueous trichloroacetic acid at 0 to 4 C. Trichloroacetic acid was removed by washing the supernatant solution five times with two volumes of diethyl ether in a separatory funnel. The aqueous extract, containing phosphate esters, was loaded on the ion exchange column (Dowex-1 × 8, 140 to 325 wet mesh, 1.1 by 50 cm resin bed), and phosphate esters were eluted from the column with a gradually increasing concentration of formic acid and ammonium formate (Peterson and Sober, 1959; Wilson and Harris, 1966). Net synthesis of phosphate esters was detected by measuring total phosphate in fractions of the effluent from the ion exchange column (Bartlett, 1959). Portions of the effluent were dried in stainless steel planchets, and ^{32}P incorporation in phosphate esters was detected by radioassay in an automatic beta counter.

Precipitation, relative humidity, air temperature 6 inches above the ground, and soil temperature at a depth of 1 inch were recorded. Soil water potentials were measured in the laboratory with a thermocouple psychrometer (Campbell et al., 1966). In the case of very dry soil samples, the thermocouple was positioned over a −10 atmosphere KCl solution, cooled with a 7.5 milliampere current for 30 minutes,
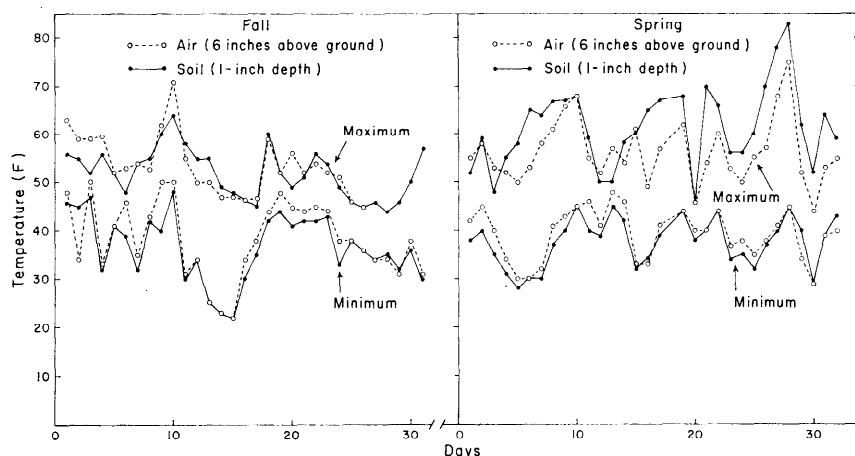
and then rotated to the soil sample to record the voltage output. Samples for measuring water potential were taken from within the ¼-inch of soil immediately below the seed samples.

Results

Loss of ^{32}P from Seeds

A radioassay was made of orthophosphate fractions from the ion exchange column to estimate the amount of ^{32}P washed from seeds by rain. Rain washed as much as 95% of the ^{32}P from seeds on the soil surface and as much as 72% from seeds at a depth of 1 inch (Table 1). Therefore, no attempt was made to compare ^{32}P incorporation in seeds planted on the soil surface. Qualitative comparisons were made of ^{32}P incorporation in seeds at a depth of 1 inch. Loss of ^{32}P from seeds did not interfere with measurements of net synthesis of phosphate esters.

Environment

Throughout the 32-day periods in the fall and spring, daily maximum temperatures (Fig. 1) were within the range in which crested wheatgrass seeds germinate (Ellern and Tadmor, 1967). However, daily minimum temperatures fell to 32 F, or lower, during 9 days in the fall and 7 days in the spring. Temperatures were lower and less favorable for germination in the fall than in the spring.

Surface moisture conditions were often favorable for germination when the relative humidity was 100% (Fig. 2). Relative humidity increased to 100% on 16 occasions in the fall and 13 in the spring. Even the longest period of continuous 100% relative humidity (56 hours) was too short for seeds to germinate at the prevailing temperatures. The average duration of the 100% humidity periods was 11 hours in the fall and 5 hours in
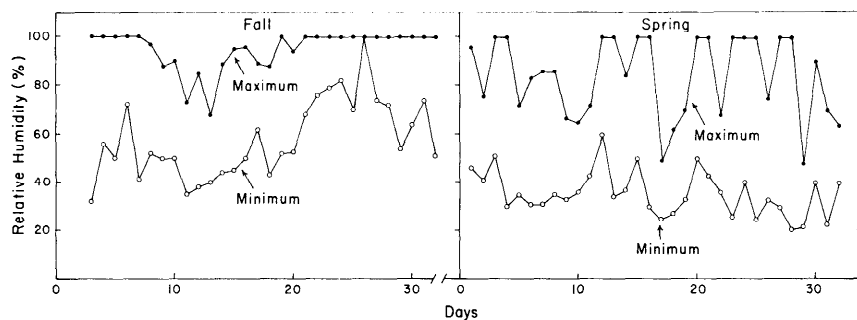

FIG. 2. Daily maximum and minimum relative humidities, during experimental periods beginning in late October, 1967, and early March, 1968.

the spring. The daily minimum humidities indicate that seeds on the soil surface were often exposed to severe drying conditions.

Precipitation and soil water potential are reported in detail in connection with seed metabolism and germination. Steep water potential gradients frequently existed in the surface soil. In one instance the gradient was −500 atmospheres per inch. In the absence of precipitation and during periods of low humidity, surface soil dried rapidly. For example, water potential of surface soil decreased from −4 atmospheres to −300 atmospheres in one 2-day period.

Fall Planting on the Soil Surface

In the absence of precipitation during the first 2 days, the water potential of surface soil decreased from −60 atmospheres at planting time to −300 atmospheres. The concentrations of phosphate esters in seeds on the soil surface were the same as in seeds before they were planted, indicating that no net synthesis had taken place (Fig 3).

Rainfall of 0.17 inch during the 3- to 8-day period provided moisture for seed metabolism. An increase in concentration of UDP-hexose[2] and adenosine triphosphate was detected on the eighth day even though the water potential of surface soil had decreased to −200 atmospheres.

In the absence of precipitation during the 9- to 16-day period, the water potential of surface soil decreased to −400 atmospheres. UDP-hexose and adenosine triphosphate were largely broken down. The increase in adenosine monophosphate probably resulted from dephosphorylation of adenosine triphosphate.

[2] The following abbreviations are used: NAD, nicotinamide adenine dinucleotide; AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; UDP-hexose, uridine diphosphate hexose; P_i, orthophosphate. Other phosphate esters are depicted as the parent compound with -P representing phosphate.
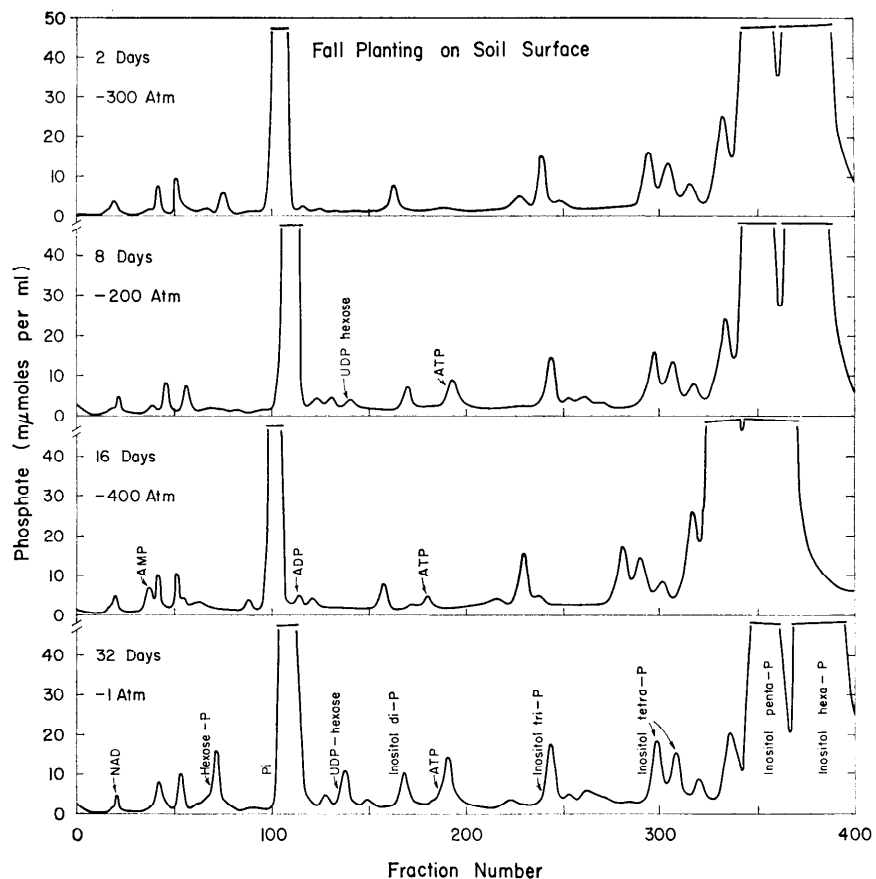

FIG. 3. Phosphate esters from crested wheatgrass seeds (4 g dry weight) planted in the fall on the soil surface. Soil water potentials in atmospheres are given for each sampling date. Phosphate esters were eluted from the ion exchange column with a gradient of formic acid and ammonium formate. The abscissa represents number of successive 10-ml fractions collected from the ion exchange column. The ordinate represents total phosphate (millimicromoles/ml) in each of the fractions. The concentration of a phosphate ester in seeds is proportional to the area under the peak for that phosphate ester. Orthophosphate, inositol penta-P, and inositol hexa-P are not fully plotted on the figures.

The 17- to 32-day period included 0.48 inch of precipitation. Although a relative humidity of 100% prevailed 45% of the time, its duration without interruption was short compared with the time needed for seeds to germinate. During this period, seeds synthesized hexose-P, UDP-hexose, adenosine triphosphate, and traces of other phosphate esters. None of the seeds on the soil surface had germinated at 32 days, but approximately 1% had germinated by the following March.

Laboratory germination tests indicated that approximately 10% of the seeds lost germinability during overwintering on the soil surface. Seeds left on the soil surface from October to March produced seedlings that appeared as large and vigorous as those from seeds stored in the laboratory for the same period. This indicated that failure of seeds to germinate in the field was due to adverse environments and not to loss of germinability.

Fall Planting at 1-Inch Depth

The water potential of soil at the 1-inch depth was −60 atmospheres at the time of planting and decreased to −70 atmospheres during the first 2 days. In these dry conditions, seeds incorporated traces of ^{32}P in hexose-P (Fig. 4). The radioactivity in fractions 220 to 240 represents an impurity in the ^{32}P with which seeds had been treated. Net synthesis of phosphate
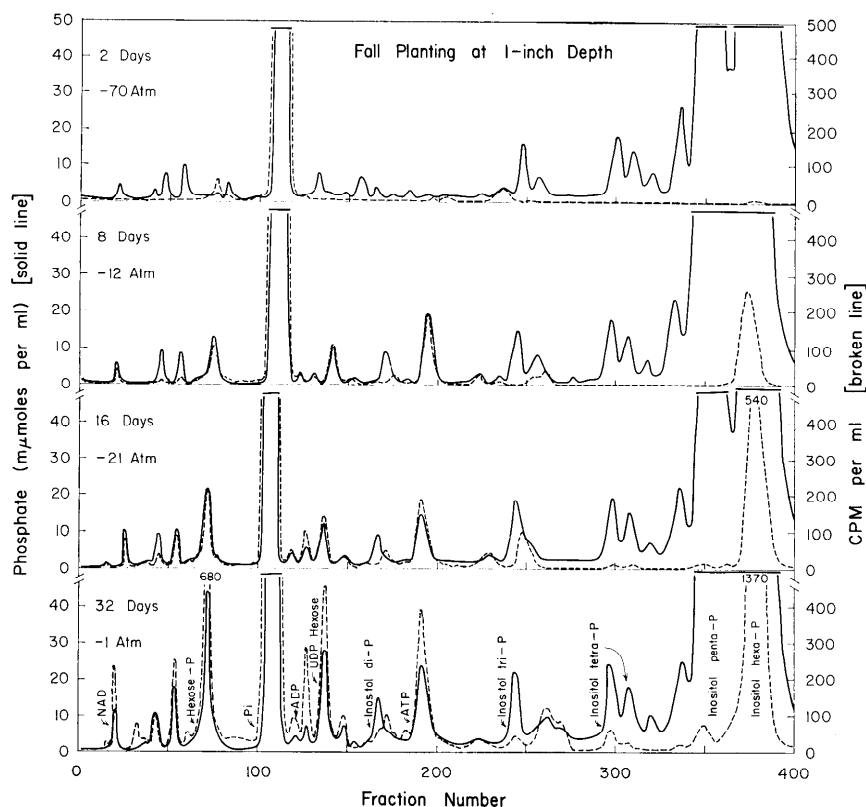
FIG. 4. Phosphate esters from crested wheatgrass seeds (4 g dry weight) planted in the fall at a depth of 1 inch. The solid line represents total phosphate in each fraction. The broken line represents radioactive phosphate (counts/minute/ml) that was absorbed by seeds and utilized for synthesizing phosphate esters. The numbers above hexose-P and inositol hexa-P indicate the maximum counts/minute/ml in fractions containing these phosphate esters.

esters, as indicated by total phosphate measurements, was not detected during the first 2 days.

As a result of precipitation during the 3- to 8-day period, soil water potential at the 1-inch depth increased to −12 atmospheres. Seeds incorporated ^{32}P in nicotinamide adenine dinucleotide, hexose-P, UDP-hexose, adenosine triphosphate, inositol hexaphosphate, and other phosphate esters. Total phosphate measurements indicated a marked increase in the concentrations of hexose-P, UDP-hexose, and adenosine triphosphate.

In the absence of precipitation during the 9- to 16-day period, water potential of soil at a depth of 1 inch decreased to −21 atmospheres. Little change in concentrations of phosphate esters occurred during this period.

Precipitation during the 17- to 32-day period increased soil water

potential to −1 atmosphere. Seeds incorporated additional ^{32}P in phosphate esters and synthesized increased amounts of hexose-P, UDP-hexose, and adenosine triphosphate. At 24 days none of the seeds had germinated. At 32 days, 40% of the seeds had germinated but had not emerged. Moisture conditions at the 1-inch depth remained favorable during the winter months, but low temperatures delayed emergence of seedlings until February.

Spring Planting on Soil Surface

In the absence of precipitation during the first 2 days, water potential of surface soil decreased from −4 atmospheres at time of planting to −300 atmospheres. No synthesis of phosphate esters was detected (Fig. 5).

Precipitation of 0.31 inch moistened the soil surface during the first part of the 3- to 8-day period. However, any phosphate esters that

may have been synthesized during favorable moisture were broken down as the surface soil dried to a water potential of −500 atmospheres.

Precipitation of 0.23 inch, preceding the sampling of seeds at 16 days, moistened the surface soil to a water potential of −1 atmosphere. Seeds synthesized adenosine triphosphate.

Precipitation of 0.28 inch fell during the 17- to 24-day period, but none fell in the 25- to 32-day period. This resulted in drying of surface soil to −800 atmospheres. Adenosine triphosphate was largely broken down during the dry period. As in the fall experiment, the increase in adenosine monophosphate suggested that adenosine triphosphate had been dephosphorylated. None of the seeds on the soil surface germinated.

Spring Planting at 1-Inch Depth

Soil water potential was −4 atmospheres at time of planting and decreased to −5 atmospheres in 2 days. Seeds synthesized hexose-P, UDP-hexose, and adenosine triphosphate (Fig. 6).

On the eighth day, soil water potential at the 1-inch depth was −4 atmospheres. Seeds incorporated ^{32}P in many phosphate esters. The concentrations of hexose-P, UDP-hexose, and adenosine triphosphate markedly increased. None of the seeds had germinated at 8 days.

Precipitation during the 9- to 16-day period increased the water potential of soil at a depth of 1 inch to −2 atmospheres. Seeds synthesized high concentrations of hexose-P, UDP-hexose, and adenosine triphosphate. At 16 days, 50% of the seeds had germinated. Seedlings had emerged at 32 days.

Orthophosphate and Inositol Hexaphosphate

Orthophosphate and inositol hexaphosphate (not fully plotted in figures 3 to 6) were present in seeds in concentrations of 10 and 80 μmoles phosphate per gram dry weight, respectively, and did not measurably change during the 32-day experimental periods. Hy-
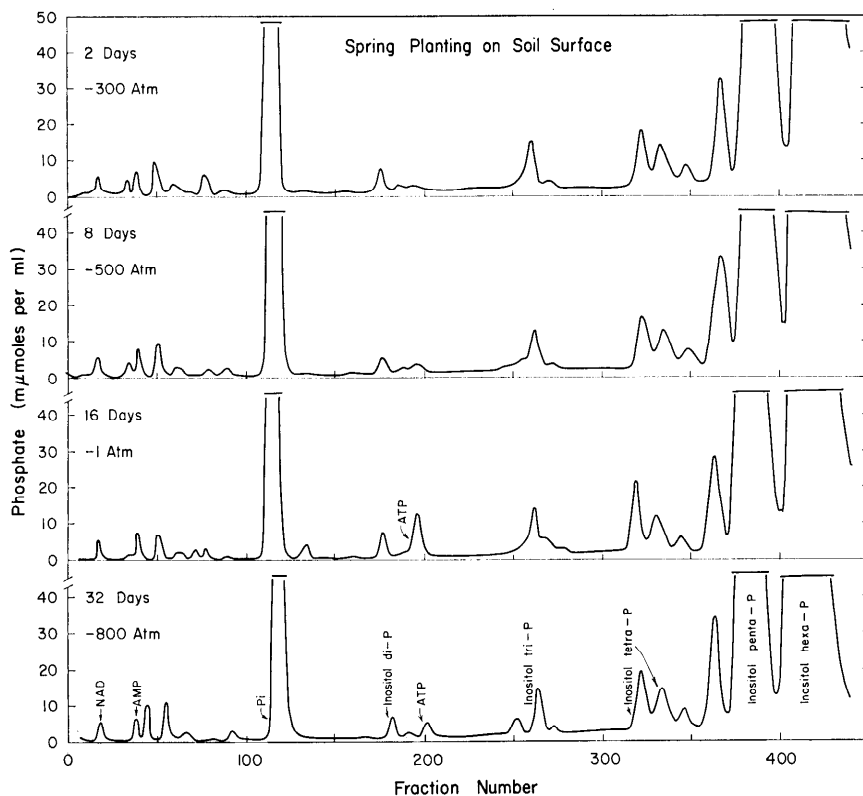
FIG. 5. Phosphate esters from crested wheatgrass seeds (4 g dry weight) planted in the spring on the soil surface. Details are given in title to figure 3.

drolysis of inositol hexaphosphate provides orthophosphate for early seedling growth. In a laboratory test at 73 F, inositol hexaphosphate was almost completely broken down during 12 days of seedling growth.

Metabolism of ^{32}P by Microorganisms

Soil microorganisms can infect seeds and metabolize the ^{32}P with which seeds are treated. The presence of ^{32}P-labeled phosphate esters from microorganisms can thus produce experimental artifacts that would interfere with the measurement of ^{32}P-labeled phosphate esters from seeds (Wilson, in press). Dead seeds were planted to evaluate the seriousness of microbial contamination. In the fall, microorganisms did not incorporate detectable radioactivity except in a compound eluted in fractions 120 to 130 (Fig. 7). The radioactivity in fractions 210 to 220 is an impurity that was present in the commercially available ^{32}P. In the spring, microorganisms incorporated traces of

^{32}P, but not in amounts great enough to interfere with the interpretation of data obtained with living seeds. No net synthesis of phosphate esters, as indicated by total phosphate measurements, was de-tected in the experiment with dead seeds.

Discussion

Measurements of both environment and seed responses to environment are needed to understand more precisely the conditions that are essential for successful seeding of semiarid rangelands. This study has dealt with the question: what seed measurements will meaningfully reflect the effects of environment? The measurements should be (1) representative of the metabolic activity of seeds, (2) indicative of the readiness of seeds to germinate, and (3) sensitive to changes in the environment. Measurement of the synthesis and breakdown of adenosine triphosphate appears to fit these criteria. Synthesis of adenosine triphosphate is a key process; few of the biosynthetic processes that lead to germination can proceed without it. The gradual increase in adenosine triphosphate in seeds in favorable moisture suggests that it might serve as an indicator of readiness of seeds to germinate. The synthesis of adenosine triphosphate was sensitive to extreme changes in environment. It was the first phosphate ester formed after brief periods of precipitation, and was
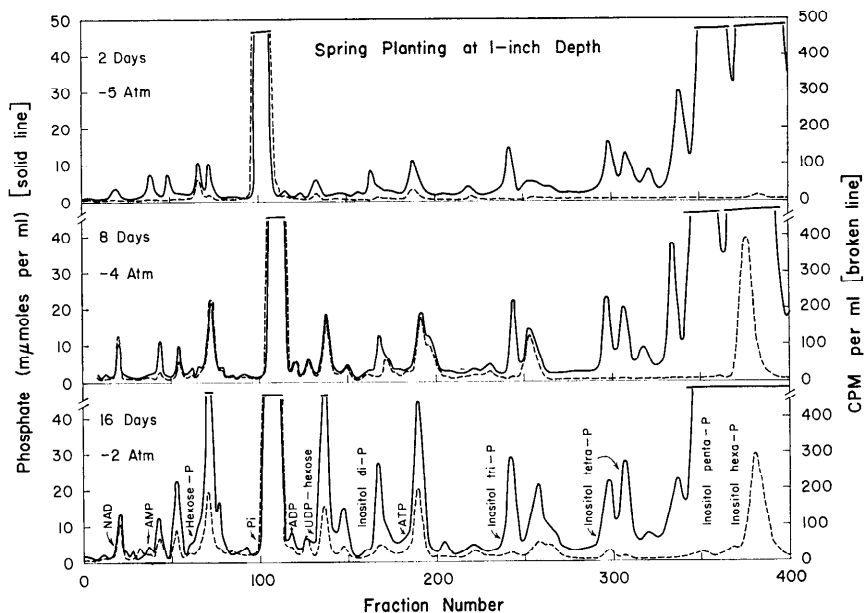

FIG. 6. Phosphate esters from crested wheatgrass seeds (4 g dry weight) planted in the spring at a depth of 1 inch. Details are given in titles to figures 3 and 4.
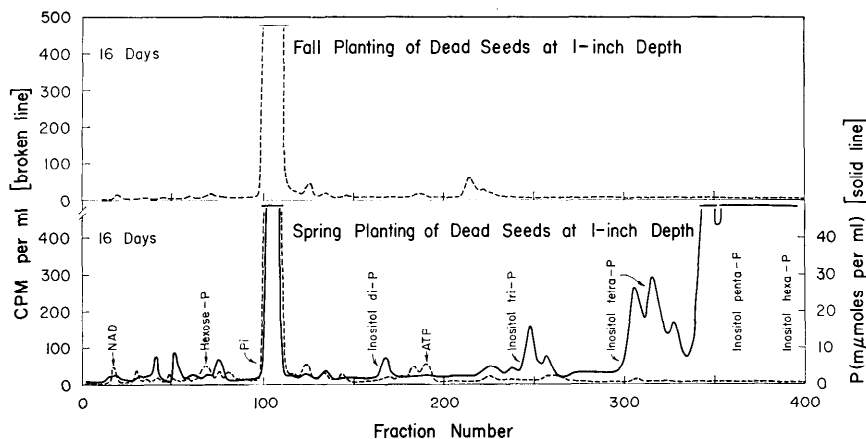
Fig. 7. Phosphate esters from dead crested wheatgrass seeds (4 g dry weight) planted at a depth of 1 inch in the fall and spring. The radioactivity peaks indicate traces of ³²P incorporation in phosphate esters by microorganisms associated with seeds. Orthophosphate (P_i) is the form of radioactive phosphate with which seeds were initially treated.

man big blue grass (*Poa ampla*) have been observed during winter months, when the soil surface was moist for long periods and when temperatures were sometimes favorable.

almost completely broken down during periods of severe drought.

The synthesis and breakdown of adenosine triphosphate was not especially sensitive to moderate drought. This was illustrated by the lack of change in concentration of adenosine triphosphate as the soil at a depth of 1 inch dried from −12 to −21 atmospheres in the fall. This result was not unexpected, because crested wheatgrass seeds can synthesize adenosine triphosphate at water potentials as low as −130 atmospheres (Wilson, in press). This also explains why adenosine triphosphate synthesized during a brief storm in the fall could still be detected on the eighth day, even though the surface soil had dried to −200 atmospheres.

Although low temperature delayed the germination of drilled seeds in favorable moisture, it probably was not the limiting factor in preventing the germination of broadcast seeds. During most days,

temperatures were within the range required for germination of crested wheatgrass seeds (Ellern and Tadmor, 1967). Our laboratory tests indicated that crested wheatgrass seeds can germinate at a constant temperature of 34 F.

Generally unfavorable and rapidly fluctuating soil moisture was probably the most important factor in preventing the germination of broadcast seeds. The synthesis and breakdown of adenosine triphosphate suggest that intermittent storms and dry periods resulted in frequent starting and stopping of germination processes.

Results of this study suggest that broadcast crested wheatgrass seeds will not germinate under the environmental conditions that prevailed during the spring and fall experiments reported here. In additional studies on this site (Nelson et al., 1970), fair germination of broadcast wheatgrasses and excellent germination of broadcast Sher-

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How much does the general public know about rangelands, their importance to the national welfare, or what is being done or should be done to improve them? For that matter, how well informed is the average school teacher on this subject, or State and county officials, legislators, and many others in positions of influence? Here is a vast opportunity for an important service to the public. (F. G. Renner, J. Range Manage. 14:119. 1961)