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EFFECT OF DROUGHT STRESS ON TRANSPIRATION RATES AND LEAF AREAS OF *ASTRAGALUS TENNESSEENSIS*, A NEAR ENDEMIC TO CEDAR GLADES

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ABSTRACT

Transpiration rates of *Astragalus tennesseensis* Gray seedlings exposed to increasing drought stress decreased accordingly, and at the permanent wilting percentage of cedar glade soil (6.5) water loss was 120 mg/dm² of leaf area/h. Transpiration rates of mature plants and of seedlings were measured before and after exposure to 2 mos of 3-, 6-, 9-, 12-, 15-, 18- and 21-day watering cycles. All seedlings exposed to drought cycles of 12 days or longer died, but mature plants survived. Rates of water loss per unit of leaf area were not reduced by drought preconditioning. However, when mature plants were subjected to drought cycles of 12 days or longer for 2 mos, they lost more leaves than they produced; therefore, water economy was achieved by reduction in the rate of water lost per plant.

INTRODUCTION

Astragalus tennesseensis Gray (Tennessee milk vetch) is an herbaceous polycarpic perennial legume that grows on cedar glades of northern Alabama and middle Tennessee (Baskin *et al.*, 1972) and on a single gravel terrace prairie in Tazewell County in north-central Illinois (Betz, 1976). The limited geographical distribution of the species as well as the fact that most populations consist of only a few individuals make this a rare species, and it is included on the Smithsonian Institution's

1978 list of recommended threatened plant species of the United States (Ayensu and DeFilippis, 1978).

The ecological life cycle of this narrowly endemic species and certain environmental parameters of its cedar glade habitat have been described (Baskin *et al.*, 1972). Within the cedar glades, *A. tennesseensis* usually grows in the transition zone between the open glade and glade thickets or woods. In this zone, light intensity, temperature and soil and atmospheric moisture are intermediate between those of open glades and glade woods. However, plants of *A. tennesseensis* are subjected to moisture stress during the summer because soil moisture in its habitat often drops below the permanent wilting percentage. Instead of going dormant, reproductively mature plants as well as seedlings and juveniles remain vegetatively active throughout the summer. In autumn the stems and leaves die, at which time vegetative buds at the caudex produce an overwintering rosette of leaves. The new stems begin to elongate the following March, and if plants are going to flower, buds are initiated at this time.

Zohary (1954) has found that many of the most important species in the common plant communities of the Near East deserts are never dormant; instead, they are active the whole year. In comparing the hydroecological behavior of these species, Zohary (1954) found that when they were exposed to permanent or

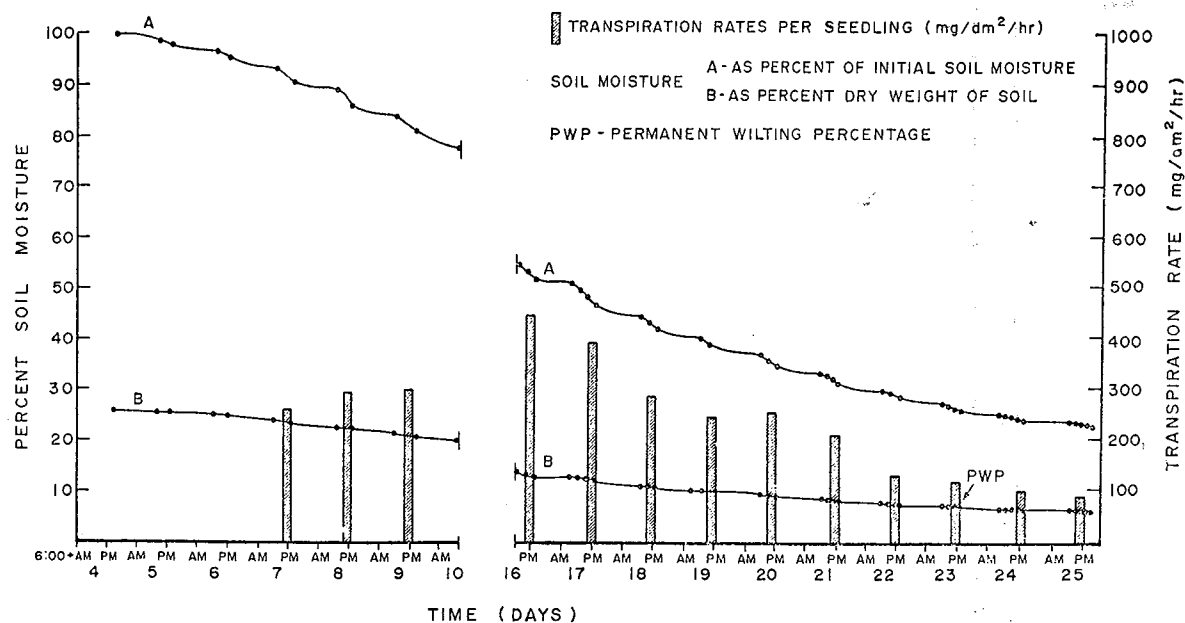


FIG. 1. Transpiration rates of *Astragalus tennesseensis* seedlings in relation to soil moisture.

seasonal drought, they were quite economical in their use of water, in both the wet and dry season. Efficient use of water by these species was shown by changes in transpiration intensity and by reduction of the transpiring surface during drought periods.

Since *A. tennesseensis* grows in a seasonally-arid habitat, an investigation was undertaken to determine how it achieves hydro-economy. This study included the determination of (1) transpiration rates of seedlings over a range of soil moisture conditions from field capacity to below the permanent wilting point, (2) transpiration rates at field capacity of seedlings and mature plants before and after drought preconditioning, (3) changes in leaf area during drought and (4) changes in water loss per plant before and after drought stress.

GENERAL METHODS

Twenty-four reproductively mature plants of *A. tennesseensis* of near uniform size were collected from a cedar glade near Couchville in Davidson County, Tennessee on 25 March 1967 and planted in cedar glade soil in 15-cm-diameter plastic pots. Seedlings were started from seeds on 11 March 1967 by planting eight scarified and leached seeds (Baskin and Quarterman, 1969) in each of 29 10-cm-diameter pots filled with cedar glade soil; seedlings were later thinned to six per pot. Mature plants and seedlings were grown in a greenhouse under natural photoperiod and were watered at 1-3 day intervals to keep the soil moist. The mature plants grew normally and subsequently flowered in late April at the same time as did plants in the field. When transpiration studies were started on 4 May 1967, seedlings had five to six leaves which corresponded to the number of leaves that seedlings of the same age usually had in the field.

When transpiration measurements were made, pots of soil containing seedlings and mature plants were first watered to field capacity and allowed to drain for 12 h. Then the pots were wrapped with two layers of plastic film and placed in a growth chamber on a 12-h photoperiod. During the light period, the temperature was 26°C and the relative humidity was approximately 50%, while during the dark period the temperatures was 6°C and the relative humidity was near 100%. Light intensity at plant level in the growth chamber was 10.8 Klx. Transpiration rates were determined by weighing the plants at

intervals and calculating the weight of water lost. At the end of the measurements, leaf areas were determined by weighing blue prints of the leaves and relating the weight of the leaves to a known area/weight ratio of the blue print paper. Since leaves have equal numbers of stomates on the upper and lower surfaces, the area was doubled for each leaf to give the total transpiring surface area. Transpiration rates were expressed as mg of water lost/dm² of leaf area/h and as water lost/plant/h.

EXPERIMENTAL PROCEDURES AND RESULTS

Transpiration rates of seedlings

Transpiration rates were measured simultaneously for five pots of seedlings throughout an 18-day period. Measurements were made at 2- to 4-h intervals during the light period, when the temperature was 26°C. The amount of water in the soil at each reading was determined at the conclusion of the experiment by subtracting the wet weight of the plants plus the dry weight of the rest of the phytometer from the wet weight of the phytometer at the time of measurement. The amount of water (grams) in the soil was expressed as a percent of water present at the beginning of the experiment (100%) as well as the percent dry weight of the soil.

The highest transpiration rate per seedling (450 mg/dm²/h) occurred when soil moisture content was 16% of the dry weight of the soil and 53% of the initial soil moisture (Fig. 1). As soil water decreased further, transpiration rates decreased. At the permanent wilting percentage (6.5), which was 26% of the initial soil moisture, 120 mg of water were lost/dm²/h. The lowest transpiration rate per seedling (90 mg/dm²/h) occurred when soil moisture was 6% of dry weight and 23% of initial soil moisture. Thus, there was a decrease of 80% in the transpiration rate as the soil moisture dropped from 16 to 6% of the dry weight and from 53 to 23% of the initial soil moisture; therefore, non-drought-conditioned seedlings of *A. tennesseensis* greatly decreased their transpiration rates as soil moisture decreased.

Effect of drought preconditioning on transpiration rates

Transpiration rates were measured at field capacity for 24 mature plants and for 24 pots of seedlings. Then, three mature plants and three pots of seedlings were selected randomly for leaf surface area determinations. The remainder of the plants were returned to the greenhouse and randomly placed on 3-, 6-, 9-, 12-, 15-, 18- or 21-day watering cycles. These plants were watered to field capacity on the first day of each respective cycle and were not watered again until the required number of days for each cycle had elapsed. No treatment was given in which plants were watered every day because a preliminary experiment showed that when plants were watered every day the soil stayed too wet and many of them died from root rot. Dead leaves were removed from each plant at 9-day intervals. After 2 mos of exposure to the various watering regimes, transpiration rates again were determined at field capacity for seedlings and mature plants.

TABLE 1: *Transpiration rates (mean ± SE) of Astragalus tennesseensis plants before and after they were subjected to drought cycles.*

	Drought cycles (days)	Water loss (mg/dm ² /h)	
		Mature plants	Seedlings
Before drought		290± 18	282±21
After drought	3	357± 41	442±47
	6	327± 26	403±57
	9	382± 90	377±27
	12	326± 65	— ^a
	15	349± 70	—
	18	362±118	—
	21	364± 95	—

^aPlants died

TABLE 2. *Changes in leaf area of Astragalus tennesseensis plants exposed to drought cycles.*

Age	Leaf area before drought cycles (dm ²)	Drought cycles (days)	Total leaf area/plant (dm ²) ^a	Total leaf area lost/gained/plant (dm ²)	Net leaf area lost or gained/plant (dm ²)
Mature	3.53	3	5.75	0.76	+1.46
	4.00	6	5.53	0.84	+0.69
	4.10	9	5.78	1.23	+0.45
	2.96	12	4.25	1.56	+0.27
	3.59	15	4.42	2.10	-1.27
	3.17	18	3.81	2.03	-1.39
	2.89	21	3.33	1.97	-1.53
Seedling	0.37	3	1.15	0.23	+0.55
	0.29	6	1.08	0.43	+0.36
	0.30	9	1.07	0.60	+0.17
	0.46	12	0.86	0.86	-0.46
	0.33	15	0.75	0.75	-0.33
	0.36	18	0.72	0.72	-0.36
	0.37	21	0.62	0.62	-0.37

^aInitial leaves plus those produced during the experiment

All mature plants survived the watering regimes, but only seedlings watered every 3, 6 or 9 days survived. A comparison of transpiration rates before and after seedlings and mature plants were subjected to the various watering cycles shows that transpiration rates per unit of leaf area were not decreased by drought preconditioning (Table 1). In fact, transpiration rates were higher for both seedlings and mature plants after the 2-mo growth period under the various watering cycles than before it began.

Changes in leaf area

Changes in leaf area were determined for the plants used to study the effect of drought preconditioning on subsequent transpiration rates. After transpiration rates were measured for the drought-treated plants, leaf areas were determined and then the leaves were dried to constant weight at 80°C in order to determine the ratio (144.8) of milligrams dry weight of leaves per decimeter square of leaf area. The weight of leaves harvested plus the weights of leaves that died during the experiment gave the total dry weight of leaves per plant. Total leaf areas produced and lost per plant were calculated by using the weight-area ratio. Initial leaf areas of seedlings and mature plants used in the drought preconditioning experiment were calculated by dividing the amount of water lost/plant/h (Table 3) by the rate of water loss (mg/dm²/h) (Table 1) before the watering cycles began. Initial leaf area, leaf area lost and total leaf area were used to calculate the change in net leaf area per plant.

TABLE 3. *Changes in water loss/plant/h of Astragalus tennesseensis plants after exposure to drought cycles.*

Age	Drought cycles (days)	Water loss (mg/plant/h)		
		Before	After	% change
Mature	3	1,024±167	1,520±154	+ 48.4
	6	1,157±285	1,470±163	+ 27.1
	9	1,190±111	1,320±248	+ 10.9
	12	857±176	700±229	- 18.3
	15	1,042± 95	650±173	- 37.6
	18	918±158	563±131	- 38.7
	21	837±162	420± 58	- 49.8
Seedling	3	104± 16	353± 46	+239.4
	6	81± 11	245± 33	+202.5
	9	85± 9	205± 16	+141.2

During the 2-mo growth period in the greenhouse, all plants produced and lost leaves regardless of the length of the watering cycles (Table 2). Mature plants and seedlings on 3, 6 and 9 day cycles produced more leaves than they lost; therefore, they exhibited a net gain in leaf area. On the other hand, mature plants on cycles of 15 or more days and seedlings on cycles of 12 or more days showed a net loss of leaf area. Whereas seedlings exposed to 12-, 15-, 18- and 21-day drought cycles lost all their leaves and then died, mature plants exposed to 15-, 18- and 21-day drought cycles lost all their leaves and then produced new ones at the basal nodes.

Changes in water loss per plant

Although 2 mos of exposure to various periods of soil moisture stress did not reduce the rate of water loss (Table 1), there were changes in the amount of water lost/plant/h (Table 3). Decreases or increases in water loss per plant are correlated with the net loss or gain of leaf area per plant. Mature plants and seedlings that received water every 3 to 9 days had a net gain of 0.17 to 1.46 dm² of leaf area and an increase of 11 to 239% in mg of water lost per plant at the end of the experiment. Conversely, mature plants that received water every 12 to 21 days showed a net loss of 0.33 to 1.53 dm² of leaf area and a decrease of 18 to 50% in water lost per plant.

DISCUSSION

Drought preconditioning of *A. tennesseensis* plants did not cause a reduction in transpiration rates per unit of leaf area. Similarly, in seedlings of *Tecomella undulata* Seem, a xeric tree species in India, differences in soil moisture produced by different intervals of watering did not greatly influence the transpiration rate per unit area of leaf surface when plants were rewatered (Lahirie and Kharabanda, 1966). After drought preconditioning, mature plants and seedlings of *A. tennesseensis* showed higher rates of water loss per unit of leaf area than they did before the watering cycles began (Table 1). Seedlings of *T. undulata* also had higher transpiration rates after exposure to 2, 4 and 6 day watering regimes than they exhibited initially. In this species, there was a graded rise in transpiration rates; minimum rates occurred in plants that received 2-day watering cycles and maximum rates in plants that received 6-day cycles.

During drought stress, mature plants of *A. tennesseensis* reduced their transpiration rates by losing leaves. The phenomenon of reduction of surface area by leaf abscission has been observed in xeric species such as *Tecomella undulata* (Lahirie and Kharabanda, 1966), *Heliotropium rotundifolium* Sieber ex Lehm., *Atriplex halimus* Phil., and *Astragalus spinosus* C. Christensen (Evenari and Richter, 1937). Generally, the oldest leaves are lost first and the water in dying leaves is translocated to younger ones at the apical meristems (Oppenheimer, 1960). In some species of the Near Eastern deserts, Orshan (1954) found that larger leaves produced during the wet season were replaced by smaller ones at the beginning of the dry season. By this process *Poterium spinosum* L., *Artemisia monosperma* Del., and *Helianthemum ellipticum* Pers. lost between 60 and 85% of the total dry weight of their leaves. Similarly, the transpiring surface of *Zygophyllum dumosum* Boiss. was reduced by 87% (Zohary and Orshan, 1954). The extent and timing of the reduction of leaf area depends upon moisture conditions. If moisture conditions are favorable, even after the beginning of the dry season, only a portion of the leaves may fall from some species or the time of falling may be delayed for as much as a month or more, depending upon how quickly the soil becomes dry (Zohary, 1961).

In a seasonally-arid habitat, long term economical use of water reserves in the soil is essential for survival of an actively growing species. One aspect of water economy is the ability of a species to reduce its

rate of water loss when soil moisture becomes limiting. Seedlings of *A. tennesseensis* greatly reduced their transpiration rates as soil moisture (dry weight basis), decreased from 16 to 6.5% (the permanent wilting percentage) (Fig. 1). However, if a plant has no other method of controlling rate of water loss as the soil dries, then it may deplete its water supply and die before the end of the dry season. Even if there are showers, the resumption of former transpiration rates will soon deplete the moisture supply again.

Plants of *A. tennesseensis* also reduce their leaf surface area as soil moisture decreases; therefore, long before soil moisture is depleted, older leaves on each plant are dead. This method of achieving water economy conserves soil moisture. Controlled loss of leaves allows *A. tennesseensis* plants to achieve a permanent reduction in transpiration rates during the whole dry season.

Mature plants of *A. tennesseensis* that lost all of their leaves initiated new ones from buds at the caudex. However, seedlings that lost all of their leaves did not produce new ones and died. This lack of leaf production in severely drought-stressed seedlings may be due to the absence of buds at the caudex of the small plants and/or low reserves of carbohydrates and water in the roots. Field studies indicate that many seedlings die during summer. Thus, whereas 83% of the newly germinated *A. tennesseensis* seedlings marked in the field in March and April 1966 were alive on 19 June 1966, only 22% of them were alive on 25 September 1966 (Baskin *et al.*, 1972). The inability of seedlings to produce new leaves when all the existing ones are killed by drought may be one reason why seedling survival is poor in the cedar glade habitat and consequently why *A. tennesseensis* populations are small.

Another aspect of the drought tolerance of *A. tennesseensis* is the ability of water-stressed plants to accumulate proline (Baskin and Baskin, 1974). After 7 days of drought the quantity of proline ($\mu\text{g}/\text{gm}$ dry wt plant material) in plant leaves was 3,174, whereas in nondrought stressed plants it was 84. The quantity of proline in drought stressed plants that were subsequently watered every day for 7 days was 118, showing that proline is metabolized when drought stress is alleviated. A number of plant species have been reported to accumulate proline during periods of water stress (*e.g.*, Barnett and Naylor, 1966; Waldron and Teare, 1974; Boggess *et al.*, 1976; Huang and Cavalieri, 1979). It has been proposed that the accumulation of proline may be a metabolic adaptation that confers some survival value to plants under water stress by acting as a protective agent for enzymes (Schobert and Tschesche, 1978) and/or as a storage compound of reducing equivalent (Jäger and Meyer, 1977) and of nitrogen for growth after stress has ended (Barnett and Naylor, 1966). The accumulation of proline during water stress has prompted comparative studies of drought resistant and drought sensitive varieties and species. Singh *et al.* (1972) studied 10 varieties of barley under drought stress and found a positive correlation between drought resistance and proline accumulation. Similarly, Hubac and Guerrier (1972) reported that the more drought resistant of the two *Carex* species they studied accu-