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IS LEAF SIZE A FACTOR IN DETERMINATION OF THE RATE OF TRANSPIRATION IN *CANNA INDICA* AND *EUPHORBIA MILII*?

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Two ornamental plants, namely *Canna indicia* and *Euphorbia milii* were subjected to four watering frequencies viz. daily, weekly, biweekly and monthly, each with five watering regimes (1.25%, 2.5%, 5%, 10% and 20%). After six weeks of watering, leaf area and transpiration rate of the seedlings were determined. The leaves were larger in *C. indica* ranging from (105 mm^2 – 436 mm^2) than in *E. milii* (36 mm^2 – 142 mm^2) while transpiration rates were higher in *E. milii* ($1.78x10^4$ $mol/m^{-2}/sec^{-1}$ – $2.56x10^{-3}$ $mol/m^{-2}/sec^{-1}$) than in *C. indica* ($2.48x10^{-4}$ $mol/m^{-2}/sec^{-1}$ – $3.70x10^{-5}$ $mol/m^{-2}/sec^{-1}$). Leaf area has no effect on the rate of transpiration in these two plants, but absence of evidence of transpiration on the adaxial surface of leaves of *E. milii* suggests that other anatomical features such as stomata rather than the leaf area might determine the rate of transpiration in plants.

Keywords: *Canna indica*, *Euphorbia milii*, leaf area, transpiration rate

Plant leaves forms the major surfaces on which photosynthesis and transpiration take place. This is possible because the leaves are equipped with exchange paths called stomata. As carbon dioxide moves into the leaves via the stomata, water vapour escapes out to the atmosphere. The former is to facilitate photosynthesis while the latter is a result of transpiration.

Some kinds of trees have large leaves and others of trees have small. For instance, in some trees that grow in wet places and those grow in the tropical rainforests have very large leaves. Other trees that grow in dry places, especially on mountain sides, often have small leaves; this is an adaptation to conserve or save water. Needles of conifers or "Christmas trees" and spines of cactus are actually very small. This type of leaves is also for conserving or saving water from escaping to the atmosphere through the stomata.

Perennial plants with relatively small leaves had moderate transpiration rates and leaf temperatures close to air temperature. Desert perennials with relatively large leaves had leaf temperatures well below air temperature along with the greatest accompanying transpiration rates of over 20 micrograms per square centimeter per second, but also had correspondingly low temperatures for maximum photosynthesis. The lower leaf temperatures measured in these large-leafed species are an exception to the more common pattern of desert plants whereby a smaller leaf size prevents overheating and leads to reductions in transpiration and increased water-use efficiency. The contribution of a larger leaf size to a lower leaf temperature, and thus a higher rate of photosynthesis for these large-leafed species, may represent an adaptive pattern previously unrecognized for desert plants (Smith 1978).

Transpiration takes place mainly in the leaves of plants, though other parts also possessed stomata and thus transpired. The plant leaves vary in size from plant to plant. The question on whether the leaf size (small or large) has any effects on the rate at which a plant transpire, need to be answered? In an attempt to provide answers to this question, earlier works on some ornamental plant species (AbdulRahaman 2009), *Agave americana* and *Aloe vera* (AbdulRahaman and Oladele 2017) and *Jatropha curcas* and *J. gossypifolia*

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(AbdulRahaman *et al*. 2017) reported that transpiration rates in these plants vary between and within large and small leaves. The rate of transpiration was not restricted to the size of the leaf; even within a species, the rate of transpiration differed between the large and small leaves of the plants.

The current effort is to further clarify earlier claims by comparing the leaf size and transpiration rate between and within two ornamental plant species, namely *Canna indica* and *Euphorbia milii* with morphologically large and small leaves respectively, which may or may not influence the rate of their transpiration. Meanwhile, the present study elucidated the effect of leaf size on the transpiration rate of *C. indica* and *E. milii*.

MATERIALS AND METHODS

Collection and identification of study specimens

The seeds of *C. indica* Linn. Liliaceae [Cannaceae] (African arrowroot, Indian shot, Arrowroot, Canna) and stem cuttings of *E. milii* Des Moul. Euphorbiaceae (Crown of thorns, Christ plant, Katie's crown) were collected from mature parent plants in their natural habitats. Study materials were identified in the Herbarium Unit of Department of Plant Biology, Faculty of Science, University of Ilorin, Ilorin, Nigeria.

Experiments in the greenhouse

This experiment (i.e. propagation of seeds of *C. indica* and stem cuttings of *E. milii* to seedlings) was conducted in a Screen house. Oven-dried soil (at temperature 105°C -110°C) of known measurements (Table 1) was distributed in bottom-perforated plastics in which the seeds, offsets and cuttings were sown. Water was supplied by using a plastic measuring cylinder of 100 ml. Depending on the watering regimes (1.2, 2.5, 5, 10 and 20% moisture content); quantity of water supplied was measured and applied based on the watering intervals (daily, weekly, biweekly and monthly). Twenty watering treatments (i.e. watering frequencies and regimes) were used to propagate each species. The soil and water were weighed and measured out in the different proportions (Table 1) adopting the method of Walter (1979). Each watering regime was replicated 15 times, 2 seeds of *C. indica* or stem cuttings of *E. milii* were planted in a plastic i.e. 300 plastics per species were used. All plastics were placed at the same level inside the screen house to expose the seeds and stem cuttings to all other factors like sunlight and air except water.

Table 1: Soil and water regimes used for raising the study materials

Soil (g)	Water (g)	% Moisture content (Water regime)
1600	400	20
1800	200	10
1900	100	5
1950	50	2.5
1975	25	1.25

Water stress treatments

Water stress (soil water-holding treatment) was imposed by withholding water from plants (i.e. cuttings and seedlings of the study materials) from the sowing stage/period for 1 week (7 d), 2 weeks (14 d) and 1 month (30 d) watering intervals, in a sunlit greenhouse. The soil relative water content (SRWC) was divided into four experimental treatments (Table 4) in order to provide different degrees of water stress preconditioning or to obtain a relatively stable water moisture gradient. Each of these treatments (SRWC) contains five different regimes of watering. Having four watering frequencies or intervals-daily, weekly, biweekly and monthly (each containing 1.25 cc, 2 cc, 5 cc, 10 cc and 20 cc watering regimes) -would yield a more complete picture of how moisture change affects the development of plants. Water stress imposition started from planting of seeds, offsets and cuttings to seedling level in order to detect the effects of the stress at both germination and post germination stages.

Experiments in the laboratory

Table 2: Leaf area and transpiration rate of *Canna indica* propagated with different percentage moisture contents

Watering regimes (%)	Mean leaf area (mm^2)	Transpiration rate ($mol/m^2/Sec^{-1}$)	
Daily		Abaxial	Adaxial
2.5	219[a]	$3.70x10^{-5}$c	$4.03x10^{-5}$c
5	436[a]	$4.72x10^{-5}$c	$3.88x10^{-5}$c
10	272[a]	$2.48x10^{-4}$b	$7.29x10^{-5}$c
20	312[a]	$5.30x10^{-5}$c	$3.76x10^{-5}$c
Weekly			
10	202[a]	$1.54x10^{-4}$b	$6.69x10^{-5}$c
20	291[a]	$6.68x10^{-5}$c	$1.14x10^{-4}$b
Biweekly			
10	105[b]	$7.59x10^{-5}$c	$1.28x10^{-4}$b
20	151[b]	$4.41x10^{-5}$c	$1.05x10^{-4}$b

Means with the same letters along the columns are not significantly different at $p < 0.05$

Table 3: Leaf area and transpiration rate of *Euphorbia milii* propagated with different percentage moisture contents

Watering regimes (%)	Mean leaf area (mm^2)	Transpiration rate ($mol/m^2/Sec^{-1}$)	
Daily		Abaxial	Adaxial
1.25	79[b]	$2.23x10^{-3}$a	-
2.5	142[a]	$8.38x10^{-4}$b	-
10	104[a]	$2.56x10^{-3}$a	-
20	86[b]	$2.03x10^{-3}$a	-
Weekly			
2.5	60[c]	$2.23x10^{-4}$b	-
5	105[a]	$8.19x10^{-4}$b	-
20	90[ab]	$1.78x10^{-4}$b	-
Biweekly			
2.5	79[b]	$7.93x10^{-4}$b	-
10	53[c]	$3.61x10^{-4}$b	-
Monthly			
2.5	36[d]	$4.40x10^{-3}$a	-
20	77[b]	$2.70x10^{-3}$a	-

Means with the same letters along the columns are not significantly different at $p < 0.05$
Key: - = no transpiration

The seedlings of *C. indica and E. milii* that eventually reached 6 weeks duration after germination in the greenhouse were taken to the laboratory for leaf area measurements and determination of transpiration rate.

Mean leaf area

The leaf area was also determined as L x B x 0.75 (Moll and Kamprath 1977, Abayomi and Adedoyin 2004).

Where: L = Length, B = Breadth

Samples of leaves used were taken from different parts of the plant body, i.e. upper, middle and lower parts. A sample size of 35 was used for each species.

Determination of transpiration rate

A cobalt chloride paper method was used to determine the transpiration rate of each specimen (Obiremi and Oladele 2001, Dutta 2003). Strips of filter paper of 2cm x 6cm dimension were cut and immersed in 20% cobalt chloride solution. The strips were thoroughly dried in an oven. The property of cobalt paper is that they are deep blue when dried, but in contact with moisture they turn pink. The blue, dried strips were placed in a sealed, airtight polythene bag and weighed (W1) using Mettler balance. It was transferred quickly to the plastic containers and affixed with a string to the marked small branch (of the plant) with leaves. Two dried cobalt papers were placed on the leaf, one on the upper and the other one on the lower surface of a thick, healthy leaf, and were covered completely with glass slides (this is to determine the transpiration rate from the two surfaces of a dorsiventral leaf, Dutta 2003). The time (in seconds) taken for the strips to turn pink was noted. Once turned pink, the bag was quickly untied and sealed again, and transferred to the laboratory and weighed (W2). Weight of water transpired was determined as W2 minus W1. The surface area of leaves used was measured (i.e. as described in the mean leaf area above). Transpiration rate was expressed as $mol/m^{-2}/sec^{-1}$.

Statistical analysis

All data generated were reported and analyzed using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). A probability value of 0.05 was used as benchmark for significant difference between parameters.

RESULTS

Consequence of varying watering regimes and frequencies is that some seeds and cuttings did not germinate or sprout. Where seeds and cuttings germinated or sprouted, the seedlings die back before the expiration of the six weeks of watering. These categories of seedlings could not be subjected to leaf area measurement and determination of transpiration rate (Tables 2 and 3).

Canna indica

Leaf size

The leaves of plants in a non-water-stressed of daily watering regimes were large while those in water-stressed plants were small. Leaf area was large (436 mm^2) in seedlings in 5 cc daily watering regime and small (105.80 mm^2) in seedlings of 10 cc weekly watering regime plants. Leaves of seedlings of daily watering regimes were generally larger than in other watering intervals and regimes (Table 2). There were significant differences at $p<0.05$ in mean leaf area in seedlings of *C. indica* of different watering regimes.

Transpiration rate

Water-stressed plants transpired lesser than non-water-stressed plants, especially on the adaxial leaf surface (Table 2). On abaxial leaf surface, the higher transpiration rate ($2.48x10^{-4}$ $mol/m^{-2}/sec^{-1}$) was recorded in 10cc daily watering regime seedling while the lower rate ($3.70x10^{-5}$ $mol/m^{-2}/sec^{-1}$) occurred in 2.5cc daily watering regimes. On adaxial surface, higher rate of transpiration ($1.28x10^{-4}$ $mol/m^{-2}/sec^{-1}$) occurred in 10cc biweekly watering regime and lower rate of $3.76x10^{-5}$ $mol/m^{-2}/sec^{-1}$) in 20cc daily watering regime plants. Transpiration rate was higher on the abaxial surface than on the adaxial surface. There was no significant difference at $p<0.05$ in rate of transpiration on both leaf surfaces in seedlings of *C. indica* of all watering regimes.

Euphorbia milii

Leaf size

Leaves in daily watering regimes were large while those in other water-stressed regimes were small. Seedlings of 2.5 cc daily watering regime plants possess larger leaves (142 mm^2) while smaller leaves (36 mm^2) are in seedlings of 2.5 cc monthly watering regime plants (Table 3). There were no significant differences at $p<0.05$ in seedlings of all watering regimes in *E. milii*.

Transpiration rate

There was no clear cut difference between the water-stressed and non-water-stressed plants with regard to rate of transpiration. On abaxial leaf surface, the higher transpiration rate of $4.40x10^{-3}$ $mol./m^{-2}/sec^{-1}$ occurred in seedlings propagated with 2.5 cc monthly watering regime and lower rate of $1.78x10^{-4}$ $mol./m^{-2}/sec^{-1}$ occurred in seedlings of 20 cc weekly watering regime. The seedlings in all watering regimes did not transpire on the adaxial surface because they lack stomata on this surface (Table 3). Transpiration rate was higher in daily watering regimes than in other watering regimes except the monthly watering regimes. There was no significant difference at $p<0.05$ in rate of transpiration on both leaf surfaces in seedlings of *E. milii* of all watering regimes.

DISCUSSION

Leaf formation and leaf area were affected by reductions in water potential. Water stress resulted in a reduction in the total amount of leaf area developed (Yang *et al*. 2007, Abdul Jaleel *et al*. 2008a, Abdul Jaleel *et al*. 2008b, Sankar *et al*. 2008). In this present study, the leaf areas and leaf productions reduced with increasing water stress in the two species studied. Leaf areas were generally larger in *C. indica* (105 mm^2 – 436 mm^2), than in *E. milii* (36 mm^2 – 142 mm^2). Meanwhile, in the two species, reduction in leaf area with a reduction in water availability was observable. That is leaves were smaller in water-stressed plants than in non-water-stressed plants. This is an adaptation to water stress and it was in line with earlier findings as stated above. Mean leaf area shows significant difference at $p< 0.05$ between the four watering frequencies in the two plant species.

Mean leaf area also showed some relationships with availability of water and the rates of transpiration. The reduction in leaf area ratio was associated with a significant increase in water use efficiency (Norby and O'Neill, 1991). Lazaridou and Koutroubas (2004) reported in their work with berseem clover that leaf area and transpiration rate were lower in plants under drought than under irrigation. Their results indicated that berseem clover reduced substantially the plant water losses by decreasing the transpiration rate and the leaf area. Also, Bindi *et al*. (2005) reported that both transpiration rates, which is assumed to be proportional to the gas exchange capacity of the plant, and leaf area development rate did not decrease until the fraction of the transpirable soil water (FTSW) declined to about 0.35. The corresponding decrease in the leaf size as observed *C. indica* and *E. milii* may also suggest a response to low water availability and the soil and thus probably to cut down transpiration.

Naturally, one would predict that the large leaf will transpire more quickly than the small leaf. However, it will be interesting to see that each leaf transpire at the same rate per square centimeter of leaf surface. Perhaps the small leaves tend to be younger and more fleshy, and the older leaves have a thicker cuticle. There were perhaps the same number of stomata on a small leaf as on a large leaf and the space between the stomata increases as the leaf grows. Perhaps larger leaves have more stomata and more stomata are formed in the spaces as a leaf gets bigger. Each of these hypotheses could lead one to a different prediction of the 'rate of transpiration per square centimeter' in large compared to small leaves.

However, there were instances where large leaf areas seem to favour high transpiration rate while small ones resulted in a low rate of transpiration. Eavis and Taylor (1979) in their experiment with soybean concluded that total transpiration increased linearly with leaf area and also that transpiration rate decreased linearly as soil water content decreased. This was evident in each of the two species as follows: In *C. indica*, small leaf areas (105 mm^2 and 151 mm^2) in seedlings with 10 cc and 20 cc biweekly watering regimes gave low transpiration rates ($7.59x10^{-5}$ mol./m^{-2}/sec^{-1} and $4.41x10^{-5}$ mol./m^{-2}/sec^{-1}) respectively. While, large leaf areas (272 mm^2, 202 mm^2 and 291 mm^2) in 10cc daily, and 10cc and 20cc weekly watering regime seedlings gave high transpiration rates ($2.48x10^{-4}$ mol./m^{-2}/sec^{-1} and $1.54x10^{-4}$ and $1.14x10^{-4}$ mol./m^{-2}/sec^{-1}) respectively. In *E. milii*, small leaf areas (60 mm^2, 90 mm^2 and 53 mm^2) in 2.5 cc weekly, 20 cc weekly and 10cc biweekly watering regime seedlings resulted in low rates of transpiration ($2.23x10^{-4}$ mol./m^{-2}/sec^{-1}, $1.78x10^{-4}$ mol./m^{-2}/sec^{-1} and $3.61x10^{-4}$ mol./m^{-2}/sec^{-1}) respectively. While, large leaf areas of 104mm^2 in 10cc daily watering regime seedlings gave high rates of transpiration of $2.56x10^{-3}$ mol./m^{-2}/sec^{-1}. Sometimes the rates of transpiration observed in large and small leaves could be linked to stomatal features present in such leaves. For example, in *E. milii*, large leaves (86 mm^2 and 77 mm^2) gave high transpiration rates (2.03x10-

^{3} mol./m^{-2}/sec^{-1} and 2.70x10^{-3} mol./m^{-2}/sec^{-1}) in 20 cc daily and 20 cc monthly watering regime seedlings respectively. While small leaves (60 mm^{2} and 53 mm^{2}) gave low transpiration rates (2.23x10^{-4} mol./m^{-2}/sec^{-1} and 3.61x10^{-4} mol./m^{-2}/sec^{-1}) in 2.5 cc weekly and 5 cc biweekly watering regime seedlings respectively. Summarily, the leaves were larger in *C. indica* ranging from (105 mm^{2} – 436 mm^{2}) than in *E. milii* (36 mm^{2} – 142 mm^{2}) while transpiration rates were higher in *E. milii* (1.78x10^{-4} mol /m^{-2}/sec^{-1} – 2.56x10^{-3} mol/m^{-2}/sec^{-1}) than in *C. indica* (2.48x10^{-4} mol/m^{-2}/sec^{-1} – 3.70x10^{-5} mol/m^{-2}/sec^{-1}).

However, leaf area alone could not determine the rate of transpiration, because in many cases, large leaves gave a low rate of transpiration and vice versa. If vividly observed in such situations, the determinant factor would be the type or kind of stomatal features (such as stomatal complex types, stomatal density, stomatal index and stomatal size) present in such leaves that actually influenced the rate of transpiration in plant species studied (AbdulRahaman 2009, AbdulRahaman and Oladele 2017, AbdulRahaman *et al*. 2017). For instance, there was no evidence of transpiration in the adaxial surface of the *E. milii*, and yet cumulatively, it has a higher rate of transpiration. This occurrence suggests that other anatomical features such as presence of stomata on the leaf surfaces rather than the leaf area might determine the rate of transpiration in plants (Oyeleke *et al*. 2004, AbdulRahaman 2009, AbdulRahaman and Oladele 2009, Saadu *et al*. 2009).

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