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Leaf size and transpiration rates in *Agave americana* and *Aloe vera*

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Abstract. Influence of the leaf area on transpiration rate was investigated in *Agave americana* and *Aloe vera*. After six weeks of irrigation, the leaf area in *A. americana* ranged from 141 mm^2 to 522 mm^2, while in *A. vera* it ranged from 16 mm^2 to 381mm^2. The transpiration rate varied from 1.05×10^{-5} mol m^{-2} sec^{-1} to 1.14×10^{-4} mol m^{-2} sec^{-1} on the abaxial and adaxial leaf surfaces, respectively, in *A. americana*. In *A. vera*, the rate of transpiration varied from 6.17×10^{-5} mol m^{-2} sec^{-1} on the abaxial surface to 4.70×10^{-4} mol m^{-2} sec^{-1} on the adaxial surface. Although the leaves are larger in *A. americana* than in *A. vera,* the transpiration rate was higher in *A. vera* than in *A. americana*.

Key words: *Agave americana, Aloe vera*, leaf size, transpiration rate

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Introduction

The leaf presents a surface through which photosynthesis and transpiration occur. This is due to the presence of chlorophyll and stomata on the leaf surfaces. As carbon dioxide diffuses into the leaf through the stomata, chlorophyll traps sunlight energy and water comes from the soil. Combined, all these form carbohydrates. Water vapour escapes outward into the atmosphere also through the stomata. The former process is called photosynthesis, while the latter is transpiration.

Describing the water flow from the soil through the plants to the atmosphere remains a formidable scientific challenge despite all years of research. This is not surprising, given the high dimensionality and the degree of nonlinearity of the soil-plant system, which evolves in space and time according to complex internal physical, chemical and biological laws enacted by external hydroclimatic variability (Katul & al. 2007). Transpiration is a process, where water containing in plants in liquid form is converted to vapour and released into the atmosphere. Much of the water taken up by plants is released through transpiration (Ferree & Hall 1980; Tanner & Beever 2001; Oladele 2002; Burghardt & Riederer 2003; George & al. 2007; Metselaar & Lier 2007). To maintain the vital flow of water and minerals, water evaporates as invisible water vapour from the leaves into the air. As water evaporates from the leaves, more water comes into the root to replace it. In fact, water is pulled in a continuous stream through the plant, from root to leaf, by capillary action – a wick or suction effect, known as transpiration tension. However, transpiration is one of the major causes of water stress in plants. If plants lose more water to the atmosphere through their stomata, and sometimes through lenticels and cuticles, than what they can absorb from the soil, then they face acute water stress (Parker 2005). The rate at which plants lose water to the atmosphere differs, but in most plants

90 to 450 kg of water transpire (The Columbia Encyclopedia 2004). Thus transpiration returns massive volumes of water from the ground to the atmosphere and is a very important part of the general water cycle on Earth (Parker 2005). It has been calculated that nearly ⅔ of water in the water cycle passes through plants. This comes to maintain 99 % of the atmospheric water balance. By transpiration, plants also participate in the soil water circulation (Wu & al. 2005). Since transpiration usually takes place in the leaves, the present research work elucidates the effect of leaf size on the rate of transpiration in *A. americana* and *Aloe vera*.

Material and methods

Collection and identification of study specimens

Offsets (or pups) of *A. americana* and *A. vera* were collected from mature parent plants in their natural habitats (Table 1). Study materials were identified in the Herbarium Unit at the Department of Plant Biology, Faculty of Science, University of Ilorin, Ilorin, Nigeria.

Experiments in the greenhouse

Propagation and raising of offsets of *A. americana* and *A. vera* to seedlings was conducted in a greenhouse. Oven-dried soil of known measurements (Tables 2 and 3) was distributed in bottom-perforated plastic containers in which the offsets and cuttings were placed. Water was supplied by using a plastic measuring cylinder of 100 ml. Depending on the watering regimes, quantity of the supplied water was measured and applied on the basis of watering intervals (Tables 3 and 4). Twenty watering treatments (i.e. watering frequencies and regimes) were used to propagate or raise each species; each watering regime was replicated fifteen times, two offsets were planted in each plastic container, i.e. 300 plastic containers per species were used. In the greenhouse, all factors remained constants, except for water. All plastic containers were placed at the same level to expose the offsets inside the plastics to all other factors, such as sunlight, except for water.

Meanwhile, since the above regimes cannot be practically used to raise the study materials, a conversion method was adopted. A factor 20 was used to multiply each of the above regimes of soil and water (Table 2), so as to reach the other regimes (Table 3) used for propagation of seeds, offsets and cuttings. Thus, 10 000 g of soil was used instead of the original 500 g as proposed by Walter (1979).

Water stress treatments

Water stress (soil water-holding treatment) was imposed by withholding water from plants (i.e. offsets and seedlings of the study materials) as from the sowing period at one week (7 days), two weeks (14 d) and one 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. The 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 offsets to seedling level, in order to detect the effects of the stress at both germination and post germination stages.

Table 1. List of studied species .

Species	Family	Common names	Origin
Agave americana L.	*Agavaceae*	American Century Plant, Century Plant, Maguey.	Mexico
Aloe vera (L.) Burm.f.	*Xanthorrhoeaceae*	Caribbean Aloe, Aloe Vera, Curacae Aloe, Lily of the Desert, Plant of Immortality, Medicine Plant, Elephant Gall.	Africa (North, East and South).

Table 2. Soil and water regimes for raising the study materials.

Soil (g)	Water (g)	% Moisture content (water regime)
80	20	20
90	10	10
95	5	5
97.5	2.5	2.5
98.75	1.25	1.25

Table 3. 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

Leaf anatomical studies

The seedlings of *A. americana* and *A. vera* that eventually reached six weeks duration after germination in the greenhouse were taken to the laboratory for photographs, leaf area measurements, determination of transpiration rate and anatomical studies. Transpiration and anatomical studies were carried out on leaves mainly because of the presence of stomata which have a greater water economy effect.

Mean leaf area

The leaf area was also determined as L×B×0.75 (Moll & Kamprath 1977; Abayomi &Adedoyin 2004), where: L = length and B = breadth. Samples of leaves were taken from different parts of the plant body, i.e. upper, middle and lower parts. A sample size of 35 parts 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 & Oladele 2001; Dutta 2003). Strips of filter paper of 2 cm × 6 cm dimensions were cut and immersed in 20 % cobalt chloride solution. The strips were thoroughly dried in an oven. The cobalt paper is deep blue when dried, but in contact with moisture turns pink. The blue dried strips were placed in a sealed, airtight bag and it was weighed (W1) using a Mettler balance. They were transferred quickly to the plastic containers and affixed with a string to a marked small branch (of the plant) with leaves. Two dried cobalt papers were placed on the leaf, one on the upper and the other on the lower surface of a thick, healthy leaf, and were covered completely with glass slides, in order to determine the transpiration rate from the two surfaces of the leaf (Dutta 2003). The time (in seconds) taken for the strips to turn pink was noted down. Once the papers turned pink, the bag was quickly untied and sealed again, and transferred to the laboratory for a second weighing (W2). The weight of transpired water was determined as W2 minus W1. The surface area of the used leaves was measured (as described in the mean leaf area above). Transpiration rate was expressed as mol m^{-2} sec^{-1}.

Table 4. Watering frequencies and regimes used for raising seedlings of the study materials.

Watering intervals	Soil moisture content (%)
Daily	1.25
	2.5
	5
	10
	20
Weekly	1.25
	2.5
	5
	10
	20
Biweekly	1.25
	2.5
	5
	10
	20
Monthly	1.25
	2.5
	5
	10
	20

Statistical analysis

All generated data were reported and analyzed by 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 the parameters.

Results

Leaf size

Agave americana. The leaves in all 20 cc of daily, weekly, biweekly and monthly watering regime seedlings were larger than in all other regimes. Leaf sizes differed from one regime to another. Larger leaves (522 mm^2) were found in 20 cc daily watering regime seedlings, while smaller ones (72 mm^2) were in the seedlings of 20 cc weekly regimes (Table 5). There were no significant differences at p<0.05 in seedlings of all watering regimes in *A. americana*.

Aloe vera. There was no clear-cut difference between the size of leaves of the water-stressed and non water-stressed plants (Table 5). However, larger leaves (381 mm^2) were observed in seedlings of 1.25 cc daily

Table 6. Transpiration rate of studied species propagated with different percentage moisture

Watering regimes (%)	Transpiration rate (mol m-2 sec-1)			
	Agave americana		*Aloe vera*	
	Abaxial	Adaxial	Abaxial	Adaxial
Daily				
1.25	2.91×10-5de	3.01×10-5d	8.66×10-5c	1.13×10-4b
2.5	1.68×10-5e	1.70×10-5e	8.64×10-5c	1.48×10-4b
5	3.46×10-5d	3.23×10-5d	2.22×10-4b	1.49×10-4b
10	4.93×10-5d	5.05×10-5cd	–	–
20	1.05×10-5e	1.22×10-5e	–	–
Weekly				
2.5	2.82×10-5de	3.00×10-5d	–	–
5	1.28×10-5e	1.32×10-5e	1.11×10-4b	1.47×10-4b
10	4.21×10-5d	4.44×10-5d	6.17×10-5c	8.67×10-5c
20	7.67×10-5c	1.14×10-4b		
Biweekly				
10	3.50×10-5d	3.65×10-5d	1.93×10-4b	2.74×10-4b
20	3.55×10-5d	2.30×10-5de	3.39×10-4a	4.70×10-4a
Monthly				
10	6.61×10-5c	6.44×10-5c	–	–
20	2.42×10-5de	2.59×10-5de		

Mean values with the same letters along the columns are not significantly different at p<0.05

watering regime, while smaller leaves (16 mm^2) were observed in seedlings of 20 cc biweekly regime. There were no significant differences at $p<0.05$ in seedlings of all watering regimes in *A. vera*.

Transpiration rate

Agave americana. There was no clear difference in transpiration rate between the water-stressed and non water-stressed plants. On the abaxial leaf surface, a higher transpiration rate (7.67×10^{-5} mol m^{-2} sec^{-1}) was recorded in the 20 cc weekly watering regime seedlings, and a lower rate (1.05×10^{-5} mol m^{-2} sec^{-1}) in the 20 cc daily watering plants on the abaxial surface. On the adaxial surface, the rate of transpiration was higher (1.14×10^{-4} mol m^{-2} sec^{-1}) in the 20 cc weekly watering regime, while it was lower (1.22×10^{-5} mol m^{-2} sec^{-1}) in the 20 cc daily watering regime. Transpiration rate was higher on the adaxial surface than on the abaxial in most seedlings, except in the 5 cc daily, 20 cc weekly, 20 cc biweekly, and 10 cc monthly watering regime seedlings (Table 6). This could be rather due to high stomatal density on the adaxial than on the abaxial surfaces of those plants. There was no significant difference at $p<0.05$ in the transpiration rate on both leaf surfaces in seedlings of *A. americana* at all watering regimes.

Aloe vera. Water-stressed plants at 10 cc weekly and biweekly watering regimes had lower rates of transpiration than non water-stressed plants. On the abaxial leaf surface, a higher rate of transpiration (3.39×10^{-4} mol m^{-2} sec^{-1}) occurred in the 20 cc biweekly watering regime plants and a lower one (6.17×10^{-5} mol m^{-2} sec^{-1}) in the 20 cc weekly watering regime plants. On the adaxial surface, it was higher (4.70×10^{-4} mol m^{-2} sec^{-1}) in the 20 cc biweekly watering regime seedlings, and lower (8.67×10^{-5} mol m^{-2} sec^{-1}) in the 20 cc weekly watering regime seedlings. Meanwhile, the transpiration rate was relatively higher on the adaxial than on the abaxial surface at most regimes (Table 6), this could be due to the vertical orientation of the leaves of this species, unlike in other species, where the abaxial and adaxial surfaces physically and texturally differed. There was no significant difference at $p<0.05$ in the transpiration rate on both leaf surfaces in seedlings of *A. vera* at all watering regimes.

Table 6. Transpiration rate of studied species propagated with different percentage moisture

Watering regimes (%)	Transpiration rate (mol m-2 sec-1) *Agave americana* Abaxial	*Agave americana* Adaxial	*Aloe vera* Abaxial	*Aloe vera* Adaxial
Daily				
1.25	2.91×10-5de	3.01×10-5d	8.66×10-5c	1.13×10-4b
2.5	1.68×10-5e	1.70×10-5e	8.64×10-5c	1.48×10-4b
5	3.46×10-5d	3.23×10-5d	2.22×10-4b	1.49×10-4b
10	4.93×10-5d	5.05×10-5cd	–	–
20	1.05×10-5e	1.22×10-5e	–	–
Weekly				
2.5	2.82×10-5de	3.00×10-5d	–	–
5	1.28×10-5e	1.32×10-5e	1.11×10-4b	1.47×10-4b
10	4.21×10-5d	4.44×10-5d	6.17×10-5c	8.67×10-5c
20	7.67×10-5c	1.14×10-4b		
Biweekly				
10	3.50×10-5d	3.65×10-5d	1.93×10-4b	2.74×10-4b
20	3.55×10-5d	2.30×10-5de	3.39×10-4a	4.70×10-4a
Monthly				
10	6.61×10-5c	6.44×10-5c	–	–
20	2.42×10-5de	2.59×10-5de		

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

Discussion

Leaf formation and leaf area were affected by reductions in water potential. Water stress resulted in a reduction in the total developed leaf area (Narayana & al. 1991; Delgado & al. 1992; Mencuccini & Grace 1994; Alves 1998; Alves & Setter 2002; Chaves & al. 2003; Gomez-del-Campo & al. 2002; Wang & Gao 2003; Banon & al. 2004; Sobeih & al. 2004; Sivilotti & al. 2005; Lebon & al. 2006; Rosado & al. 2006; Aranjuelo & al. 2007; Dias & al. 2007; Gazanchian & al. 2007; Reynolss & al. 2007; Yang & al. 2007; Abdul Jaleel & al. 2008a; Abdul Jaleel & al. 2008b; Sankar & al. 2008). In the study, the leaf areas and leaf productions have reduced with the increasing water stress in the two studied species. Leaf areas were generally larger in *A. americana* (72–522 mm^2) and smaller in *A. vera* (16–381 mm^2). In all species, reduction in the leaf area with reduction of water availability was observable, i.e. the leaves were smaller in water-stressed than in non water-stressed plants. This is an adaptation to water stress and it was in line with earlier findings as stated above. The mean leaf area has shown a significant difference at $p<0.05$ between the four watering frequencies in both plant species.

There were some relationships between the mean leaf area and transpiration rates in both study plants. The mean leaf area also showed some relationships with the availability of water and the rates of transpiration. Reduction in the leaf area ratio was associated with a significant increase in water use efficiency (WUE) (Norby & O'Neill 1991). Lazaridou & Koutroubas (2004) reported in their work on Berseem Clover that leaf area and transpiration rate were lower in plants under drought than under irrigation. Their results indicated that Berseem Clover has reduced substantially the plant water losses by decreasing the transpiration rate and the leaf area. Also, Bindi & al. (2005) reported that both transpiration rate, 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.

Naturally, one would predict that a large leaf will transpire more quickly than a small leaf. However, it will be interesting to note that each leaf transpires 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 are more woody and have a thicker cuticle. Perhaps, there were the same number of stomata on a small leaf as on a large one 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 as compared to small leaves.

There were instances where the large leaf areas seem to favour high transpiration rate, while the small ones produced a low rate of transpiration. Eavis & Taylor (1979), in their experiment with soybean, concluded that total transpiration increases linearly with the leaf area and also that transpiration rate decreases linearly with the decrease of soil water content. This was evident in each of the two species as follows: in *A americana*, small leaf area of 161 mm^2 and 72 mm^2 in 2.5 cc and 20 cc weekly watering regime seedlings gave low rates of transpiration of 3.00×10^{-5} mol m^{-2} sec^{-1} and 7.67×10^{-5} mol m^{-2} sec^{-1}, respectively. In *A. vera*, small leaf areas of 42 mm^2 in 2.5 cc daily watering regime seedlings gave low transpiration rates of 8.64×10^{-5} mol m^{-2} sec^{-1}. Large leaf areas of 381 mm^2 and 95 mm^2 in 1.25 cc daily and 20 cc weekly regimes resulted in high transpiration rates of 1.13×10^{-4} mol m^{-2} sec^{-1} and 8.67×10^{-5} mol m^{-2} sec^{-1}, respectively.

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 could be the type or kind of stomatal feature(s) present in such leaves that actually influence the rate of transpiration in the same plant species. AbdulRahaman (2009) has observed that stomata density, stomata index and stomata size had a certain influence on the rate of transpiration in *Canna indica* and *Euphorbia milii.* But the same cannot be said about *Agave americana* and *A. vera.*

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