

# Growth of Moonshine Yarrow on a Limited Moisture Budget<sup>1</sup>

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## Abstract

Yarrow is an herbaceous perennial that is highly recommended for landscape gardens in many parts of the United States. However, performance data of yarrow produced under limited irrigation conditions is lacking. During 2004 and 2005, we studied the growth and physiology of yarrow (*Achillea* 'Moonshine') maintained as well irrigated controls or irrigated when there was a 30 or 60% depletion of moisture in the growing substrate. Plants irrigated at the 60% moisture level had the lowest predawn water potential, stomatal conductance and transpiration rate. Cell osmotic potential and relative water content data suggest that yarrow might be able to withstand prolonged exposure to drought. In 2005, net assimilation rate of well irrigated plants (0.317 mg/cm<sup>2</sup>/d) was almost twice as high (0.179 mg/cm<sup>2</sup>/d) as that of plants maintained at the 60% moisture level. This suggests that a 60% moisture depletion level had a very significant impact on carbon assimilation. In 2005, leaf area of plants irrigated at the 30% moisture depletion level showed only a 15% decline compared to well irrigated plants, while those irrigated at the 60% moisture depletion level showed a 47% decline in leaf area.

**Index words:** environmental stress, herbaceous perennial, water relations.

**Species used in this study:** *Achillea* 'Moonshine'.

## Significance to the Nursery Industry

Production costs of nursery production systems are reduced when marketable ornamental plants are produced under minimum irrigation requirements. Yarrows are recommended highly for use in landscape gardens, but information on the tolerance of the plant to limited irrigation during nursery production virtually is nonexistent. This research shows that growing yarrow at a 60% moisture depletion level severely impacts growth and development. Plants that were irrigated daily had twice the assimilation rate of plants irrigated when the growing substrate moisture depletion level reached 60%. Because greenhouse plants of moonshine yarrow that were grown on a 30% moisture depletion level showed limited loss of leaf area, horticulturists might want to consider growing yarrow on allowable soil moisture depletion levels of 30%. Furthermore, moonshine yarrow may be able to withstand prolonged exposure to moisture deficits.

## Introduction

Dwindling water supplies in the United States are causing municipalities to aggressively legislate for water conservation in the nursery industry (15). Water management districts even mandate the irrigation water allotment that can be used for containerized ornamental plant production (2). A major challenge for the nursery industry is to identify ornamental plants that can be produced on a limited moisture budget. Identification of plants that can be produced with a limited amount of water will be facilitated if horticulturists know the drought adaptation mechanisms of those plants.

Yarrows (*Achillea* sp.) are herbaceous perennials with flowers that range in color from white to yellow to red (9). Many cultivars of yarrow are equally valuable as garden accent plants or as cut flowers. The plant is speculated to be best suited to cottage rather than formal gardens (10). Yarrows are recommended highly for landscape gardens in temperate moist to semi-arid regions of the United States (14), but information on the drought tolerance of several yarrow cultivars is virtually nonexistent. *Achillea* 'Moonshine' is a compact yarrow with deeply divided foliage and bright lemon-yellow flowers (14). How moonshine yarrow responds to limited irrigation during nursery production is unknown.

Morphological and physiological responses that represent plant adaptations to drought include, decreased leaf surface area (7), altered dry matter partitioning (5), and reduced leaf gas exchange (8). The maintenance of favorable plant water status, such as high water potential (1) and high relative water content might also indicate a plant's fitness for drought (13). The extent to which these responses are expressed during stress might indicate the relative degree of plant stress tolerance (12), but information on those responses is absent for moonshine yarrow. The objectives of this study were to quantify the short term effects of drought on water potential, transpiration, stomatal conductance, cell osmotic potential, relative water content and biomass production and partitioning of greenhouse-grown *Achillea* 'Moonshine'.

## Materials and Methods

Two greenhouse experiments were conducted at New Mexico State University, Las Cruces, N.M (elev. 1183 m (3883 ft); lat. 32°16'4"N; long. 106°46'18"W). The experiment was first conducted in 2004 and repeated in 2005.

*Experiment 1. Plant materials.* On June 2, 2004, seeds of *Achillea* 'Moonshine' (moonshine yarrow) were sown in plastic flats. Plants were removed from the original containers on July 1, 2004, and repotted into #1 (3.8 liter) plastic containers. Containers were filled with a growing substrate consisting of peat, composted bark, sphagnum peat, perlite and a wetting

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agent containing 0.01% available phosphorus (Scotts Advantage, Scotts, Marysville, OH). Seedlings were drip-irrigated to container capacity every two days and fertilized weekly with Peters solution (20N–8.7P–16.6K (20–20–20 plus 0.05%, Mg and 0.05% Fe) (Scotts) at 568 mg N/liter (ppm).

**Initial harvest.** On July 20, 2004, four seedlings were harvested destructively to assess initial seedling traits. By July 20, 2004, leaf fresh weight was about 12 g per pot. Initial seedling data also were used to calculate net assimilation rate (NAR) and relative growth rate (RGR) at the end of the experiment. Leaves were severed 2 cm above the growing substrate surface and their surface area was measured with a leaf area meter (LI 3000; LICOR, Lincoln, NE). All remaining plant parts, stems and roots were water-washed free of debris and oven dried at 65C (149F) for 14 days.

**Irrigation treatment and experimental design.** On the same day that plants were initially harvested, three irrigation treatments were initiated with the remainder of the plants. Control plants were irrigated daily with tap water ( $\approx 1$  liter; EC = 0.65 dS/m) to container capacity. Plants in the two other moisture treatments were irrigated in cycles. A drought cycle ended when there was a 30 or 60% decrease in average weight of three pots in each treatment. To determine initial pot weight at the start of a drought cycle, each plant was irrigated, allowed to drain for 2 h, and then weighed. Irrigation then was withheld from plants in the drought treatment until the combined weight had decreased by 30 and 60% due to evapotranspiration. Plants in the drought treatment were weighed daily to gauge the end of a drought cycle. A new drought cycle was started by irrigating plants to container capacity with the fertilizer solution. The new weight of the indicator pots was used as the new initial weight.

Control plants were fertilized weekly. Moisture-stressed plants were fertilized at the end of each drought cycle to maintain plant nutrient status. All plants were fertilized with Peters (20N–8.7P–16.6K (20–20–20 plus 0.05%, Mg and 0.05% Fe) (Scotts) at 568 mg N/liter (ppm). During physiological measurements, leaf temperature averaged  $22 \pm 4$ C ( $73.4 \pm 14$ F). Maximum/minimum temperature in the greenhouse averaged  $35 \pm 2$ C ( $95 \pm 4$ F)/ $12 \pm 1$ C ( $54 \pm 1$ F). Maximum/minimum relative humidity averaged  $73 \pm 8$ %/ $19 \pm 1$ %. Photosynthetically active radiation at canopy level averaged  $964 \pm 167$   $\mu\text{mol}/\text{m}^2/\text{s}$ . Plants did not receive artificial radiation. Environmental data were determined with a steady state porometer (LI-1600; LI-COR, Lincoln, NE). The experimental design was a randomized complete block design with three irrigation treatments and three replications. The experimental unit was a single plant in a pot.

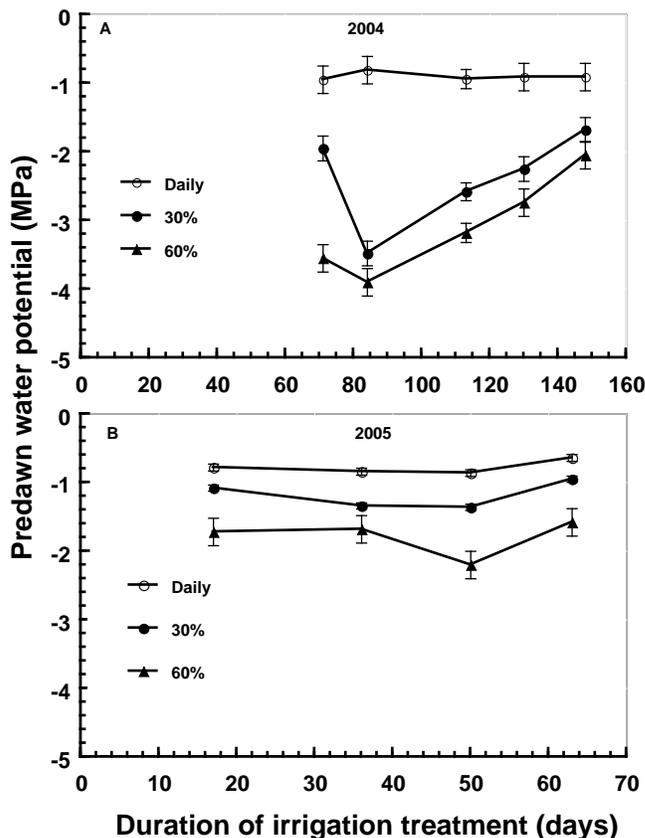
**Plant water relations.** Predawn leaf water potential ( $\Psi_{pd}$ ) was measured on young fully expanded leaves with a pressure chamber (Model 3005; Soil Moisture Equipment, Santa Barbara, CA). We measured  $\Psi_{pd}$  only on the days when the end of a drought cycle coincided for plants in both deficit irrigation treatments (30 and 60%). Plants in 30% moisture treatment completed nine drought cycles while those in the 60% treatment completed five drought cycles.

Between 11:00 and 14:00 HR, transpiration and stomatal conductance were measured on the youngest, fully expanded leaf with a steady-state porometer (LI-1600; LI-COR). Measurements were made at the end of drought cycle on

September 29, October 12, November 10, November 27, and December 15. On those dates, the end of a drought cycle coincided for the 30 and 60% moisture treatments.

**Cell osmotic potential and relative water content.** For cell osmotic potential measurements, a young fully expanded leaf was selected, excised, sealed in a zip lock plastic bag, placed on ice, immediately transported to the laboratory and stored in a freezer at  $-20$  C ( $-4$ F) in the dark for 3–5 d. Leaves were taken from the freezer, rolled, placed into a Markhart leaf press (Model LP-27; Wescor, Logan, UT) and pressed to squeeze out cell contents. A 10  $\mu\text{L}$  aliquot of the cell contents was transferred onto paper discs (SS-033 sample disc, Wescor). Discs were then placed in a self calibrating vapor pressure osmometer (Vapro model 5520; Wescor, Logan, UT) to determine cell osmolality. Values for cell osmolality (mmol/kg) were converted to cell osmotic potential ( $-\text{MPa}$ ) using van't Hoff's equation.

A young fully expanded leaf was selected for relative water content measurement. The leaf was excised from the plant, sealed in a zip lock plastic bag, placed on ice, and immediately transported to the laboratory. Each leaf was weighed to determine fresh weight (FW) and rehydrated in deionized water overnight. Each leaf was blotted with lintless paper to remove excess moisture, re-weighed to obtain turgid weight (TW), and then dried for 10 h at 85C (185F). Dry weight (DW) was recorded, and relative water content (RWC) was determined using the formula  $\text{RWC} (\%) = [(FW - DW) / (TW - DW)] \times 100$ .



**Fig. 1.** Predawn water potential of *Achillea* 'Moonshine' irrigated daily, or after 30 or 60% moisture depletion in the growing substrate in (A) 2004 and (B) 2005. Error bars represent the standard error.

$-DW) / (TW - DW)] \times 100$ . Cell osmotic potential and RWC data were collected at the end of a drought cycle for the 30 and 60% moisture treatments on October 12, November 10, November 27, and December 15.

**Final destructive harvest.** All plants were destructively harvested on December 15, 2004 (99 days of drought treatment). Leaves, stems, and roots were dried at 65C (149F) for 14 d.

**Experiment 2.** Seeds of *Achillea* 'Moonshine' used in 2005 were sown on November 22, 2004. Plants were removed from the original containers on January 28, 2005, and repotted into plastic pots using the same procedures outlined in 2004.

On March 6, 2005, irrigation treatments similar (irrigated daily and irrigated at 30 or 60% gravimetric moisture loss) to those used in 2004 were initiated. On that day, three plants in each treatment were selected randomly and destructively harvested. Five plants in each treatment were retained for drought experiments. Plants in 30% moisture depletion treatment completed nine drought cycles while those in

the 60% moisture regime completed four. During drought treatment, plants were fertilized as in experiment 1. During physiological measurements, leaf temperature averaged  $22 \pm 1C$  ( $73 \pm 4F$ ). Maximum/minimum temperature in the greenhouse averaged  $36 \pm 2C$  ( $97 \pm 3F$ )/ $14 \pm 4C$  ( $57 \pm 7F$ ). Maximum/minimum relative humidity averaged  $91 \pm 8\%$ / $28 \pm 4\%$ . Photosynthetically active radiation at canopy level averaged  $1689 \pm 200 \mu\text{mol}/\text{m}^2/\text{s}$ . Plants did not receive supplemental radiation.

Predawn leaf water potential, stomatal conductance, transpiration, cell osmolality and RWC data were collected using procedures outlined in experiment 1. Data for  $\Psi_{pd}$ , stomatal conductance, and transpiration were collected on March 23, April 11, April 25, and May 8. Cell osmolality and RWC data were collected on March 25, April 12, April 26, and May 10. On May 11, 2005 (67 days of drought treatment), all plants were destructively harvested. Leaves, stems, and roots were dried at 65C (149F) for 14 d. The experimental design, plant water relations, transpiration, stomatal conductance, cell osmolality, relative water content and final destructive harvest measurements were recorded as described for 2004.

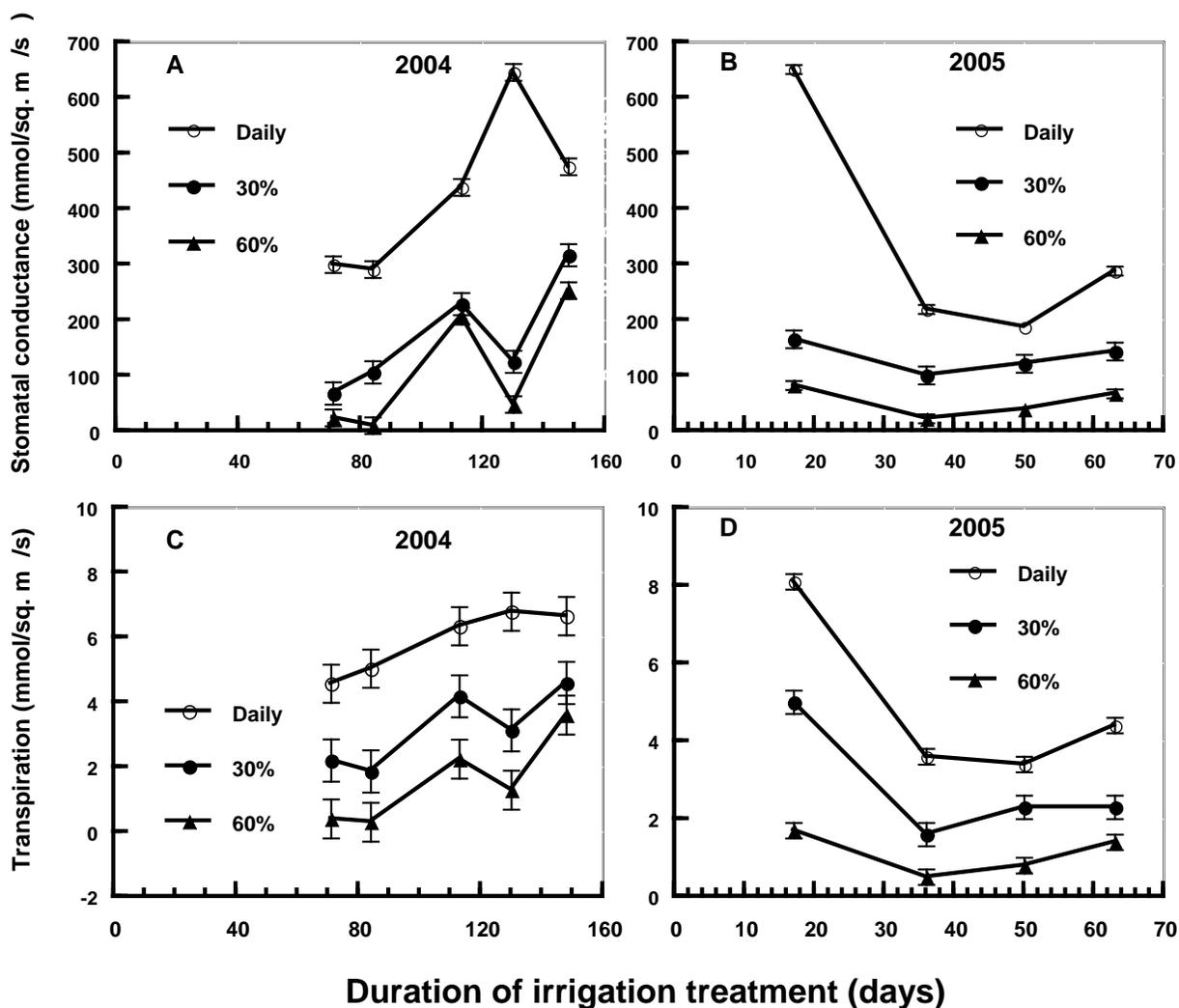


Fig. 2. Stomatal conductance of *Achillea* 'Moonshine' irrigated daily, or after 30 or 60% moisture depletion in the growing substrate in (A) 2004 and (B) 2005 and transpiration rates of *Achillea* 'Moonshine' subjected to three irrigation levels in (C) 2004 and (D) 2005. Error bars represent standard error.

**Statistical analysis.** Data were analyzed using SAS software for windows Version 9.1 (SAS Inst., Cary, NC). Means of leaf area, leaf weight, root weight, leaf area ratio (LAR), root to shoot dry weight (DW) ratio, net assimilation rate (NAR) and relative growth rate (RGR) were separated using Fisher's least significant difference (LSD) at  $P \leq 0.05$  after analysis of variance. The relationship of leaf water potentials, transpiration, stomatal conductance, relative water content and cell osmolality with drought cycle was analyzed using repeated measures in the Proc Mixed procedure of SAS to assess species, drought treatment, drought cycle effects and all interactions. Net assimilation rate was calculated by using the equation of (11):  $NAR = (W_2 - W_1) / (T_2 - T_1) \times (\log L_2 - \log L_1) / (L_2 - L_1)$ , where  $W_1$  was the DW determined from four plants before irrigation treatments started ( $T_1$ ),  $W_2$  was the DW at harvest ( $T_2$ ), and  $L_2$  and  $L_1$  were the leaf surface area at  $T_2$  and  $T_1$  respectively. Relative growth rate (RGR) was calculated as:  $RGR = (\ln W_2 - \ln W_1) / (T_1 - T_2)$ , where  $W_2$  was the DW at harvest ( $T_2$ ) and  $W_1$  was the DW before irrigation treatments began ( $T_1$ ).

## Results and Discussion

Drought treatment and drought cycles affected  $\Psi_{pd}$  in 2004 and 2005 (Fig. 1). The magnitude of treatment differences in  $\Psi_{pd}$  depended on drought cycle only in 2004 (Fig. 1A). Plants receiving daily irrigation kept  $\Psi_{pd}$  near  $-0.90$  MPa in 2004, and  $-0.80$  MPa in 2005. Plants irrigated at 60% moisture

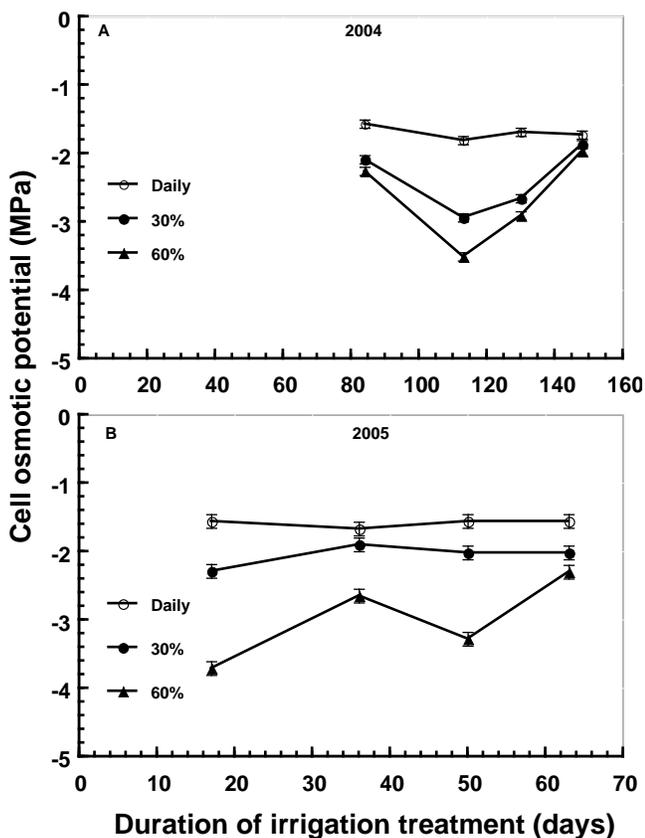


Fig. 3. Cell osmotic potential of *Achillea* 'Moonshine' irrigated daily, or after 30 or 60% moisture depletion in the growing substrate in (A) 2004 and (B) 2005. Error bars represent standard error.

depletion had the lowest  $\Psi_{pd}$  (Fig. 1). The low  $\Psi_{pd}$ , especially in plants irrigated after 60% moisture depletion, suggests that foliar tissues were not fully rehydrated at the end of drought cycle due to the limited availability of moisture in the growing substrate (6).

Moisture regime and drought cycles affected stomatal conductance and transpiration in both years (Fig. 2), while the interaction between moisture regime and drought cycles was significant only for plants grown in 2005 (Fig. 2B and D). Stomatal conductance rates decreased with an increase in moisture depletion level and minimum stomatal conductance rates was recorded for plants irrigated after 60% moisture depletion (Fig. 2A and B). While very small changes in growing substrate moisture level can trigger stomatal closure (4), plants irrigated daily clearly offered less resistance to moisture loss. On the other hand, the closure of stomates in plants exposed to moisture stress might be one strategy that yarrow plants use to tolerate low moisture environments. As was noted for stomatal conductance, transpiration rates decreased with an increase in moisture depletion levels and the lowest transpiration rates were observed for plants in the 60% moisture treatment (Fig. 2).

An increase in cell solutes that is triggered by exposure to water deficits lowers the water potential at which stomatal closure occurs (16). Additionally, cell solutes may play a significant role in the maintenance of water inflow into the tissue. The influx of water into the tissue will maintain turgor and enable the plant to continue growth despite being

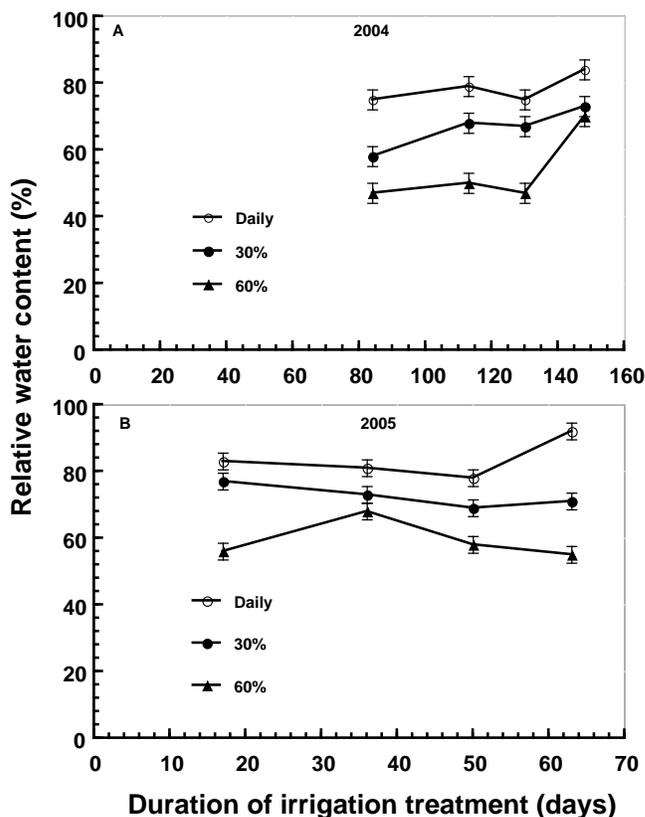


Fig. 4. Relative water content of *Achillea* 'Moonshine' irrigated daily, or after 30 or 60% moisture depletion in the growing substrate in (A) 2004 and (B) 2005. Error bars represent standard error.

**Table 1. Growth and development parameters of *Achillea* ‘Moonshine’ subjected to three irrigation treatments during 2005.**

Moisture depletion level (%)	Leaf area (cm <sup>2</sup> )	Leaf weight (g)	LAR (cm <sup>2</sup> /g)	Root weight (g)	NAR (mg/cm <sup>2</sup> /d)	RGR (mg/g/d)
Control	3049a <sup>2</sup>	41.9a	73a	71.8a	0.317a	0.029a
30	2580b	30.8b	84a	53.2b	0.216b	0.021b
60	1619c	23.0c	71a	28.6c	0.179b	0.020b

<sup>2</sup>Means within a column followed by similar letters are non significant at  $P \leq 0.05$  using Fisher's LSD.

challenged with moisture deficits (3). We found that plants irrigated at 30 and 60% moisture depletion had lower cell osmotic potentials than those irrigated daily (Fig. 3). At the end of the growing season in 2004, we observed that cell osmotic potential in all moisture levels was similar, but this was less so for plants grown in 2005 (Fig. 3). One possible explanation for this difference in years is that the longer growing period in 2004 may have allowed for the greater movement of water in cell tissues because of the greater accumulation of cell osmolytes. Environmental factors have a direct impact on the degree of solute accumulation (16). This could be a significant drought adaptation mechanism for yarrow plants exposed to prolonged moisture deficits.

That prolonged exposure to moisture deficits might have triggered water movement into the cell is evident from the relative water content (RWC) data (Fig. 4). Moisture treatment affected RWC in both years (Fig. 4). But, drought cycle was statistically significant only in 2004 (Fig. 4A), the year of the more prolonged drought treatment. Furthermore, the interaction between moisture level and drought cycle was not statistically significant for RWC neither in 2004 nor in 2005. This suggests that it is the length of treatment exposure rather than the severity of treatment that contributed to the change in cell water relations. Carbon assimilation may be halted when RWC reaches 70% because the increase in cell solutes inhibits enzymatic activity in the chloroplast (3). So, we expect carbon assimilation to be minimally impacted only in plants irrigated daily because this treatment consistently had RWC values that exceeded 70%.

In 2005, leaf area of plants irrigated at the 30% moisture level showed a 15% decline compared to the controls, while those irrigated at the 60% moisture depletion level showed a 47% decline in leaf area (Table 1). While irrigation treatment affected leaf weight, the total leaf area per unit dry leaf mass (LAR) was unaffected. Plants in the control treatments had higher net assimilation rate (NAR) and relative growth rates (RGR). Taken together, those data mean that yarrow maintains normal developmental patterns when challenged with drought, but the physiological capacity to assimilate carbon is diminished. This could be a desirable trait for plants maintained in managed landscapes because the progress of traits associated with plant aesthetics, such as leaf development, will be normal. Furthermore, if a slight decrease in leaf area is tolerable, then horticulturists in arid regions might want to consider growing yarrow plants at the 30% moisture depletion level.

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