

# Daily and Seasonal Osmotic Changes in a Tropical Treeline Species

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## ABSTRACT

The influence of environmental factors on osmotic properties in leaf tissue of *Polylepis sericea*, a widespread tropical treeline species in the Venezuelan Andes was studied under field and laboratory conditions.

Daily osmotic changes were determined with thermocouple psychrometers, and the degree of seasonal adjustments was estimated by analysis of pressure-volume ( $P-V$ ) curves. Measurement of diurnal variations in water potential components and soluble carbohydrates revealed osmotic adjustment between 0.6 and 1.0 MPa during the predawn hours. A linear relationship was found between minimum osmotic potential and leaf temperature indicating that temperature may significantly affect the magnitude of the predawn osmotic potential drop. Analysis of  $P-V$  relationships indicated that there was a seasonal adjustment, from the end of the wet season (November) to the middle of the dry season (February), of approximately 0.5 MPa due probably to a net increase in osmotically active solutes.

Laboratory experiments revealed that lowering the preconditioning plant temperature decreased both the leaf osmotic potential and the supercooling point and increased the leaf soluble carbohydrate content.

The adaptive significance of these mechanisms for trees growing at high elevations in tropical regions where freezing temperatures may occur any night of the year is discussed.

**Key words:** *Polylepis sericea*; Osmotic adjustment; Cold resistance mechanisms.

## INTRODUCTION

Members of the arborescent growth form *Polylepis* (Rosaceae), a genus restricted to the tropical high Andes, are found at elevations up to 5200 m (Simpson, 1979). This ability to grow at altitudes where even nonarborescent vascular plants are rare suggests the existence of special physiological adaptations which permit *Polylepis* to withstand extremely rigorous conditions. In the Venezuelan Andes, *Polylepis sericea* WEDD grows above timberline, reaching altitudes of 4600 m (Arnal, 1983). At such altitudes freezing temperatures may occur any night of the year, although remaining below zero for only a few hours. In this tropical high elevation climate, diurnal temperature variations are far more pronounced than seasonal ones and plants are regularly exposed to fluctuations from summer to winter-like conditions in less than 24 h.

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*P. sericea* trees seldom grow as isolated individuals. They tend to form small forest 'islands' above the continuous forest limit (3200 masl in the Venezuelan Andes), generally near massive rock outcroppings (Walter and Medina, 1969; Monasterio, 1980; Arnal, 1983). These sites may be considered thermal refuges as freezing temperatures are less frequent than in the surrounding Paramo vegetation dominated by caulescent giant rosette plants of the genus *Espeletia* (Azocar and Monasterio, 1980; Rada, 1983). Nevertheless, *P. sericea* is still exposed to night-time temperatures which are low enough to cause tissue freezing and metabolic disturbances in plants not well adapted to these conditions.

In temperate zones, plants are exposed to seasonal changes in temperature. These plants have developed resistance mechanisms to low temperatures such as bud dormancy, leaf abscission, and tolerance of extracellular ice formation during the unfavourable period. In contrast, high elevation arborescent growth forms in the tropics must resist freezing temperatures and at the same time maintain continuous growth or at least a continuously high level of physiological activity. Avoidance mechanisms such as freezing point depression or supercooling would confer selective advantages in cold tropical regimes where temperature seasonality does not exist and minimum subfreezing temperatures are not very low (Larcher, 1973). Freezing point depression provides, for evergreen woody species, a reliable degree of protection against frost in regions with a mild winter (Larcher, 1970, 1973).

The purpose of this study is to evaluate one of the cold resistance mechanisms in *P. sericea* growing at high elevations in the Venezuelan Andes. To accomplish this we investigated daily and seasonal active changes in osmotic potential (osmotic adjustment) and the role these changes may play in lowering the freezing point of active tissues. The relationship between freezing point depression, osmotic potential, soluble carbohydrates and supercooling was also investigated in the laboratory.

## MATERIALS AND METHODS

The study was carried out using 2–4 m tall members of *P. sericea* populations from two sites in the Piedras Blancas Paramo (approximate coordinates lat. 8° 37' N, long. 70° 12' W) at an elevation of 3900 m. The mean annual temperature is approximately 3.7°C and the difference in mean monthly temperature between the coldest and warmest months is only 2.7°C. The annual precipitation of 800–900 mm falls mainly between April and December. These trees are evergreen, can attain 5–10 m in height, have a twisted stem with layers of thin, red exfoliating sheets, and compound leaves. The leaflets are less than 1.0 cm wide, dark green above and densely covered with silvery trichomes on the underside. Leaves and flowers are continuously produced throughout the year despite seasonal changes in monthly precipitation (P. Berry, personal communication).

Daily patterns of environmental variables, leaf osmotic potential and soluble carbohydrates were measured on eleven occasions between 1982 and the beginning of 1984. Leaf and air temperatures were measured with 36-gauge copper–constantan thermocouples. Relative humidity was measured with an Assman psychrometer installed at 1.5 m. The absolute humidity difference between the leaf and the air was determined from the leaf temperature and the wet and dry bulb temperatures obtained from the psychrometer. Mature leaves from the same position on the shoot were collected throughout the day at approximately 3, 2 and 1 h intervals at night-time, daytime and predawn, respectively. For leaf osmotic potential ( $\psi_{\pi}$ ) determinations, samples were carefully wrapped in aluminum foil, frozen in liquid nitrogen, placed in small tubes sealed with a rubber stopper, and kept on ice while transported to the laboratory. The leaf samples were placed in a Wescor Sample Chamber (model C52) and after reaching equilibrium (approximately 1 h) the tissue osmotic potential was measured with a Wescor HR-33T Dew Point Microvoltmeter operating in the dew point mode. The osmotic potential of at least two replicate samples was measured. If there was a difference greater than 0.25 MPa between the two replicates, additional readings were taken. This system was frequently calibrated with filter paper discs soaked in known solutions of sodium chloride.

Daily patterns of leaf water potential ( $\psi_L$ ) were obtained on two occasions during representative wet and dry season days. A pressure chamber was used to measure  $\psi_L$  of four to five leaves which had been placed in plastic bags immediately after excision. Differences between replicates never exceeded

0.25 MPa. The turgor pressure potential ( $\psi_p$ ) was calculated subtracting  $\psi_\pi$  from  $\psi_L$  ( $\psi_L = \psi_\pi + \psi_p$ ), assuming leaf matric potential to be zero. For the determination of the soluble sugar concentration levels, leaf material was collected at the same time intervals as those for the osmotic potential. These samples were placed in polyethylene bags, completely sealed and immediately put on ice and brought back to the laboratory. They were oven dried at 40°C for 3 d and then ground. The soluble sugar concentration was determined by the anthrone method described by Allen (1974).

In order to evaluate the magnitude of seasonal adjustments, leaf water potential components and the modulus of elasticity were estimated in the laboratory by means of the pressure-volume technique (Tyree and Hammel, 1972). Small branches containing mature expanded leaves were cut at mid-day and the stems placed under water where they were immediately recut. The leaves were allowed to saturate fully overnight under a polyethylene cover. The following day the submerged ends were cut again, the leaves quickly weighed and the initial balancing pressure determined with a pressure chamber. The leaves were allowed to transpire freely and fresh weight and balancing pressure determinations were continued until several points on the linear portion of the pressure-volume curve had been obtained. Curves were analysed using plots of  $1/\psi_L$  versus relative water content (*RWC*). A regression of the form  $1/\psi_L = b_0 + b_1 \cdot RWC$  was fitted to the linear portion (Tyree and Richter, 1981, 1982). The parameters obtained from the pressure-volume curves include: the osmotic potential at full saturation ( $\psi_\pi^{100}$ ), osmotic potential at turgor loss pressure ( $\psi_\pi^0$ ), relative water content at turgor loss pressure (*RWC*<sup>0</sup>), the bulk modulus of elasticity ( $\epsilon$ ) and the number of osmoles per unit of dry weight ( $N_s/Dw$ ). The bulk modulus of elasticity was determined using Warren-Wilson's (1967) and Tyree, Cheung, MacGregor, and Talbot's (1978) methods. The number of osmoles of solute in a leaf ( $N_s$ ) was calculated by the equation  $N_s = \psi_\pi^{100} \cdot V_0 / RT$ , where  $\psi_\pi^{100}$  = osmotic potential at saturation,  $V_0$  = symplasmic water volume,  $R$  = the universal gas constant and  $T$  = °Kelvin (Parker, Pallardy, Hinckley, and Teskey, 1982).

Laboratory experiments were conducted to investigate the relationship between  $\psi_\pi$ , carbohydrate levels, freezing point depression and supercooling under controlled conditions. Small trees were excavated with roots and soil intact, transported to the laboratory and placed in growth chambers with temperature and irradiance control. After one day of acclimation at 10°C, the temperature was slowly decreased down to -10°C in approximately 3°C steps. After 2-3 h at each preconditioning temperature, samples of expanded leaves were obtained for carbohydrate and  $\psi_\pi$  determinations using the procedures previously outlined. To investigate the supercooling characteristics of leaves subjected experimentally to different temperature conditions, leaf samples were also taken at each interval and immediately enclosed in small airtight tubes to avoid changes in tissue water potential. Copper-constantan thermocouples were inserted in the leaves and changes in leaf temperatures were continuously monitored with a strip chart recorder fitted with an electronic 0°C reference. The tubes were then placed in an aluminum cylinder and immersed in a refrigerated alcohol bath. The cylinder acted as a heat sink and temperature stabilizer during the cooling process (Quamme, Stushnoff, and Weiser, 1972). The cooling rate of about 10°C h<sup>-1</sup> was used, similar to that observed after sundown in the field. Tissue temperature decreased rather uniformly and supercooling occurred followed by an exothermic reaction. The supercooling point was considered to be the temperature at which the heat of fusion was released.

## RESULTS

Figure 1 shows daily patterns of climatic variables, leaf water potential, osmotic potential and soluble carbohydrate content for two representative days during the wet and dry seasons. During the daytime, leaf temperature and vapour pressure difference between leaf and air (*VPD*) were higher on the dry as compared to the wet season day. At night, minimum leaf temperatures were similar while the *VPD* remained higher during the dry season day. Water potential minima occurred around solar noon, however,  $\psi_\pi$  reached a minimum at 05.00 h and 06.00 h in the wet and dry season days, respectively, coinciding with minimum leaf temperatures.  $\psi_L$  showed full recovery by 20.00 h on both days. Between this time and predawn  $\psi_L$  tended to decrease by 0.2 to 0.4 MPa. This drop in  $\psi_L$  followed the significant decrease in  $\psi_\pi$  which occurred during the night and particularly at predawn. The pattern of changes in levels of soluble carbohydrates seemed to be the mirror image of the  $\psi_\pi$  pattern. Carbohydrates increased after sunset and peaked at 05.00-06.00 h, coinciding approximately

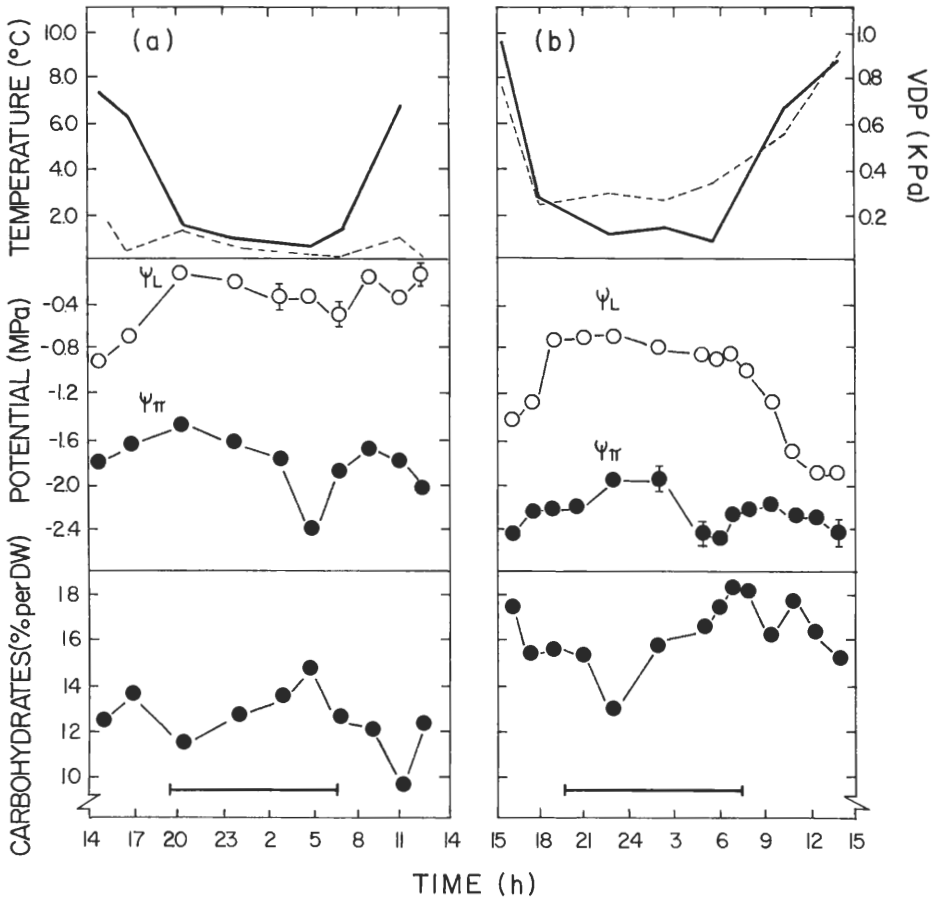


FIG. 1. Daily courses of leaf temperature (—), vapour pressure difference between leaf and air (---), leaf water potential ( $\psi_L$ ), psychrometric measure of osmotic potential ( $\psi_\pi$ ) and soluble carbohydrate levels during (a) a wet season day (20 December 1983) and (b) a dry season day (13 February 1984). The vertical bars for  $\psi_L$  and  $\psi_\pi$  represent the standard errors ( $n = 4$  to  $5$ ). The absence of a bar indicates that the standard error is smaller than the symbol. The horizontal segments in the lower part of each panel indicate the night-time period.

with the rather large predawn depression in  $\psi_\pi$ . The data in Fig. 1 show that as the night progressed,  $\psi_\pi$  declined, at least in part from the observed accumulation of osmotically active sugars in leaves. The time of occurrence of minimum  $\psi_\pi$  and maximum soluble carbohydrates did not differ significantly among the dry and wet season days. The absolute values were, however, very different. During the dry season day, the maximum  $\psi_\pi$  and  $\psi_L$  values were about 0.6 MPa lower than on the wet season day, probably indicating that the soil water potential had decreased by a similar amount. The lower night-time  $\psi_\pi$  seemed to be independent of seasonal changes in precipitation and evaporative demand as minimum values were approximately the same (about  $-2.6$  MPa) for both days.

Representative daily osmotic potentials and leaf temperature patterns for three different wet season days are shown in Fig. 2. Minimum night-time  $\psi_\pi$  always coincided with the lowest recorded temperatures. Osmotic potentials were more negative, by as much as 0.8 MPa, for those nights with the lowest leaf temperatures. The figure also shows how  $\psi_\pi$

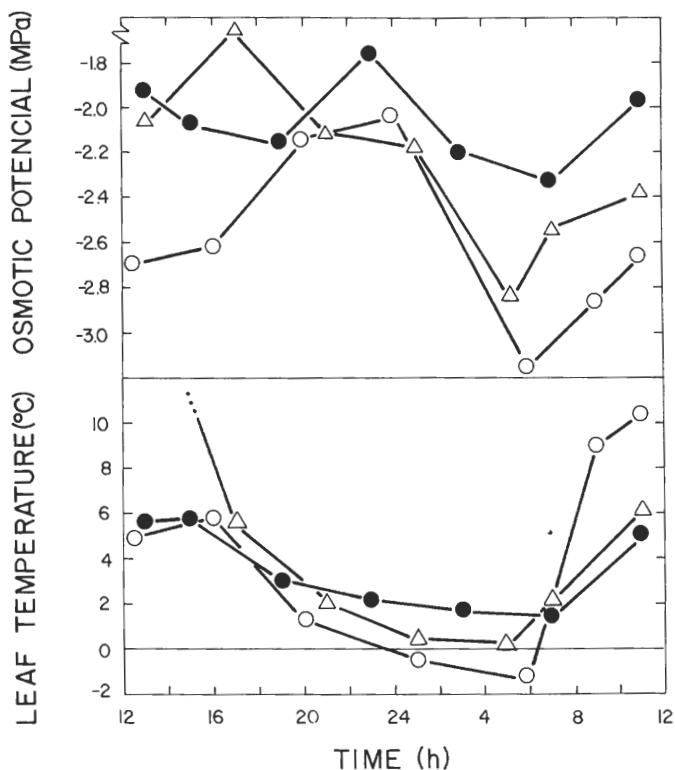


FIG. 2. Daily courses of osmotic potential (psychrometric measurement) and leaf temperature during 28 September 1982 (●); 3 November 1982 (○) and 8 December 1982 (△).

decreased abruptly from midnight to the predawn hours (lowest temperature hour) resulting in changes from 0.6 MPa for 28 September 1982, to 1.1 MPa for 3 November 1982.

In order to evaluate the relationship between leaf temperature and  $\psi_{\pi}$ , a linear regression analysis between the lowest  $\psi_{\pi}$  obtained at night-time and its corresponding leaf temperature was performed (Fig. 3). A strong linear relationship was found between  $\psi_{\pi}$  and leaf temperature ( $r = 0.90$ ;  $P < 0.01$ ), suggesting that leaf temperature may somehow affect the magnitude of the predawn drop in  $\psi_{\pi}$ .

Osmotic adjustments in response to water deficit can give rise to either partial or full turgor maintenance (Turner and Jones, 1980). Figure 4 shows the relationship between turgor potential and total leaf water potential for the same wet and dry season data depicted in Fig. 1. Full turgor maintenance would imply an active decrease in  $\psi_{\pi}$  with a decrease in  $\psi_L$ , resulting in a  $\psi_P/\psi_L$  relationship with practically no slope, characteristic of plants that are capable of strong osmotic adjustment and full turgor maintenance (Cline and Campbell, 1976; Davies and Lakso, 1979). For *P. sericea*, a slope statistically significant from zero but with differing  $y$ -intercepts was observed for both days. However, the turgor pressures obtained at a particular water potential under drier conditions were higher than under wet conditions. These results suggest that daily osmotic changes did not fully maintain turgor on a daily basis although seasonal changes in leaf water potential components did occur. In order to evaluate the importance of seasonal changes in water potential components, pressure-volume ( $P$ - $V$ ) curves were calculated from twigs obtained during the wet and dry

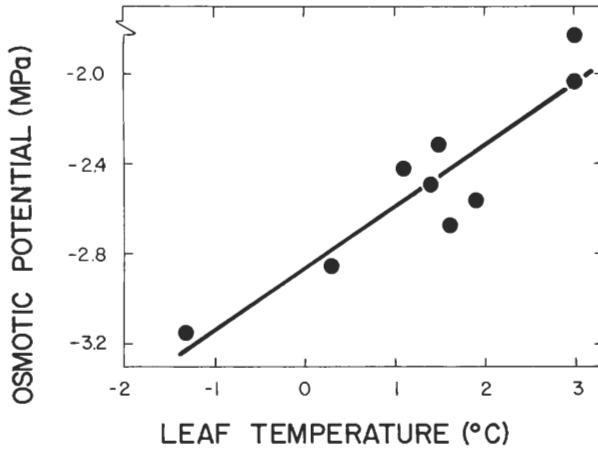


FIG. 3. Minimum night-time osmotic potential in relation to minimum leaf temperature. The solid line represents a linear regression fitted to the data.

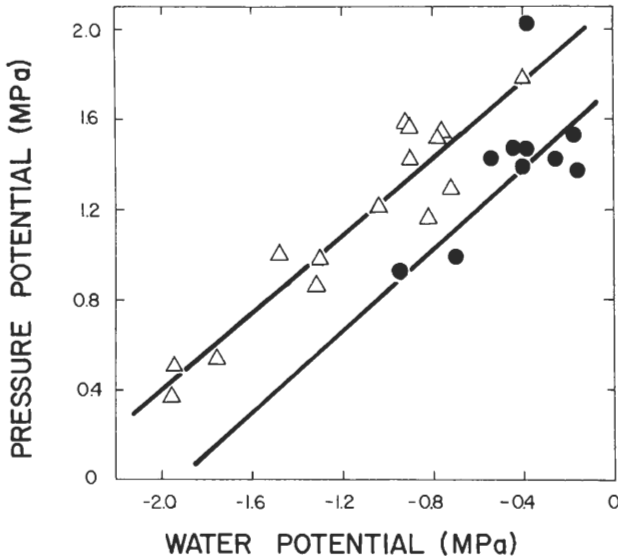


FIG. 4. Pressure potential versus leaf water potential during a dry season day (13 February 1984;  $\Delta$ ) and a wet season day (20 December 1983;  $\bullet$ ). Pressure potential was calculated from  $P-V$  curves for these two days. The lines are regressions fitted to the data ( $r = 0.94$ ,  $P < 0.01$  for  $\Delta$  and  $r = 0.84$ ,  $P < 0.01$  for  $\bullet$ ).

seasons. Representative  $P-V$  curves for both weather periods (Fig. 5), exhibited two characteristic phases: an initial non-linear portion at high values of  $RWC$  and a linear relationship at lower values. Several water relation parameters for representative wet and dry season conditions are tabulated in Table 1. There were differences in both the behaviour and magnitude of several important parameters. The osmotic potential at full saturation and at turgor loss decreased as much as 0.5 MPa from wet to dry season conditions. There was also an increase of the number of osmoles per leaf dry weight ( $N_s/Dw$ ) comparing the wet months to the dry ones. For example, for 8 December 1982, a wet month, a value of 0.05 was observed, as compared to 18 January 1983 where a value of 0.12, more than twice the value for December, was obtained.

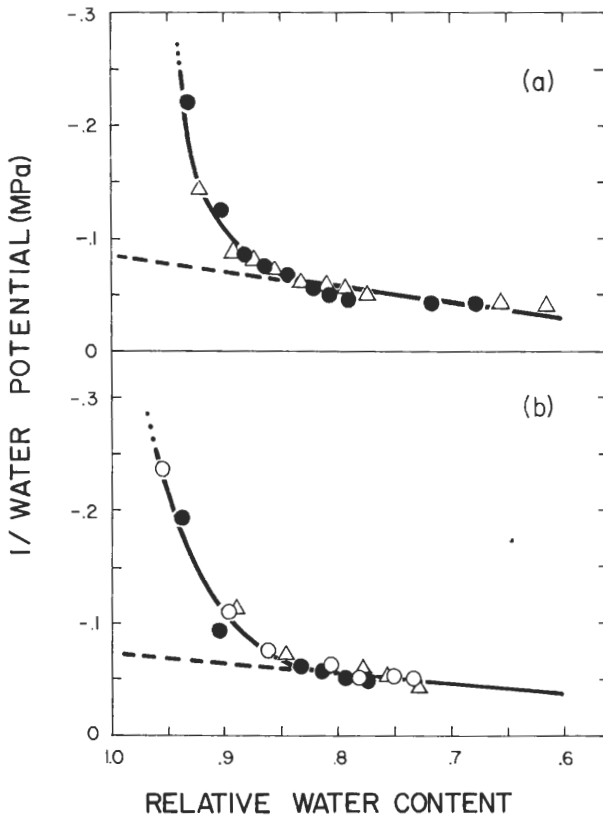


FIG. 5. Representative pressure-volume curves for *P. sericea* twigs obtained during a wet season day (a) and a dry season day (b). Sampling days are the same as in Figs 1 and 4. The symbols on the curves are from two different twigs in (a) and three different twigs in (b).

TABLE 1. Parameters obtained from P-V curves

$\psi_{\pi}^{100}$  = osmotic potential at saturation;  $\psi_{\pi}^0$  = osmotic potential at turgor loss pressure;  $RWC^0$  = relative water content at turgor loss pressure;  $N_s/Dw$  = Number of osmoles  $\text{kg}^{-1}$  dry weight;  $\epsilon$  = modulus of elasticity obtained from Warren-Wilson's ( $\epsilon_1$ ) and Tyree and Hammel's ( $\epsilon_2$ ) methods in MPa;  $Ppt$  = mean monthly precipitation (mm);  $Et$  = mean monthly evapotranspiration (mm) (for October 82  $Ppt = 80.4$  and  $Et = 67.3$ ).

	$Ppt$	$Et$	$\psi_{\pi}^{100}$	$\psi_{\pi}^0$	$RWC^0$	$N_s/Dw$	$\epsilon_1$	$\epsilon_2$
November 82	59.1	71.7	-1.16	-1.40	0.909	0.0575	9.10	3.43
December 82	19.0	69.7	-1.13	-1.38	0.909	0.0469	8.89	2.70
January 83	6.4	84.4	-1.55	-1.85	0.851	0.1156	11.25	3.60
February 83	9.3	108.5	-1.63	-2.13	0.820	0.1128	4.47	2.95

The laboratory results (Fig. 6) show very clear trends in the three leaf physiological properties related to cold resistance mechanisms. As the preconditioning temperature was lowered from  $10^{\circ}\text{C}$  to  $-5^{\circ}\text{C}$ , the osmotic potential decreased by  $0.5$  MPa, the supercooling point was lowered by about  $3^{\circ}\text{C}$  and the carbohydrate concentrations tended to increase linearly. A  $0.5$  MPa decrease in  $\psi_{\pi}$  would result in a  $0.41^{\circ}\text{C}$  lowering of the freezing point, as derived from Van't Hoff's and Raoult's equations:  $\Delta Tf = \psi_{\pi}/1.222$ , where  $\Delta Tf$  is the change in

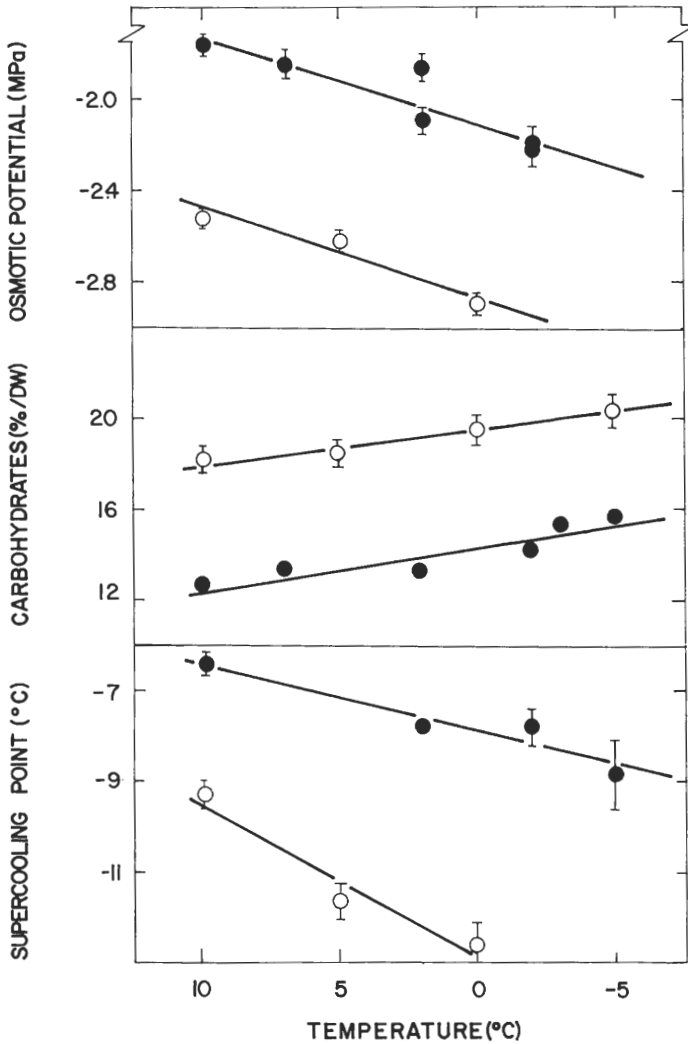


FIG. 6. Leaf osmotic potential ( $\psi_{\pi}$ ), sugar concentration levels and supercooling points obtained from leaf samples as preconditioning temperature was decreased in approximately 5 °C steps under controlled conditions. Plant material was obtained 20 February 1984 (●) and 9 April 1984 (○).

the freezing point and  $\psi_{\pi}$  is the osmotic potential in MPa. The supercooling point was actually depressed by about 3.0 °C. Similar functions but with different  $y$ -intercepts were obtained for the different sampling periods, beginning of the dry season (22 February 1984) and end of the dry season (12 April 1984), suggesting seasonal changes in the properties studied. The osmotic potential was 0.7 MPa lower in April than in February; the sugar concentration was 6% higher for April; and the supercooling points were also lower by almost 3 °C during the driest month. As the temperature was lowered to  $-10.0$  °C, the linear trends disappeared; the osmotic potential and the supercooling points abruptly increased, while the sugar concentration levels begin to decrease, coinciding with the occurrence of leaf tissue injury. For this reason, data points below  $-6.0$  °C were not included in Fig. 6.



## DISCUSSION

### *Seasonal changes in leaf water potential components*

The osmotic potentials at zero turgor measured in *P. sericea* (Table 1) are substantially higher than values which have been reported for plant species occurring in drought-prone environments (Scholander, Hammel, Hemmingsen, and Broadstreet, 1964; Hinckley, Duhme, Hinckley, and Richter, 1980). They are, however, lower than values measured in the other dominant growth-form at those high elevations in the Venezuelan Andes: the giant caulescent rosette plants belonging to the genus *Espeletia* (Goldstein, Meinzer, and Monasterio, 1984). The giant rosette species possess a range of water storage capacities. Species with a large storage capacity do not exhibit pronounced changes in leaf water potential and have osmotic potentials at zero turgor of  $-1.0$  to  $-1.2$  MPa. *Espeletia* species that have a smaller water storage capacity and therefore, undergo stronger  $\psi_L$  fluctuations, have  $\psi_\pi^0$  of about  $-1.5$  to  $-2.0$  MPa, similar to the range of values measured in *P. sericea*.

We have not measured changes in soil water potential in sites where *P. sericea* occurs. In any case, the rocky nature of the substrate would make it difficult to obtain representative values using moisture release curves or soil thermocouple psychrometers. More than 25 years of precipitation records, however, do exist for one weather station located very close to the study sites. These records indicate that there are four months where the precipitation is lower than the evaporation (December to March), with a relatively small year to year variation.

The leaf water potentials at zero turgor and at full saturation, determined from analysis of the  $P$ - $V$  relationships, were used to estimate seasonal water potential component changes. There is an osmotic adjustment of about 0.5 MPa from the wet to the dry season (Table 1). We doubt, however, that such an osmotic adjustment would be sufficient to allow positive turgor maintenance in *P. sericea* under severe drought conditions. The coincidence of high evaporative demand and low soil water availability conditions such as those prevalent on 13 February 1984 are infrequent even during the short dry season. Although these results are not conclusive, the seasonal osmotic pattern observed here probably did not arise from changes in tissue elasticity, but from an increase in osmotically active solutes. Values of tissue elasticity did not show a clear trend from wet to dry season and the use of different calculation procedures did not provide consistent results either. On the other hand, the amount of osmotically active solutes, calculated from the  $P$ - $V$  methods, did show a clear trend (Table 1) increasing progressively from the end of the wet season (November) to the middle of the dry season (February).

### *Short term osmotic adjustments and temperature effects*

Recently, considerable interest has been shown in the study of short term active changes in osmotic potential. The decrease of  $\psi_\pi$  in most of these studies was largely induced by a transient water stress associated with relatively high evaporative demands. An active increase in the concentration of cell solutes may maintain positive turgor potentials above thresholds for stomatal closure and growth cessation (Hsiao, Acevedo, Fereres, and Henderson, 1976), and therefore be of adaptive value to plants that, potentially, may experience water stress.

Changes in the magnitude of the differences between thermocouple psychrometric determinations of leaf osmotic potential and  $\psi_L$  was used in this study as a measure of active  $\psi_\pi$  changes. Increasing differences between  $\psi_\pi$  and  $\psi_L$  as the night progressed, for example, would mean that active osmotic changes were occurring. Wenkert, Lemon, and Sinclair (1978) used  $P$ - $V$  curves to demonstrate short term adjustments in leaves of *Glycine max*. The

authors generated the curves from leaves sampled at various time intervals during a diurnal course. To avoid changes in osmotic properties as a consequence of the resaturation process, they did not saturate their leaf samples. Plants, however, may adjust the solute contents of their cells during the rapid dehydration occurring in the development of a  $P-V$  curve. This may obscure the observed osmotic patterns in very osmotically responsive species. Diurnal solute adjustments appear to be clearly documented for maize (Acevedo, Fereres, Hsiao, and Henderson, 1979), apple trees (Davies and Lakso, 1979) and pearl millet (Henson, Mahalakshmi, Bidinger, and Alagarswamy, 1982). Turner (1974) and Roberts, Strain, and Knoerr (1980), on the other hand, found that short term  $\psi_{\pi}$  fluctuations were largely accounted for by changes in turgor and that active osmotic changes were minimal. Species, therefore, seem to differ in their ability to undergo short term  $\psi_{\pi}$  changes.

The night-time  $\psi_{\pi}$  drop in *P. sericea* did not result from water loss since only minor  $\psi_L$  changes were observed during this period. There was a large drop in  $\psi_{\pi}$  before sunrise (between 05.00 h and 06.00 h) while the leaf water potential remained practically constant and the soluble carbohydrate levels increased suggesting unequivocal evidence of short term osmotic adjustments. With *P. sericea*, osmotic changes are more pronounced during nights with low leaf temperatures than those with high leaf temperatures (Fig. 2). To date, most work indicates that active changes are water stress induced, while in *P. sericea*, leaf temperature seems to be the environmental factor influencing the degree of osmotic adjustment. Tyree *et al.* (1978) also found a relationship between  $\psi_{\pi}$  and temperature, however, the changes were seasonal rather than daily. In their study, *Tsuga canadensis* leaves showed a marked decrease in  $\psi_{\pi}$  as winter approached and temperatures fell below 0°C.

Turner and Jones (1980) define osmotic adjustment as a lowering of the  $\psi_{\pi}$  arising from the net accumulation of solutes in response to water deficits or salinity. The present study, however, indicates that their definition could be broadened to include responses to stresses such as low temperature which may influence the plant's active osmotic changes.

The  $\psi_{\pi}$  decrease in *P. sericea* observed at predawn is, depending on the minimum night-time temperature, in the order of 0.4 to 1.2 MPa. The freezing point depression for such osmotic potentials is approximately 0.5 to 1.0°C. This additional degree in freezing protection may be, by itself, important from an ecological standpoint because in the environment where this species grows, daily temperature fluctuations are not very pronounced and minimum subfreezing temperatures usually remain very close to 0°C.

In a previous work (Rada, 1983) a strong relationship was observed between the supercooling point and 50% leaf tissue damage estimated according to the triphenyl tetrazolium chloride method (Steponkus and Lanphear, 1967), indicating that *P. sericea* does not have the ability to tolerate freezing of its tissues. Leaves of *P. sericea* supercool in the range of -6.0 to -8.0°C. This poses a question about the adaptive significance of the freezing point depression observed in this species since supercooling seems to be effective below the lowest calculated freezing point temperature of the leaf tissue (-2.8°C corresponding to -3.2 MPa). The supercooled state, however, is extremely labile and can seldom be maintained more than a few hours (Larcher, 1973). The temperature induced changes in the freezing point of *P. sericea* leaves, therefore, may serve as a 'security valve' to prevent tissue freezing and damage when supercooling is no longer effective.

The ecological importance of the freezing point depression mechanism described in this work may also be examined in terms of the relationships observed in the laboratory experiments. If the lowering of the supercooling point as a function of the decrease in the freezing point depicted in Fig. 6, holds under natural conditions, then preconditioning will produce a larger decrease in the supercooling point than in the freezing point. Zachariassen (1982) working with insect hemolymph, has found that supercooling point depression is

associated with the accumulation of low molecular weight cryoprotective substances, such as glycerol, sorbitol, mannitol and others, but can also be related to sugars such as glucose and trehalose. The increase in the soluble carbohydrate levels observed at night-time in *P. sericea* may well also indicate that these sugars act as a cryoprotectant that may help decrease the supercooling point or make it more effective under natural open conditions.

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