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# WATER RELATIONS OF DWARF MISTLETOES (ARCEUTHOBIUM SPP.)

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Abstract At low and high levels of moisture stress a tissue water potential gradient was measured between ponderosa and lodgepole pines and their respective dwarf mistletoes (A. vaginatum and A. americanum). The gradient steepened with stress and can be explained by excessive transpiration and respiration of the parasite at moderate to severe stress levels.

## INTRODUCTION

Dwarf mistletoes are obligate stem parasites causing substantial mortality and growth losses in western U.S. conifer forests. An estimated 3.2 billion board feet are lost each year in this region (Shea and Howard 1969) where all but one of the North American taxa are found (Hawksworth and Wiens 1972).

Infestations on xeromorphic pines throughout the semi-arid Southwest indicate good fitness of the parasite to environmental moisture stress. However, some species are not present throughout the host range and appear excluded from stands characterized by extreme seasonal drought. Specific examples include the absence of A. vaginatum subsp. cryptopodum Hawks. and Wiens near the lower elevational limits of ponderosa pine (Pinus ponderosa Laws.) along the Front Range of Colorado and the absence of A. americanum Nuttal ex Engelmann near the

upper limits of commercial lodgepole pine (P. contorta Dougl.) in Colorado and Wyoming.

At lower elevations exclusion could derive from steeper plant-atmosphere vapor pressure deficits caused by higher ambient temperatures. At high sites, water stress can result from large transpirational losses caused by strong winds and low soil temperatures inhibiting water absorption.

The purpose of this study was to test the hypothesis that dwarf mistletoes are excluded from sites exposed to severe drought. Comparisons were made between host and parasite water potentials, transpiration and respiration as forest trees and experimental seedlings were subjected to increasing water stress.

## LITERATURE REVIEW

A thorough literature review was facilitated by the Hawksworth Mistletoe Index (Scharpf, Hawksworth and Erickson 1976). This is available (Fisher 1975) and will only be summarized here.

Leafy mistletoes (Viscaceae) were the focus of numerous European studies prior to World War II. Generally, these and more current studies showed much greater transpiration rates for the parasite which remained high during periods of drought when host water loss was more restricted. Prior to this study transpiration research on dwarf mistletoes was limited to injection of dye into xylem of black spruce bearing witch's brooms (Tainter and French 1973). Flow toward the brooms was indicated.

Host-parasite solute concentrations received consideration attention from Harris et al. (University of Minnesota) and European physiologists from 1915 to 1930. Although solutes tended to concentrate in mistletoe tissues, exceptions make it difficult to present a unified concept of the role of solute concentration gradients in parasitism. Furthermore, most evidence indicates that transpiring plants absorb water primarily by passive rather than osmotic mechanisms. Osmotic values alone are therefore not sufficient to predict maximum absorptive forces; negative wall pressures must also be considered.

The pressure chamber offers a reasonably simple means for measuring xylem pressure potential (P) and has been applied to mistletoes. Scholander et al. (1965) reported that several leafy mistletoes had more negative P values than hosts. Mark and Reid (1971) measured pressure potentials of A. americanum and lodgepole pine in the forest. Although nonsystemic mistletoe shoots consistently had more negative values than host foliage, no gradient was measured between host and systemic infections.

## METHODS AND MATERIALS

Specific objectives of laboratory and field studies were to measure host and parasite water potentials, transpiration, and respiration gradients under water stress conditions. Studies proceeded as follows:

### Calibration of the pressure chamber

The pressure chamber measures xylem pressure potential (P) rather than tissue water potential, a more exact measure of plant water status. For each species regression equations were calculated to correct P values to tissue water potential. These were derived from comparisons of P and water potential values determined for paired foliage samples, harvested simultaneously from identical seedlings about 0.5 meters tall. Water potential ($\Psi_{tissue}$) was determined with a wet-loop thermocouple psychrometer (Richards and Ogata 1958). The isopiestic technique (Boyer and Knipling 1965) minimized errors caused by leaf diffusive resistance and output from a dry thermocouple corrected for heat of respiration.

### Determination of water potentials of A. vaginatum and ponderosa pine in the forest.

In order to determine the magnitude and direction of water potential gradients where A. vaginatum appears established, pressure chamber measurements were made throughout the year in a ponderosa pine forest near Sugarloaf, Colorado.

The infected stand is located 2.25 miles north of Boulder Canyon and 0.5 miles west of Sugarloaf (T. IN., R. 72 W. Section 26) and is 8000 feet (2438 m) above sea level. The stand is essentially pure ponderosa pine of a 50 to 60 year age class (d.b.h. 7.6 to 17.8 cm). Understory vegetation consists mainly of herbs and grasses typical of the Upper Montane ponderosa pine forests in this area (Marr 1967) and a few shrub species: for example, Ribes cereum Dougl. and Arctostaphylos uva-ursi (L.) Spreng.

Sampling was conducted in an area of 1.2 hectares from which 10 trees were selected according to height (4.6 to 7.6 m), d.b.h. (7.6 to 17.8 cm), and intensity of infection. Using the 6-class system proposed by Hawksworth (1961) sampled trees had intensities of infection ranging from 3.0 to 4.0.

Host and parasite pressure potentials were measured on: May 5, June 24, Sept. 26, Dec. 3 and Jan. 21, beginning at 1000 hours MST and continuing to approximately 1400 hours. Two host and two parasite measurements were recorded for two separate branches of each tree. Measurements were limited to the lower half of the tree's crown.

### Determination of water potential and respiration levels of A. vaginatum and ponderosa pine under increasing soil moisture stress

Infected seedlings were transplanted from the forest to 18.9 liter

containers and grown under greenhouse conditions. Soil in the containers was about 50% sand, 25% silt, and 25% clay, determined by the hydrometer method. At the beginning of the study, seedlings were 0.4 to 0.5 meters tall and from 15 to 22 years of age.

After two years, infected transplants were placed in a growth chamber and allowed to adjust to the environment for 6 weeks. The chamber was set for a 12-hour photoperiod, provided by both cool white fluorescent and incandescent bulbs yielding a far-red to blue spectral energy of 3680 uw. cm $^{-2}$. Day temperature was 25°C, night temperature was 9°C, and humidity was held at 35%. Plants were watered to field capacity weekly.

At the end of 6 weeks, with soils at field capacity, pots were taken in random order and pressure chamber measurements were made on host and parasite. These were begun 4 hours into the photoperiod and included two needle fasicles and two mistletoe shoots from each seedling. Following initial sampling, three pots were allowed to dry while a control pot was watered to field capacity weekly. Plants were sampled 17, 22, 28, 36, and 44 days following the first measurements.

Respiration of host and parasite tissues was determined upon completing pressure chamber measurements, approximately 6 hours into the photoperiod. Measurements were made with a Clark-type oxygen sensor as suggested by Boyer, Romancier, and Ralston (1971).

Water potentials and transpiration of A. americanum and lodgepole pine

Infected seedlings were transplanted from the forest to 3.79 liter containers and grown under greenhouse conditions for one year. They were subsequently placed in an environmental growth chamber and remained there until needed for experiments. Seedlings were 0.4 to 0.5 meters tall and ranged from 12 to 18 years of age.

As needed, seedlings were removed from the chamber and soil was removed from roots with running tap water and an ultrasonic cleaner to minimize root damage. Seedlings were then placed in a second growth chamber with roots suspended in 0.25 strength Hoagland's complete nutrient solution (Hoagland and Arnon 1950) and aerated with frits.

A 16-hour photoperiod was provided by cool white fluorescent and incandescent bulbs yielding far-red to blue spectral energy of 1510 uw cm $^{-2}$. Night and day temperatures were 17.5°C and 20°C and relative humidity was 35%. After one month, roots showed new growth and no visible damage from cleaning.

Host and parasite water potentials and transpiration were measured in separate experiments as seedlings were subjected to identical cycles. Substrate water potential ($\Psi_{sub}$) was controlled by the addition of polyethylene glycol (PEG) 4000 to the nutrient solution. Substrate potential was reduced 3 bars every 48 hours to a minimum of -15 bars. The amount of PEG needed was determined with a Richards and Ogata (1958) thermocouple psychrometer.

Pressure chamber measurements were made on three needle fasicles and two parasite shoots taken from each of five infected seedlings, sampled randomly after 4 hours of light. Sampling was completed after 2.5 hours. At the end of the light period, $\Psi_{sub}$ was reduced 3 bars in all but one control container, maintained near 0 bars.

Transpiration was measured during the dark and for 8 hours following initiation of the light period. Water loss was measured by enclosing host branchlets and mistletoe shoots in separate cuvettes and determining the change in humidity of a stream of air before and after passing through the enclosures (Fig. 1).

Cuvette air temperature was maintained at 23 ± 1°C by circulating water through the side wall water jackets. Clear plastic water vessels filtered long wave radiation. Light reaching foliage had a far-red to blue spectral energy of 1548 $\mu w \cdot cm^{-2}$.

Chamber air passed through two glass tubes (1.2 m in length) before reaching foliage. One tube, with an inside diameter of 3 cm, was half filled with a saturated solution of sodium dichromate ($Na_2 Cr_2O_7 \cdot H_2O$). The second tube (I.D. 1.2 cm) was filled with desiccated calcium sulfate (Drierite). Air in equilibrium with a saturated solution of sodium dichromate has a relative humidity that varies little from 15 to 40C, and is 54.5% at 23°C. Adjustment of a thumb-screw clamp compressed the latex tubing leading from the sodium dichromate tube and controlled mixing of air from the two tubes. Produced was an air stream with a specific humidity of 31%, partitioned so that one stream passed through either of the cuvettes, while the other served as a reference line. Air flowed through the system at a rate of 1 liter per minute, determined with two flowmeters (Gilmont No. 12). Before entering the humidity sensing device, both streams of air were brought to nominally similar dry bulb temperatures after passing through copper coils (3.6 meters in length), submerged in a temperature-controlled water bath.

The humidity sensing device was a differential psychrometer of the design by Slayter and Bierhuizen (1964). It had a very rapid response time and differences of less than 0.05 mg water per liter could be determined. The psychrometer contained two tubes and each supported wet and dry thermocouples. The reference stream passed through one tube while the transpiration stream flowed through the other.

While transpiration of one species was being measured, growth chamber air was pumped (1 liter $min^{-1}$) through the cuvette enclosing the other. This prevented accumulation of water vapor and maintained the same flow rate measured during transpiration measurements.

Psychrometric data were reduced by computer, programmed to calculate vapor pressure, saturation vapor pressure, and saturation density. Printout showed the difference in water vapor ($mg \cdot liter^{-1}$) between reference and transpiration lines. Rate was calculated by multiplying $mg \cdot liter^{-1}$ by flow rate (60 liters $\cdot$ $hour^{-1}$), dividing the product by transpiration surface area (discussed below), and multiplying the quotient by 100. Transpiration was thus expressed as mg $H_2O$ transpired per $dm^2$ per hour. More details on the technique are available from

Bierhuizen and Slayter (1964).

Plant surface area was determined with the glass ball technique (Thompson and Leyton 1971). Plant parts were dipped in a pressure sensitive adhesive, weighed, covered with a uniform layer of glass beads and reweighed. Weight gain was proportional to surface area and the technique was ideally suited to the irregular surfaces of dwarf mistletoes.

## RESULTS

### Pressure chamber calibration

Results showed that host pressure potentials could be corrected to tissue water potentials by using the following equations:

Eq. 1. Ponderosa pine $Y=.703\ (X) + 2.37$ $r^2 = .85$

Eq. 2. Lodgepole pine $Y=.72\ (X) + 4.43$ $r^2 = .98$

where X is xylem pressure potential and Y is $\Psi_{tissue}$ (-bars). Values of P for the pines deviated from $\Psi_{tissue}$ as much as 5 bars (based on regression lines). Differences were greatest where $\Psi_{tissue}$ tended to be more negative than P, and at relatively low water potentials. Plotted on the same graph, regression lines and lines of equal potential cross, creating "X-shaped" curves similar to those reported for loblolly and white pines (Kaufman 1968).

Regression equations for the parasites were as follows:

Eq. 3. *A. vaginatum* $Y = .912\ (X) + 1.49$ $r^2 = .86$

Eq. 4 *A. americanum* $Y = .883\ (X) + 4.08$ $r^2 = .89$

Mistletoe pressure and tissue water potentials did not deviate as markedly from a 1:1 relationship as did those plotted for pines. Plotted on the same graph, parasite regression lines are parallel, as one would expect for morphologically similar species of the same genus.

Values of $r^2$ were no less than 0.85 for any species and proved the pressure chamber a reliable instrument for mistletoe parasitism research. Concluded was that host-parasite $\Psi_{tissue}$ differences less than 5 bars would be difficult to detect without correction of chamber readings.

### Water potentials of *A. vaginatum* and ponderosa pine in the forest

Results of field studies are shown in Figure 2. Tissue water potentials shown are mean values for host or parasite and were recorded on the days indicated. Vertical bars represent ± one standard error (SE) of the mean. Significant differences between host and parasite mean values were determined with a t-test for paired observations.

Parasite potentials were significantly (.01 level) lower than host values for all dates except May 5. Results clearly showed that host and

parasite values were fairly close when potentials were -7 to -11 bars. With increased water stress, however, parasite potentials showed greater depression. This created a steeper gradient between host and parasite.

Water potentials and respiration of A. vaginatum and ponderosa pine subjected to soil moisture stress

The control pot showed only slight variations in host and parasite potentials throughout the experiment. Parasite $\Psi_{tissue}$ was on the average 3 bars lower than the host $\Psi_{tissue}$ which remained between -8 to -10 bars. Plant responses were similar for all treated pots and indicated a steepening of the potential gradients with time. Pot no. 2 potentials are seen in Figure 3, with vertical bars representing ± SE of the mean.

Differences between host and parasite respiration rates were statistically significant (.01 level), the latter being 1.5 to 3.5 times greater. Results indicated that a respiration gradient was maintained at low as well as high water potentials.

Water potentials of A. americanum and lodgepole pine

For the control pot, host and parasite water potentials varied little throughout the study. Parasite potentials were always about 4 bars more negative than host potentials. Host values averaged -12.9 bars while parasite values averaged -16.8 bars. Host and parasite responses for each treated seedling were plotted separately and the curves showed excellent agreement from one pot to another.

The overall effect of decreasing substrate water potential on host-parasite potential gradient is seen in Figure 4, which combines data from all pots. The solid line was mathematically fitted, the line of equal potential is dashed.

Figures 2 and 3 clearly show: (1) parasite more negative than host potentials throughout the study; (2) a slight increase of the host-parasite potential gradient with increased stress; (3) potential ranges from -10 to -16.5 bars for the host and -12 to -22 bars for the parasite.

Transpiration of A. americanum and lodgepole pine

Transpiration of host and parasite was plotted for three infected seedlings exposed to substrate potentials of 0, -3, -6, -9, -12, and -15 bars. Host and parasite responses were quite similar from pot to pot and transpiration is shown only for one seedling and parasite shoot at 0 and -15 bars (Figs. 5 and 6).

At approximately 0 bars $\Psi_{sub}$, maximum transpiration was greatest for the parasite. However, total transpiration for the 8-hour period was greater for the host, determined by planimeter. Although maximum rates were similar for host and parasite at -9 bars substrate potential,

total transpiration was considerably greater for the host. The marked reduction of host transpiration at -12 and -15 bars $\Psi_{sub}$ created the widest margin between host and parasite rates. At -15 bars $\Psi_{sub}$, parasite exceeded host transpiration two to three times during the entire 8-hour period.

## DISCUSSION

Field and laboratory results indicated more negative water potentials for parasite than host and agreed with previous studies reported by Scholander et al. (1965) and Mark and Reid (1971). Laboratory studies showed a slight gradient, even at field capacity and 0 bars $\Psi_{sub}$. Absence of a measurable gradient one day in the field at low stress levels probably reflects the more limited nature of research conducted in the field on mature trees.

Results showing transpiration of the parasite greater than host under moderately high water stress are in agreement with results reported by Kamerling (1914) and Hellmuth (1971). The relative inability of mistletoes and other hemiparasites to control water loss under environmental drought is not fully understood. Deserving consideration is Hodgson's suggestion (1973) that a deficiency of abscisic acid in the parasite may cause parasite stomata to remain open under stress.

Parasite respiration values compare favorably with those reported for A. tsugense (Miller 1973). Parasite respiration exceeding host rates several times agrees with studies of Hellmuth (1971) and Kumar and Mukherjee (1969) showing mistletoe shoots to be "metabolic sinks." High respiratory activity should enable dwarf mistletoes to compete vigorously for photosynthates. Rapid metabolism of carbohydrates provides energy required for accumulation of minerals and maintenance of higher osmotic pressure (Kumar and Mukherjee 1969).

Transpiration and respiration studies help explain the potential differences measured. Water moves through a plant along a water potential gradient controlled by the rate of water loss from leaf cells. It follows that the large inbalance in host and parasite transpiration measured at moderately high levels of water stress must account somewhat for the maintenance and steeping of the host-parasite potential gradient. Solute gradients between host and parasite may direct flow at low levels of stress. However, these usually do not exceed a few bars and do not account for the rather large differences found during drought. High parasite respiration under all conditions indicates that the parasite is being provided the substrate and consequently the energy required for the endergonic processes associated with active absorption. This partially explains parasite accumulation of solutes even at high stress levels, and indicates that high parasite respiration rates may help maintain a potential gradient favorable to the parasite.

It seems highly unlikely that exclusion is derived from the inability of the parasite to maintain a potential gradient on more

droughty sites. A gradient was maintained at host potentials not yet measured in the forest. The possibility exists that the inability of the parasite to restrict water loss may cause shoots to wilt and die. The endophytic system, however, could survive and produce new shoots.

In conclusion, it is more probable that environmental moisture stress would be more forcefully expressed during the seed germination process or host penetration. Clearly, this better explains the distribution of A. vaginatum at lower elevations than the absence of A. americanum near upper host limits.

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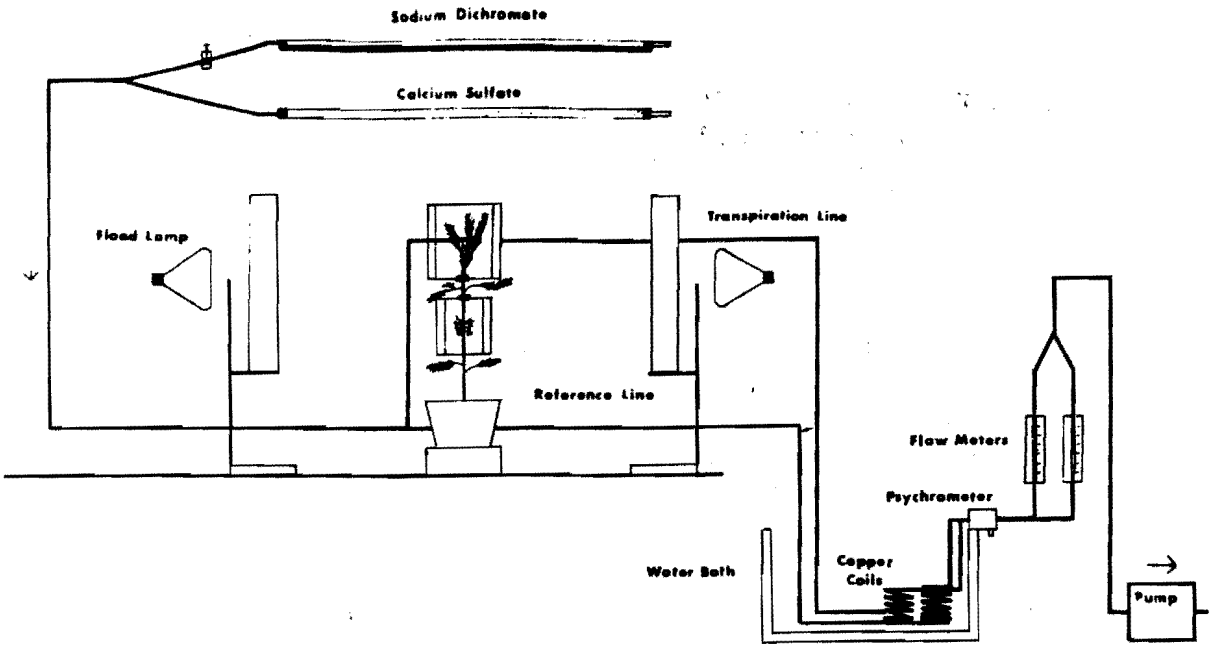


Figure 1. Schematic representation of apparatus used to determine transpiration of A. americanum and lodgepole pine.

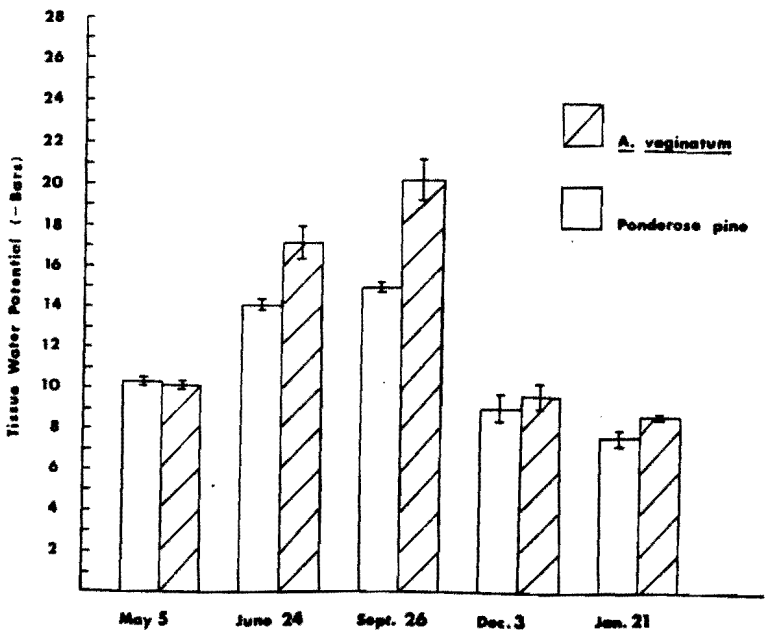


Figure 2. Tissue water potentials of A. vaginatum and ponderosa pine growing near Sugarloaf, Colorado.

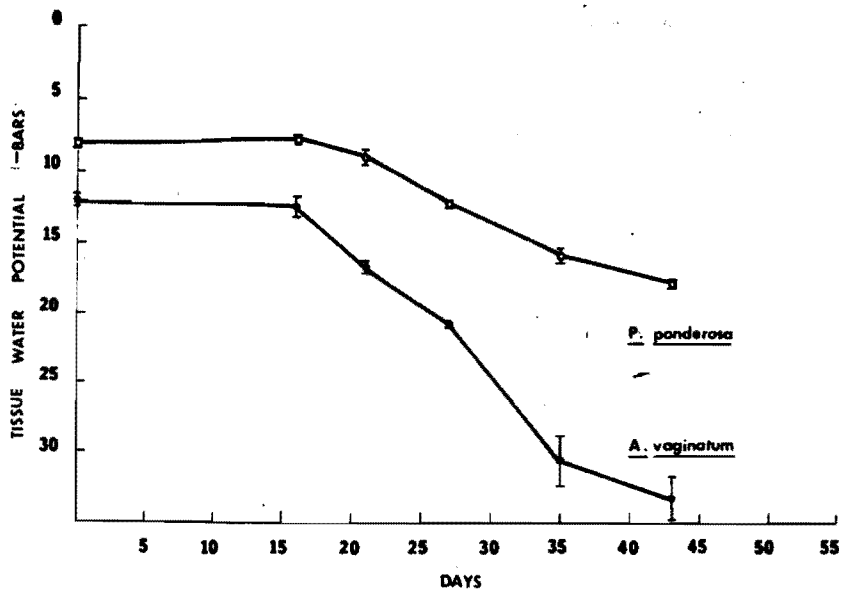


Figure 3. Effect of decreasing soil moisture on tissue water potentials of A. vaginatum and P. ponderosa.

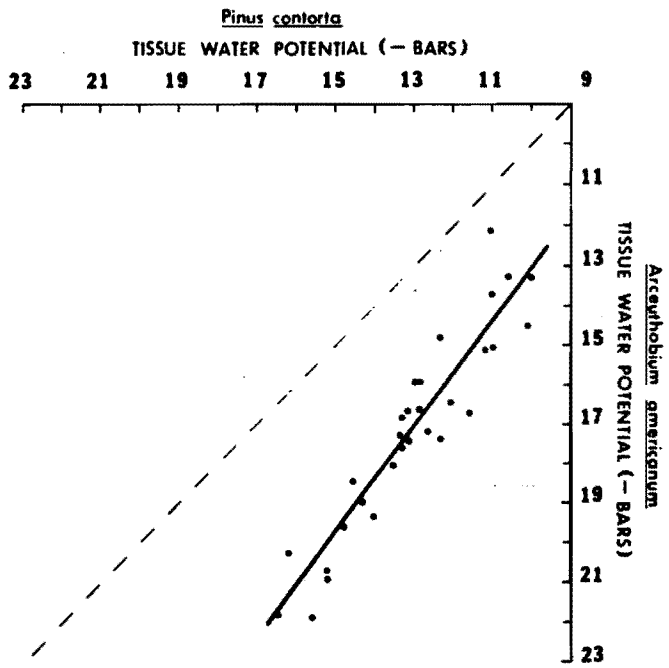


Figure 4. Effect of decreasing substrate water potential on tissue water potentials of A. americanum and host.

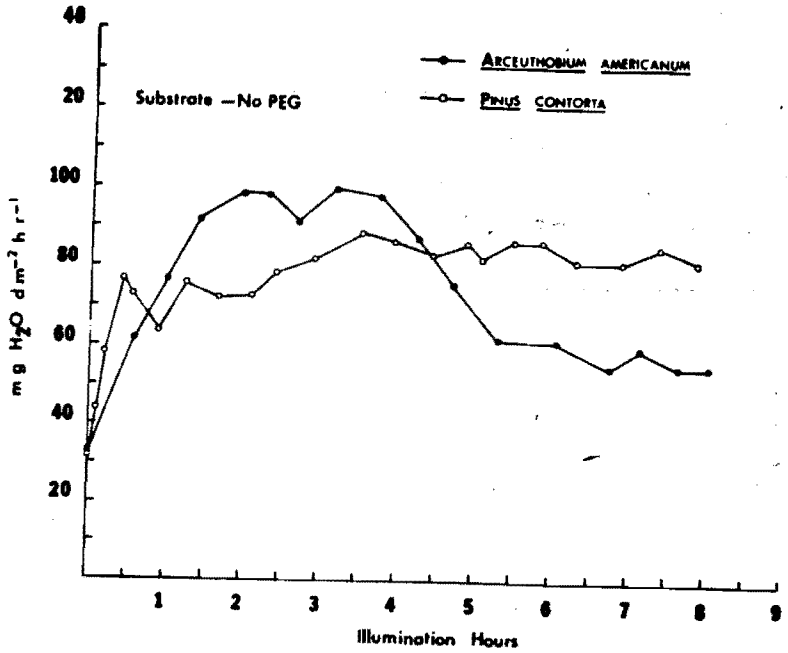


Figure 5. Transpiration of lodgepole pine and A. americanum at 0 bars substrate water potential.

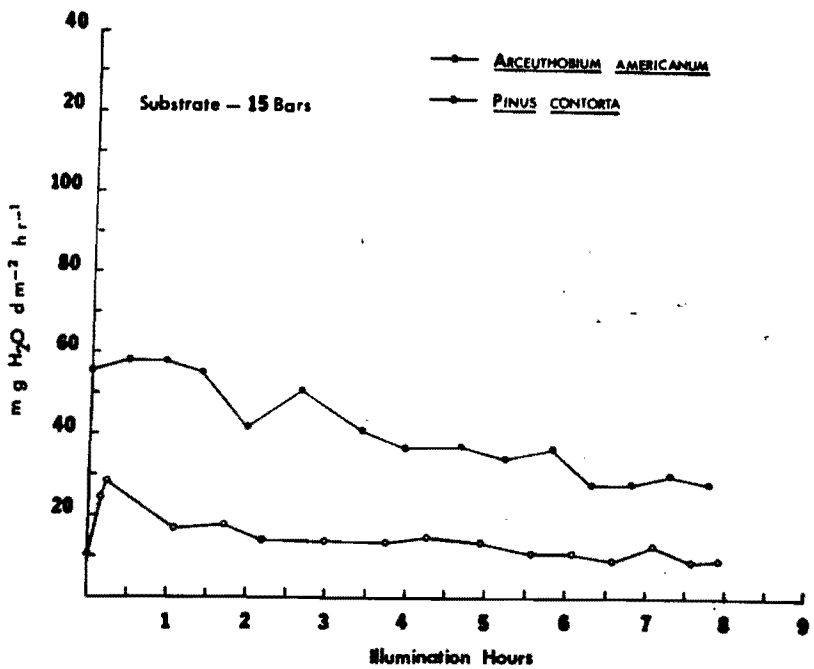


Figure 6. Transpiration of lodgepole pine and A. americanum at -15 bars substrate water potential.