

On the role of orientation in reducing photoinhibitory damage in photosynthetic-twig desert shrubs

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ABSTRACT

Quantum yields for O₂ evolution were measured in photosynthetic twigs and leaves of *Hymenoclea salsola* and *Senecio douglasii*, two common shrubs of the deserts of western North America. When exposed to long-term drought, quantum yields of leaves and twigs remained constant up to the point of leaf abscission in both species. As water stress developed further, quantum yields began to decline in twigs; the extent of this quantum yield reduction was dependent on incident photon flux density. The reduction in quantum yield in twigs, which have a near-vertical orientation, was greatly accelerated when twigs were reoriented to the near-horizontal inclination typical of leaves. The reductions in quantum yield were not rapidly reversible and are interpreted as indicating photoinhibitory damage. The results are discussed in terms of the role that a near-vertical orientation might serve in maintaining photosynthetic structures through a drought period.

Key-words: *Hymenoclea salsola*; *Senecio douglasii*; Asteraceae; desert; photoinhibition; quantum yield; water stress; twig photosynthesis.

INTRODUCTION

Desert plants are exposed to a variety of abiotic stresses which limit productivity, the most important of which is undoubtedly water stress (Ehleringer & Mooney 1983; Smith & Nowak 1990). Water stress does not typically occur in the absence of other abiotic stresses, but most often occurs in conjunction with high temperatures and excessive irradiance levels. Upon exposure to long-term water stress, plants respond with a decline in photosynthetic capacity, in part the result of adjustment to lower resource levels (acclimation response) and in part the result of damage-induced photoinhibition caused by excessive photon flux densities (Björkman & Powles 1984; Powles 1984). Over evolutionary time, plants have evolved two primary patterns for adapting to the stresses imposed by life in the desert: avoidance as typically

occurs in annuals which complete their life cycle in advance of severe stress or acclimation as typically occurs in many perennials (Ehleringer 1985). Within the broad category of perennials, species with drought-deciduous photosynthetic tissues commonly avoid extremes in environmental stresses by abscission of those tissues, whereas species with evergreen leaves acclimate to tolerate those abiotic stresses.

A major limitation to the acquisition of carbon by deciduous-leaved species is that, immediately after rains, when soil moisture levels are highest, there is no photosynthetic leaf area. Overall potential seasonal productivity is decreased by the period required to produce leaf area, because soil moisture is evaporated before plants have the opportunity to utilize that water (Comstock & Ehleringer 1986). This situation would be exacerbated further in habitats where rains were both intermittent and light. As a variation on the theme of leaf deciduousness, a large number of desert perennials have developed photosynthetic twigs, which persist into the drought period after leaves have abscised (Comstock, Cooper & Ehleringer 1988). Under conditions of low water stress, net photosynthetic rates of these twigs are positive, often approaching those of leaf tissues (Osmond *et al.* 1987; Ehleringer, Comstock & Cooper 1987; Comstock & Ehleringer 1988; Comstock *et al.* 1988). In all cases examined to date, photosynthetic twigs operate at lower intercellular carbon dioxide concentrations (c_i) than do leaves, making this tissue type more water-use efficient at all tissue water potentials (Ehleringer *et al.* 1987; Comstock & Ehleringer 1988). Twigs also differ from leaves in their orientation, with twigs exhibiting steep inclinations, whereas leaf tissues tend to be much more horizontal. However, because both photosynthetic twig and leaf diameters are small (typically < 3 mm), convective exchange is high and both tissue types have similar temperatures in the field.

In response to long-term soil moisture depletion, photosynthetic capacity declines in both leaf and twig tissues (Comstock & Ehleringer 1988). However, the rate of decline is steeper in leaf tissues than in twig tissues, and leaves are abscised at a higher water potential than are photosynthetic twigs. Thus, as water stress develops, plants are shifting from a photosynthetic tissue type that is less water-use efficient to one that is

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more water-use efficient and also shifting from a photosynthetic tissue type with low inclination (near horizontal) to one with steep inclination (near vertical). Reduced solar radiation absorption is one possible advantage to steep inclination under water stress and excess light levels have been shown to induce photoinhibitory damage in the semi-arid herb *Macropitium purpurea* (Ludlow & Björkman 1984) and aridland phreatophyte *Nerium oleander* (Björkman & Powles 1984). The purpose of this study was to address several questions relating to the maintenance of photosynthetic tissues by twig-photosynthetic species into a prolonged drought period, as typically occurs at the end of each spring. First, do leaf and/or photosynthetic tissues exhibit photoinhibitory damage when exposed to long-term water deficits? Here, photoinhibition is the long-term reduction in quantum yield associated with exposure to high light. Second, do the differences in orientation between leaf and stem have any bearing on the ability of twigs to persist into a drought period?

MATERIALS AND METHODS

Species and growth conditions

Young individuals of *Hymenoclea salsola* T. & G. and *Senecio douglasii* DC. were collected from native Sonoran Desert field sites approximately 9 km west of Oatman, Arizona, USA (lat. 34°57'N, 114°25'W). These two species are by far the most common twig-photosynthetic shrubs at this site (Comstock *et al.* 1988). Plants were transplanted into 30-cm-diameter PVC tubes that were 100 cm tall and filled with a mixture of autoclaved loam, perlite, sand and vermiculite to mimic the sandy soils in which these plants typically grow. Plants were grown in a greenhouse on a 33/20°C day/night temperature regime and received supplemental high-intensity-discharge lighting (combination of metal halide and sodium vapour) to provide a daily photon flux density (PFD) flux of 40–50 mol m⁻².

For long-term drought studies, plants were initially grown under well-watered conditions in the greenhouse soil mixture previously described. The drought sequence was designed to simulate the water deficits experienced by these plants under field conditions. Previous field observations indicated that predawn tissue water potentials varied from -0.8 MPa in both species under moist spring conditions to -4.0 and -3.4 MPa in *H. salsola* and *S. douglasii*, respectively, under midsummer drought conditions (Comstock *et al.* 1988). Plants were droughted over a 6-week period through a combination of increasing the interval between watering and decreasing the amount of water received at each watering. Predawn leaf water potentials were monitored daily with a pressure chamber (PMS Instruments, Corvallis, Oregon, USA).

Quantum yield measurements

Rates of photosynthetic O₂ evolution were measured using a Clark-type leaf electrode (model LD-2, Hansatech, Kings Lynn, Norfolk, UK). Voltage output from the electrode was monitored with a Hansatech control box (model CB1D) and signal readouts were displayed on a chart recorder. Chamber volume was calibrated daily. After calibration, the plant material placed inside and the chamber was flushed with a known gas (10% CO₂, 21% O₂, balance N₂). After the signal had stabilized, the light source was fitted to the chamber top, inlet and outlet valves to the chamber were closed, and the O₂ concentration monitored until the slope reached a constant value. After the reading had been obtained, the inlet and outlet valves were again opened and the chamber flushed with gas, before proceeding to the next lower light level.

Light was supplied to the leaf chamber using a high intensity light source (model LS-2, Hansatech), and PFD was varied between 0.04 and 1.20 mmol m⁻² s⁻¹ (400–700 nm) by use of neutral density filters. PFD incident on leaf discs was measured with a quantum sensor (model LI-185A, Licor Instruments, Lincoln, NE, USA). PFD measurements were made at the end of each quantum yield measurement, because of small variations in the intensity of the light source that could have a bearing on the quantum yield calculations. For quantum yield measurements, leaves were first exposed to a PFD of 0.2 mmol m⁻² s⁻¹ to achieve a constant photosynthetic rate. Thereafter, PFD was reduced in steps of 0.02 mmol m⁻² s⁻¹. Incident quantum yield measurements were corrected for differences in leaf absorptance. Leaf absorptances were measured with an Ulbricht integrating sphere as described previously by Ehleringer (1981). Leaf and twig areas were measured on a projected-area basis. The leaf chamber was maintained at 25°C using a refrigerated circulating water bath. To minimize temperature fluctuations within the system, all lines leading between the water bath and the leaf chamber were insulated.

As leaf water potentials declined, quantum yield measurements were made on both leaf and twig tissues of both species to examine the long-term effects of water deficit. After low predawn leaf water potentials were obtained (similar to those observed in the field), plants were rewatered to follow the recovery rate in each species.

A related experiment monitored possible changes in photoinhibition behaviour during the drought exposure. Prior to quantum yield measurements in the morning, a subsample of attached twigs were reoriented from their vertical orientation and restrained in a horizontal position for 6 h. During this period, twigs were exposed the direct rays of the sun and supplemental HID lighting (total PFD of 1.1–1.6 mmol m⁻² s⁻¹). After the 6-h exposure, quantum yield was measured on these twigs. The extent of photoinhibition induced by this twig

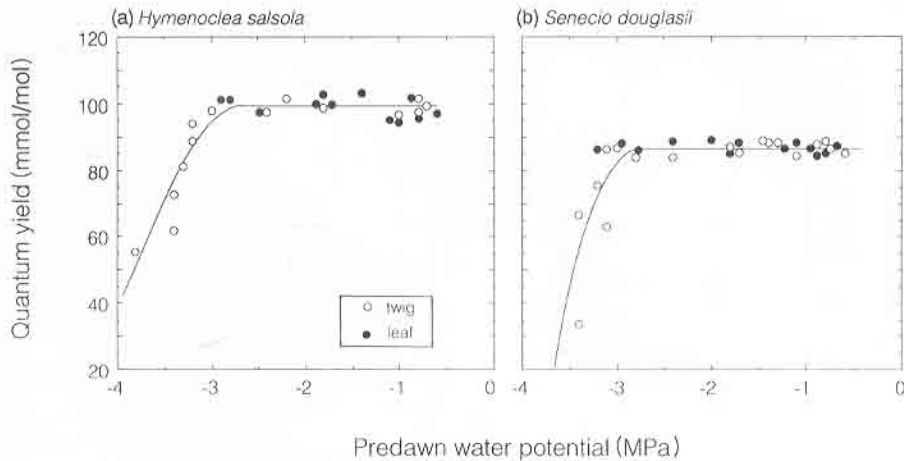


Figure 1. Absorbed quantum yield for photosynthetic O_2 evolution in leaf and green-twig tissues of (a) *Hymenoclea salsola* and (b) *Senecio douglasii* as a function of predawn leaf water potential for plants exposed to long-term water deficits.

exposure after reorientation was calculated as the ratio of the quantum yields after exposure to before the 6h exposure.

RESULTS AND DISCUSSION

Under well-watered conditions, absorbed quantum yields for O_2 evolution were 0.099 ± 0.008 and 0.099 ± 0.009 mol mol $^{-1}$ for leaves and twigs of *Hymenoclea salsola*, respectively, and 0.087 ± 0.004 and 0.087 ± 0.006 mol mol $^{-1}$ for leaves and twigs of *Senecio douglasii*, respectively. As soil water deficits increased, tissue water potentials declined in both species. At a tissue water potential of approximately -3.0 MPa in *H. salsola* and -3.2 MPa in *S. douglasii*, leaves were abscised. Over that entire range of water potentials, quantum yields remained constant in both species (Fig. 1). Similarly, quantum yields for O_2 evolution in photosynthetic-twig tissues remained constant and at their maximal values between water potentials of -0.5 and -3.0 MPa (Fig. 1). However, at tissues water potentials below the threshold for leaf abscission during the next several weeks, the quantum yields began to decline in twig tissues as water potentials continued to decrease. At a minimum tissue water potential of -3.8 MPa in *H. salsola* and -3.4 MPa in *S. douglasii*, twig quantum yields had declined to 0.055 and 0.034 mol mol $^{-1}$, respectively.

During the long-term drying cycle, leaves and twigs had been allowed to maintain their natural orientation. Previous experiments by Björkman & Powles (1984) on the desert shrub *Nerium oleander* had indicated an interaction between water deficit and light on photosynthetic performance during water stress. Based on this information, experiments were designed to determine if the different orientations of leaves (horizontal) and twigs (nearly vertical) were related to their long-term

persistence during the drought period. That is, could it be that excessive photon flux levels on leaves under water stress resulted in photoinhibitory damage and the eventual loss of those leaves, whereas twigs by virtue of a steeper inclination avoided that photoinhibitory damage and persisted longer into the drought? In a second drying cycle, twigs were reoriented in the morning to a horizontal angle (similar to that which the leaves would experience) and exposed to the diurnal variation in solar radiation for a 6-h period after which quantum yields were again measured on both leaves and twigs. Control experiments were conducted to determine if there were time-dependent changes in the quantum yield between morning and afternoon in twigs that were not reoriented; no differences in quantum yields were observed between morning and afternoon measurements on twigs that were not reoriented.

No decline in quantum yield was observed in leaf tissues between morning and afternoon in either *H. salsola* or *S. douglasii* over the entire range of water potentials in which leaves persisted. In photosynthetic twigs of *H. salsola*, there was also no decline in quantum yield after the reorientation treatment at tissue water potentials higher than -3.0 MPa, which is approximately where leaf abscission occurred (Fig. 2). However, twig tissues became progressively more sensitive to a reorientation treatment at tissue water potentials less than -3.0 MPa, such that at a water potential of -3.7 MPa reorientation resulted in a 76% reduction in the quantum yield. This reduction was a result of excess light exposure, since *H. salsola* twigs that were not reoriented exhibited no such reduction in quantum yield.

On the other hand, photosynthetic twigs of *S. douglasii* were sensitive to a reorientation treatment over the entire range of tissue water potentials (Fig. 2). At water potentials above -2.8 MPa (slightly higher than the point at which leaf abscission occurred), reorien-

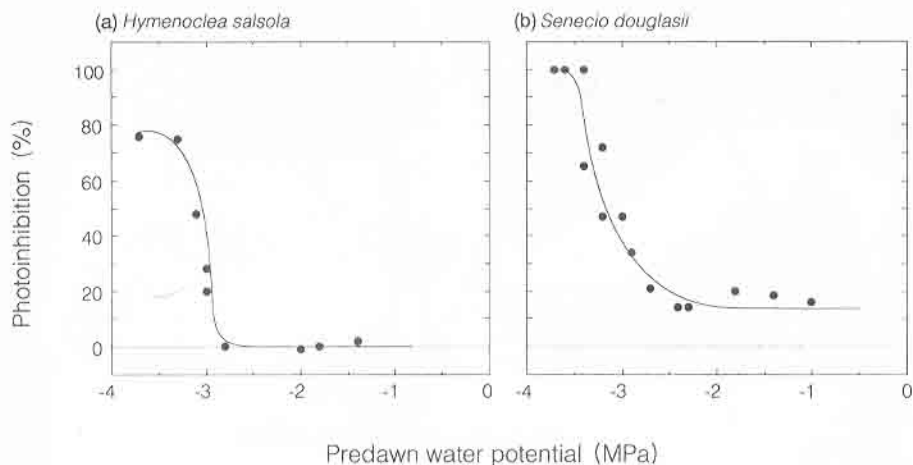


Figure 2. Reduction in quantum yield of photosynthetic O_2 evolution (photoinhibition) upon reorientation for green-twig tissues of (a) *Hymenoclea salsola* and (b) *Senecio douglasii* as a function of predawn leaf water potential for plants exposed to long-term water deficits.

tation resulted in a constant 15% reduction in photosynthetic efficiency. At tissue water potentials below -2.8 MPa, the reduction in quantum yield progressively increased such that by a water potential of -3.5 MPa, a reorientation treatment reduced the quantum yield to zero.

While experiencing water stress in excess of -3.0 MPa, photosynthetic activity in twigs reoriented to a horizontal position was quickly depressed and did not recover in either *H. salsola* or *S. douglasii*. In both species, reoriented twigs became chlorotic and died within 2–3 d following the exposure treatment.

In a third set of experiments, recovery from water stress was followed in order to determine if the observed decline in quantum yield was the result of damage to the photosystems or excess photon energy was being dissipated by an alternative pathway (Demmig *et al.* 1987; Demmig & Winter 1988). Upon rewatering, predawn leaf water potentials quickly rose to values typical of pre-stress conditions (Fig. 3).

However, the recovery in quantum yield was not immediate in either species, but required 5–8 d before quantum yield values were as high as pre-stress levels. Thus, we conclude that photosystem damage had occurred and that the observed reduction in quantum yield under stress was not temporary and followed by rapid recovery.

By virtue of differences in inclination and not absorptance, leaves absorb significantly greater photon flux densities (Fig. 4). The calculated PFD absorbed by twigs was lower than that of leaves at all hours through the day. On a daily basis, the steeper twig inclination results in a 48% reduction in PFD. There are morphological alternatives to reduce exposure of photosynthetic tissues to excessive PFD. Begg (1980) showed that leaf curling in grasses was an effective means of reducing incident

light levels in grasses under water stress. Powles & Björkman (1982) showed that rapid leaf movements in the understory herb *Oxalis oregano* were an effective means of reducing incident PFD and avoiding photoinhibitory damage. Similarly, Ludlow & Björkman (1984) observed that under water stress, paraheliotropic leaf movements in *Macropitulum purpureum* reduced incident light levels, allowing leaves to persist longer into a drought cycle. All of these mechanisms involve active movement. Yet active leaf or twig movements do not occur in either *H. salsola* or *S. douglasii*. When exposed to a combination of water stress and high PFD, the orientation of photosynthetic tissues changes through abscission. Leaves, which had low inclinations, abscised and twigs with steep inclinations persisted. Photosynthetic twigs in both species were sensitive to the photoinhibitory damage under water stress. However, the steeper inclination of twigs reduced the extent of that damage and should allow these species to persist longer into the drought period.

A viable alternative to steep inclinations for reducing incident PFD under water stress is to reduce the absorptance by adding waxes, hairs or other reflective structures to the epidermis as is common on many desert species (Ehleringer 1981; Ehleringer & Comstock 1987). Yet there is no evidence for changes in spectral characteristics in either *H. salsola* or *S. douglasii*. Instead, the plants shift from having the preponderance of photosynthetic area in horizontal tissues to vertical tissues when exposed to water stress (Comstock *et al.* 1988). Reduced absorptances are disadvantageous under periods of high soil water availability, because the reduced absorptance would reduce rates of carbon gain by reflecting quanta that might otherwise be utilized in photosynthesis. Unless high reflectance photosynthetic tissues can be abscised and replaced during mesic

periods, steeper inclinations result in less of a decrease in productivity rates. In this context, Ehleringer (1988) analysed 159 species along a precipitation gradient in western North America and observed that as precipitation levels decreased, leaf inclinations increased in those species occupying the drier sites. Only in the driest habitats did species exhibit increased leaf reflectances. A similar pattern is seen in the Sonoran Desert. Species occupying the relatively mesic, wash habitats, such as *H. salsola* and *S. douglasii*, tend to have lower leaf reflectances than species from the adjacent, drier slope habitats (Ehleringer 1981).

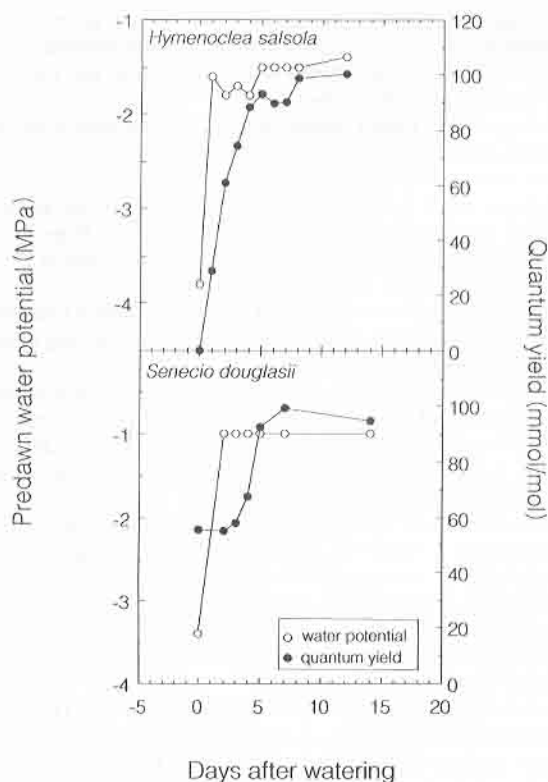


Figure 3. Time course of recovery of quantum yield of photosynthetic O_2 evolution and maximum photosynthetic rate for green-twig tissues upon rewatering after exposure to long-term water deficits.

On an annual basis, *H. salsola* and *S. douglasii* experienced large changes in soil moisture availability (Fig. 5), despite possibly having greater access to soil moisture because they occupy wash microsites which have deeper alluvial deposits and channel all sheet flow. Predawn leaf water potentials ranged between -0.5 and -3.3 MPa. Both species became mostly leafless during the stress periods between winter and summer rainy seasons. When only twig tissues are present during these water stress periods, the amount of canopy carbon gain is low (Comstock *et al.* 1988). A far more substantial fraction of annual carbon by the canopy is gained during the wet periods when both leaf and twig tissues are

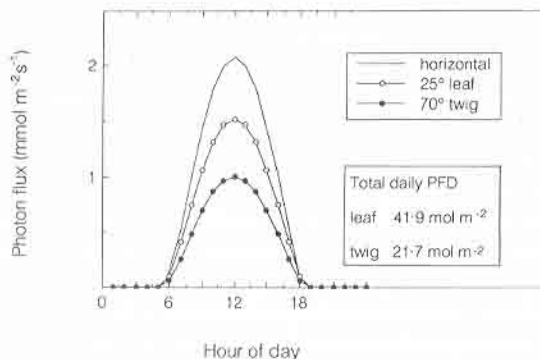


Figure 4. Calculated instantaneous and daily total photon flux densities (400–700 nm) that would be absorbed by photosynthetic tissues differing in orientation and that incident on a horizontal surface. Tissue angles of 25 and 70° for leaf and green-twig, respectively, and absorptances of 80% for both tissues are typical of values measured for *Hymenoclea salsola* and *Senecio douglasii* under field conditions. Calculations are for mid-summer conditions in the Sonoran Desert (latitude of 35°, a solar declination of 15°, atmospheric transmission coefficient of 0.8, and a solar diffuse fraction of 10%).

present and photosynthetic rates are less inhibited by water stress (Comstock & Ehleringer 1988; Comstock *et al.* 1988). Thus, from an annual carbon balance perspective, there is likely to be little advantage in relying on photosynthetic twigs for carbon gain during water stress periods even though water-use efficiencies are much higher in twigs than leaves.

What then might the purpose in maintaining photosynthetic twigs into a drought period? One unavoidable conclusion is that of an increase in water-use efficiency, yet carbon gain by shrubs under water stress is low as mentioned previously. As one possibility, we suggest that photosynthetic twigs are a mechanism for insuring that photosynthetic surface area is immediately present after the initial rains to capitalize on these brief periods

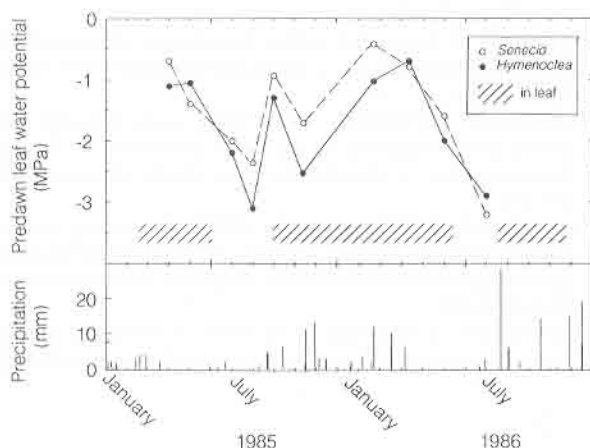


Figure 5. Seasonal courses of precipitation and predawn water potentials for *Hymenoclea salsola* and *Senecio douglasii* at a field site 20 km east of Needles, California, USA, within the Sonoran Desert.

of high soil moisture availability. Leaf area development of deciduous species in deserts (Comstock & Ehleringer 1988) and in the adjacent chaparral (Mooney 1977) often requires several weeks for complete expansion. During that period, a significant fraction of the precipitation may be lost by surface evaporation. Thus, photosynthetic twigs may provide a means whereby photosynthetic tissues are retained into the drought period and capable of providing high rates of carbon gain (Ehleringer *et al.* 1988; Comstock & Ehleringer 1988) while leaf tissues are still developing. With a steep inclination, water-stressed twigs have a greater probability of surviving the drought period without photoinhibitory damage. In support of this hypothesis, Comstock & Ehleringer (1991) observed in *H. salsola* ecotypes that the fraction of photosynthetic tissues associated with twigs increased in Sonoran Desert habitats as the duration of drought decreased and the predictability of summer rains increased. In those populations from habitats where drought duration was long and there were often years when no summer rains occurred, photosynthetic twig tissues comprised only a small fraction of the photosynthetic area and only a small component of the overall carbon gaining capacity of the canopy.

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