

The Effect of Temperature and Polyethylene Glycol-induced Osmotic Stress on Radicle Growth of *Lespedeza stipulacea*, *Lolium multiflorum*, and *Bouteloua curtipendula*

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Abstract. Seedlings of *Lolium multiflorum* Lam., *Lespedeza stipulacea* Maxim., and *Buteloua curtipendula* (Michx.) Torr. were grown for 48 hours in darkness at constant temperatures of 22°, 27°, 32°, or 37°C either in distilled water or polyethylene glycol 20,000 (PEG) solutions with osmotic potentials of -3.2, -7.7, and -9.8 bars. Radicle growth rate decreased as concentrations of polyethylene glycol increased. Radicle growth was reduced at 32° and negligible at 37° for *L. multiflorum*. All levels of PEG-induced osmotic stress reduced radicle growth at 22° and 32°, while at 27° only higher levels reduced growth of *L. multiflorum*. PEG-induced osmotic stress reduced radicle growth of *B. curtipendula* significantly at all temperatures except 37°, where the lowest level of PEG had an insignificant effect on radicle growth. With *L. stipulacea*, low levels of PEG-induced osmotic stress did not have a significant effect on radicle growth, but an osmotic stress of -9 bars reduced growth at all temperatures.

The effect of temperature on plant growth is modified and sometimes masked by other environmental parameters such as soil moisture. Emerging seedlings often are exposed to both unfavorable soil temperatures as well as low concentrations of available soil moisture. Temperature extremes also affect rate of water uptake which intensifies heat stress (2).

Every species is characterized by temperature optima for water absorption. Plants have evolved for water absorption under temperature conditions existing in their natural habitats (13, 14). Since soil temperatures in the range of 13° to 23°C are most favorable for uptake of water by temperate species, high soil temperatures endanger these plants by dehydration (11, 14). Temperature affects the rate of water absorption of *Agropyron spicatum* 'Interme', *Festuca ovina*, *Poa ampla*, and *Dactylis glomerata* seeds and seedlings (15).

Seedling emergence and ability to survive decrease as soil moisture concentrations decrease. Rain aids in emergence of *Andropogon barbinodis*, *Leptochola clubia*, and *Bouteloua curtipendula* even at low soil temperatures (12). Germination of *Bromus inermis*, *Elymus junceus*, and 4 species of *Agropyron* is delayed and germination rate reduced as moisture stress increases (9). Some species, such as *Lolium perenne*, have a superior ability to germinate under osmotic stress (10).

Seedling growth depends on osmotic concentration of the solution used, the permeability of the cells to the solute, and the toxicity of the solute in studies involving osmotic stress. Thus, the effect of artificially created osmotic stress is different than moisture stress experienced in the field (7, 17). The purpose of this investigation was to determine the response of radicle growth to PEG-induced osmotic stress and temperature.

Materials and Methods

Species, from previous studies, were chosen to be representative of a C-3 cool-season grass, *Lolium multiflorum*; a C-4

warm-season grass, *Bouteloua curtipendula*; and a legume, *Lespedeza stipulacea*. Seeds of *B. curtipendula* (sideoats grama), *L. multiflorum* (annual ryegrass), and *L. stipulacea* (Korean lespedeza) were sized according to weight (0.5 ± 0.2 , 2.7 ± 0.5 , and 2.2 ± 0.5 mg, respectively). Seeds were placed between 2 layers of Whatman #1, 9-cm filter paper moistened with 10 ml distilled water in 9-cm Petri dishes. The Petri dishes were sealed inside polyethylene bags, placed in a darkened drawer, and the seeds allowed to germinate.

Three solutions of PEG 20,000 (average molecular weight 15,000-20,000 daltons) were prepared according to the method described by Thrill et al. (17). The concentrations used were 200, 250, or 300 g/liter, and the osmotic potentials were measured with a Wescor Thermocouple Psychrometer Model PS 625 with C-52 chambers. The osmotic potentials were -3.2, -7.7, and -9.8 bars, respectively. These solutions and a distilled water control then were used to moisten filter paper placed inside 65×15 mm test tubes (8).

Two pregerminated seeds were placed inside a test tube between the wall and moistened filter paper. The test tubes then were sealed with rubber serum stoppers. Test tubes containing seeds were set into holes in an aluminum temperature gradient block to a depth of 53 mm (5). The desired temperatures were maintained by passing deionized water—heated or cooled by a Lauda/Brinkmann R-3 circulator (temperature range -20° to 100°C)—through holes drilled in one end of the block. The top, bottom, and sides of the block were insulated with 2.5, 3.5, and 5 cm thickness of Styrofoam, respectively. Black cloth was placed on top of the insulated block to ensure that seedlings were in darkness.

The aluminum block was large enough to allow 10 seedlings of each species to be exposed to each concentration of PEG and to the distilled water control. The seedlings were grown at constant temperatures of 22°, 27°, 32°, and 37°C. The experiment was a split-plot design with 3 replications over time. The bar temperature was monitored with 3 calibrated thermocouples located in tubes in the last row in each end of the block and in the middle row. The largest temperature variation observed was $\pm 1.2^\circ$ at 37°. The radicle growth was measured after 48 hr,

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Table 1. Effect of temperature and polyethylene glycol (PEG)-induced water stress on the increase in radicle length occurring in 48 hr for 3 herbaceous species.

Species	Osmotic potential (- bars)	Radicle length increase (mm)			
		22°C	Temperature		
		27°	32°	37°	
<i>Lespedeza stipulacea</i>	0	22 aB ^z	28 abB	35 bC	30 bB
	3.2	16 aAB	24 bB	36 cC	23 abB
	7.7	11 aAB	24 bB	25 bB	13 aA
	9.8	9 aA	13 abA	17 bA	10 abA
<i>Bouteloua curtipendula</i>	0	26 aB	30 aC	40 bC	27 aB
	3.2	10 aA	18 bAB	20 bB	23 bB
	7.7	10 aA	22 bB	13 aA	12 aA
	9.8	6 aA	15 bcA	17 cAB	11 abA
<i>Lolium multiflorum</i>	0	40 dC	32 cC	18 bB	1 aA
	3.2	20 cB	35 dC	10 bA	0 aA
	7.7	14 cA	22 dB	7 bA	0 aA
	9.8	10 cA	14 cA	5 bA	0 aA

^zMean separation within osmotic potential (small letters) and within temperature (capital letters) by Duncan's multiple range test, 5% level.

and the change in radicle length of the 2 seedlings per tube was averaged.

Results

The combination of temperature and PEG-induced osmotic stress had a significant effect on radicle growth. Increasing concentrations of PEG decreased radicle growth.

There was no significant decrease in radicle growth of *Lespedeza stipulacea* relative to that in distilled water at -3.2 bars. Radicle growth at -7.7 bars was comparable also to that in distilled water at 22° and 27°C. At all temperatures, radicle growth at -9.8 bars was significantly less than in distilled water. Radicle growth was significantly less at -9.8 bars than at -3.2 bars at higher temperatures (27 to 37°).

Radicle growth of *Bouteloua curtipendula* at 22°, 27°, or 32°C was significantly less for all PEG concentrations than for distilled water. The amount of radicle growth at -7.7 bars tended to improve as the temperature increased (Table 1). At 37° there was no significant difference between growth at -3.2 bars and that in distilled water.

Radicle growth of *Lolium multiflorum* at -3.2 bars was significantly greater than growth at either of the other 2 osmotic levels used at 22° and 27°C (Table 1). At 27°, radicle growth at -3.2 bars was not different from that in distilled water. Radicle growth, at 32°, was similar at all PEG concentrations and it was significantly less than in distilled water. No significant growth occurred for any of the treatments at 37°.

PEG-induced moisture stress did not cause a shift in the temperature at which maximal radicle growth occurred. But at higher PEG levels, temperature tended to have less of an effect on the amount of radicle growth that did occur (Table 1). The best temperature for radicle growth for *Lespedeza stipulacea* went from 27° to 37° to 32°C and back to 27° to 37° as the concentration of PEG increased, whereas maximum radicle growth for *Lolium multiflorum* shifted from 22° to 22° to 27° at the highest PEG concentration.

Discussion

The probability of successful grass or legume establishment is improved if seedling roots reach relatively moist soil layers before cotyledon emergence (16, 18). The decreased radicle

growth of *Bouteloua curtipendula* with increased osmotic stress may relate to the microclimatic adaptation of *B. curtipendula*. This species is found in moist microclimates and is replaced by *B. gracilis* on drier sites (3).

Lespedeza stipulacea radicles withstand high levels of PEG-induced moisture stress, which may explain why this species germinates during warm weather seeding under unfavorable moisture conditions. *Lolium multiflorum* radicles respond in an opposite manner, being inhibited at 22°C, by osmotic levels above -3.2 bars and being inhibited by high temperatures.

The evolution of a species in response to its natural origin may be important. *Lolium multiflorum* originated in areas with a Mediterranean climate, where summer drought is a major, limiting factor. Species from such an environment normally are semi-dormant during the summer, and the majority of root growth occurs during winter (4). Thus, root growth at lower temperatures and ability to obtain adequate moisture at higher temperatures is important for *L. multiflorum* survival. *Bouteloua curtipendula* originated in a continental climate where cold temperatures limit growth (1). Growth of *B. curtipendula* is limited under PEG-induced osmotic stress at lower temperatures. Ku et al. (6) found that locations with cool, dry summers had few or no C-4 grass species such as *B. curtipendula* as part of their native flora.

In attempting to correlate results obtained from laboratory studies of seedling growth with field observations, it is important to consider the interaction of environmental parameters. A seedling in a natural situation will be exposed to hot temperatures, in conjunction with limited moisture, and its ability to survive will depend on its response to these conditions.

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Anatomical Aspects of Abscission in Pistachio

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Abstract. In pistachio (*Pistacia vera* L. cv. Kerman), when flowers did not set or when young fruits were removed, pedicels and/or portions of the rachis or of the primary branches subtending these parts abscised. Inflorescence buds on fruiting shoots and those on nonbearing shoots treated with (2-chloroethyl) phosphonic acid (ethephon) also abscised. Abscission of the above organs, as well as of leaflets and compound leaves, occurred in definite zones. The first manifestation of the abscission process was transverse cell division in the abscission zone. A separation layer developed in the distal portion of the abscission zone in these organs. The newly formed cells in the proximal area of the abscission zone of pedicels, rachises, and ethephon-treated inflorescence buds became protective layers. However, the counterpart in abscising inflorescence buds on fruiting shoots was largely degraded as abscission progressed. Abscission of mature fruits of pistachio was not preceded by cell division but involved separation and collapse of cells in the fruit mesocarp and exocarp surrounding the distal portion of the pedicel.

Abscission occurs in many different places in pistachio. Leaflets abscise from the main petiole of the pinnately compound leaves, which also abscise from current-season's growth. Mature fruit abscise at the point of attachment of the ovary to the pedicel.

The pistachio is dioecious and both pistillate and staminate inflorescences are panicles. The rachis, or central axis of the inflorescence, branches repeatedly with each branch bearing numerous flowers. Abscission may occur at the following points on the inflorescence: at the base of the pedicels supporting the individual flowers or young fruits; at the points of attachment of the lateral branches to the central axis of the rachis; at the point on the lateral branch or the central axis distal to which there is no developing fruit; and at the point where the inflorescence is attached to the 1-year-old wood.

The mechanism of alternate or biennial bearing in pistachio is the abscission of inflorescence buds from current shoots while

fruits are being produced on the 1-year-old wood (6). Bud abscission begins the last of June and is most intense during July and August, the period when most seed growth and development occur (4).

Application of ethephon to fruits and foliage caused severe inflorescence bud abscission and intensified the alternate bearing problem (5). The study described here was undertaken to characterize the abscission process in the organs cited above and to provide a structural basis for future work aimed at its regulation.

Materials and Methods

Ten-year-old 'Kerman' pistachio trees in the Wolfskill Experimental Orchard, Winters, Calif. were the sources of all samples collected for histological study. Generally, at least 10 replications of each sample (e.g., rachises, inflorescence buds) were collected on a given date. Sampling began in early May when various portions of the rachis were induced to abscise by removal of young fruit. Samples were collected at 3-day intervals so progressive development of the abscission zone could be followed. Inflorescence buds on fruiting shoots were sampled at intervals during June and July. Additional buds on nonfruiting

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