Determination of the water use of two pairs of soybean isolines differing in stomatal frequency using a heat balance stem flow gauge

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Tan, C. S. and Buttery, B. R. 1995. **Determination of the water use of two pairs of soybean isolines differing in stomatal frequency using a heat balance stem flow gauge.** Can. J. Plant Sci. **75**: 99–103. Using heat-balance stem flow gauges, we were able to measure directly and continuously the sap flow rates in two pairs of soybean [*Glycine max* (L.) Merr.] isolines differing in stomatal frequency. Plants with high stomatal frequency transpired significantly more water than the low stomatal frequency plants at high soil moisture levels. Under low soil moisture levels, the water use rate decreased greatly for the high stomatal frequency plants. Plants with low stomatal frequency were able to maintain greater sap flow rates than those with high stomatal frequency. Higher leaf temperatures associated with the low stomatal frequency plants were likely due to lower transpiration rates which reduced evaporative cooling especially under well-watered conditions.

Key words: *Glycine max* (L.) Merr., transpiration, water deficits

Tan, C. S. et Buttery, B. R. 1995. **Détermination de l'utilisation de l'eau chez deux paires de lignée isogéniques de soja différant par leur fréquence stomatique, au moyen d'un régulateur de débit de la sève à bilan calorifique.** Can. J. Plant Sci. **75**: 99–103. À l'aide de régulateur de débit de la sève dans la tige par bilan calorifique, nous avons pu mesurer en direct et en continu le débit de la sève dans deux paires de lignées isogéniques de soja (*Glycine max* (L.) Merr.) différant par la fréquence stomatique. À des fortes teneurs en eau du sol, les plantes dotées d'une fréquence stomatique élevées transpiraient significativement plus que les plantes à basse fréquence stomatique. À des niveaux hydriques du sol bas, le taux d'utilisation de l'eau diminuait fortement chez les plantes à haute fréquence stomatique. Les plantes à basse fréquence stomatique étaient capables de conserver des débits de la sève plus intenses que celles à haute fréquence stomatique. Les températures foliaires plus élevées observées chez les plantes à basse fréquence stomatique étaient vraisemblablement attribuables à un moindre taux de transpiration lequel abaisserait le refroidissement par évaporation, surtout en situations bien pourvues en eau.

Mots clés: *Glycine max* (L.) Merr., transpiration, déficit hydrique

Stomatal frequency of soybean [*Glycine max* (L.) Merr.] leaves, especially on the upper surface, is much reduced in the presence of kaempferol triglucoside (K9), a flavonol glycoside also associated with reduced chlorophyll and a reduced photosynthetic rate (Cosio and McClure 1984; Buttery and Buzzell 1987; Buttery et al. 1992). Buttery et al. (1993) found that in irrigated soybeans, high stomatal frequency was associated with higher photosynthetic rate, stomatal conductance, plant weight, and bean yield. However, under non-irrigated conditions, yields of the high stomatal frequency lines dropped, while low stomatal frequency lines maintained a yield similar to that obtained under irrigation. This suggested that there was a difference in water use between the two lines under well-watered and dry conditions. There currently are no data to test this assumption, as it has been difficult to accurately and continuously measure plant water use in a wide range of soil water conditions, especially under rapidly changing conditions. Until recently the techniques available only allowed an instantaneous short-duration measurement of the plant response, making it difficult to capture the transient changes in water use. A technique was needed to provide a continuous measurement of the entire plant response, so that brief but important water use changes could be recorded and studied.

Recently, a technique was developed that is capable of continuously measuring the mass flow rate of water in herbaceous plant stems. The approach is based on a continuous application of heat to the stem, and the measurement of temperature gradients to determine the flux of heat out of the system (Baker and van Bavel 1987). This technique gives measurements within 5–10% of actual transpiration as measured by weighing (Heilman and Ham 1990; Steinberg et al. 1990). The gauges are connected to a datalogger, allowing automatic measurement and recording a sap flow at short intervals (Baker and van Bavel 1987). With this method it is possible to accurately and directly measure changes in water use in a plant as it is exposed to a wide range of moisture conditions over several days.

The objective of this study was to use the heat balance technique to determine the effect of stomatal frequency on water use of two pairs of soybean isolines subjected to a wide range of soil water conditions.

Abbreviations: K9, kaempferol triglucoside

MATERIALS AND METHODS

The experimental materials consisted of two pairs of near-isogenic soybean lines (OX921 and OX922; OX941 and OX942) which differ in the genes controlling flavonol glycosides in the leaves. OX921 and OX942 lack kaempferol triglucoside (K9) and have more stomata on the upper leaf surface (average 155 mm^{-2}) than OX922 and OX941, which contain K9 and have few stomata (average 4 mm^{-2}) on the upper leaf surfaces (Buttery and Buzzell 1987). Eight seeds of each soybean line were planted in the fall of 1992 in a growth room at the Agriculture and Agri–Food Canada Research Station in Harrow, Ontario. One seed was sown in each of 32, 12–L pots filled with potting soil containing sand, peat moss and sandy loam soil in the ratio of 1:1:2. The field capacity of the potting soil was 34.1% on a volume basis, the permanent wilting point was 8.9%. The growth room was maintained on 16-h photoperiods by cool white fluorescent and incandescent lamps with a photon flux density of 250 μmol m^{-2} s^{-1} between 400 and 700 nm at plant level. The temperature was 28 $\pm$ 0.5°C during the 16–h day, and 21 $\pm$ 0.5°C during the dark period. The long days were used to delay flowering and ensure large vegetative plants. After 30 d, six uniform plants of each soybean isoline were selected and leaves and branches of all plants were trimmed to 22 cm above the soil level for installation of stem-flow gauges. The stem-flow gauges were installed 3 d later after healing of the trimming injury had occurred so as to prevent damage to the gauge from plant sap. An electrical insulating compound (Dow-Corning compound 4) was applied to the area of the lower stem which would be covered by the gauges and to the inside of gauges. Sap flow was measured to observe the response of each soybean isoline to depletion of soil moisture by withholding watering for 4 d followed by re-watering. The 24 plants consisted of six replicates of each of the four soybean isolines. Canopy temperature was measured for three plants each on two of the isolines (OX941 and OX942).

Sap flow was measured by the heat balance method (Baker and van Bavel 1987), using 24, 10-mm–diameter stem flow gauges (Model SGA10, Dynamax Inc., Houston, TX). Data were recorded every 30 min using a Campbell CR–10 datalogger and four AM410 multiplexers. Flow analysis software was supplied by Dynamax Inc. for data logging, data analysis and retrieval, and downloading of control programs. Soil volumetric water content was measured twice a day using the time domain reflectometry technique (Model 6050X1 Trase System, Soil Moisture Equipment Corporation, Santa Barbara, CA). Canopy temperature was determined using infrared temperature sensors (Model 4000A, Everest Interscience Inc., Fullerton, CA). Data were recorded every hour using a Campbell CR-21X datalogger.

At the end of the experiment, the leaf area of each plant was determined with a LI-COR leaf area meter (Model LI-3000, LI-COR Inc., Lincoln, NE).

RESULTS

Daily sap flow rate was calculated per unit leaf area to allow comparisons between plants which have different leaf areas (Table 1). Sap flow within each pair of soybean isolines (OX921 and OX922; OX941 and OX942) differed. When soil moisture was high, the high stomatal frequency isolines (OX921, OX942) had a higher sap flow than their respective low stomatal frequency isolines (OX922, OX941). As soil moisture was depleted (day 4), sap flow in the high stomatal frequency lines dropped considerably more than sap flow in the low stomatal frequency lines. After rewatering, daily sap flow in three of the soybean lines recovered almost to earlier levels. OX942 sap flow remained significantly below previous levels (Table 1).

At the initially high soil water contents, hourly sap flow rate for OX921 and OX942 during the daytime was significantly higher than sap flow rate for OX922 and OX941 (Fig. 1). At night, sap flow rates for all lines were near zero. As soil moisture declined, a point was reached at which the sap flow rate of the high stomatal frequency line dropped below that of the low stomatal frequency line. The difference in the isoline responses to water stress is especially noticeable on day 4. The high stomatal frequency lines caused soil moisture to decrease more quickly than did the low stomatal frequency lines (Table 1, Fig. 1). After rewatering, sap flows increased to close to their previous levels. Sap flow for the low stomatal frequency lines recovered most quickly. OX942, which experienced the lowest soil moisture, was the slowest to recover (Fig. 1).

The ratio of sap flow rate between the high and the low stomatal frequency isolines in each pair varied with the soil moisture levels (Fig. 2). When the ratio was greater than 1.0, the high stomatal frequency line had the highest sap flow.

Table 1. The daily soil volumetric water content (%), sap flow rate per unit area (g m^{-2} d^{-1}) and sap flow rate per plant (g $plant^{-1}$ d^{-1}) of two pairs soybean isolines with high and low stomatal frequency during a 4–d dry-down and rewater cycle

	Volumetric water content (%)				Sap flow rate per unit area (g m^{-2} d^{-1})				Sap flow rate (g $plant^{-1}$ d^{-1})			
	Pair 1		Pair 2		Pair 1		Pair 2		Pair 1		Pair 2	
Day	OX921 (High)	OX922 (Low)	OX942 (High)	OX941 (Low)	OX921 (High)	OX922 (Low)	OX942 (High)	OX941 (Low)	OX921 (High)	OX922 (Low)	OX942 (High)	OX941 (Low)
1	29.8*ax*	31.5*ax*	30.3*ax*	31.0*ax*	1216*ax*	1030*ay*	1622*ax*	1282*ay*	893*ax*	657*ay*	1133*ax*	793*ay*
2	22.1*bx*	24.7*bx*	20.4*bx*	23.7*bx*	1233*ax*	1064*ay*	1710*ax*	1388*ay*	906*ax*	677*ay*	1193*ax*	858*ay*
3	12.5*cy*	17.9*cx*	9.2*cy*	14.2*cx*	1285*ax*	1065*ay*	1470*abx*	1571*ax*	937*ax*	667*ay*	1039*ax*	972*ax*
4	4.8*dy*	8.1*dx*	4.1*dx*	5.7*dx*	547*by*	846*bx*	434*cy*	821*bx*	400*by*	515*bx*	310*cy*	510*cx*
5	31.3*ax*	33.7*ax*	33.0*ax*	34.4*ax*	1027*ax*	987*ax*	1165*bx*	1160*ax*	749*ax*	610*ax*	821*bx*	719*bx*

*a-d*Values in columns followed by the same letter do not differ at P = 0.05 by Duncans range test.
*x-y*Values in rows within the same pair followed by the same letter do not differ at P = 0.05 by Duncans range test.

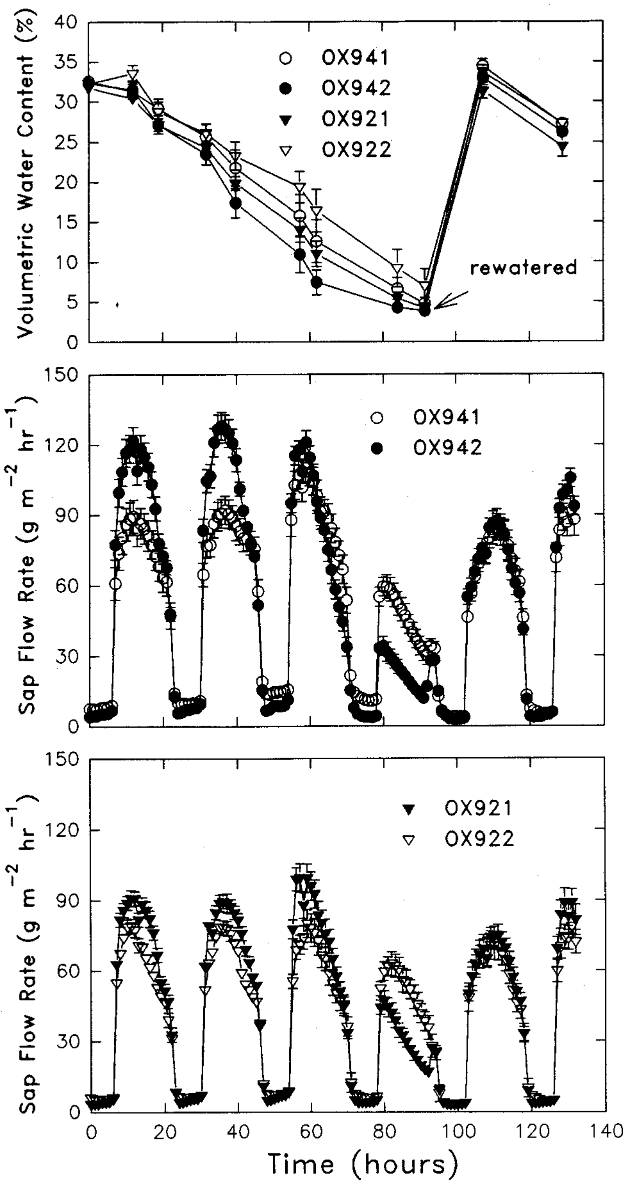

Fig. 1. The soil volumetric water content (%) and hourly sap flow rate per unit leaf area (g m^{-2} h^{-1}) of two pairs of soybean isolines with high (OX921 and OX942) and low (OX922 and OX941) stomatal frequency during a 4-d dry-down and rewater cycle. The arrow denotes rewater. Vertical lines represent standard errors with six replications.

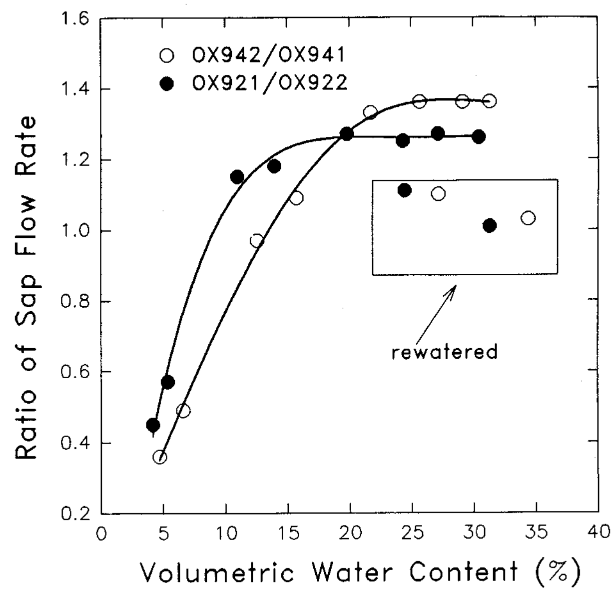

Fig. 2. The ratio of hourly sap flow rate per unit area between high (OX921 and OX942) and low (OX922 and OX941) stomatal frequency isolines in each pair versus corresponding soil volumetric water content.

When the ratio fell below 1.0, sap flow was highest for the low stomatal frequency line. The corresponding volumetric water content was around 10%. After rewatering, the ratios were lower than the initial ratios (Fig. 2), and in fact were close to 1.0, indicating similar sap flow for both high and low stomatal frequency lines.

The accumulated sap flow data demonstrated that differences existed in water use between the high and low stomatal frequency lines, but there were also differences between the two pairs of isolines (Fig. 3). During periods of high moisture, the high stomatal frequency lines had higher rates of accumulated sap flow than the low stomatal frequency lines. During the water stress period, however, the opposite was true, with the result that both members of each isoline pair ended up with similar total sap flow values. Accumulated sap flow for the two pairs of isolines were different. The OX941 and OX942 isoline pair had higher accumulation rates and a higher total sap flow than the OX921 and OX922 pair.

At high soil moisture levels, the canopy temperature of the high stomatal frequency line, OX942 was below that of the low stomatal frequency line, OX941 (Fig. 4). By the middle of day 3, when soil moisture had dropped to approximately 15%, the OX942 canopy temperature was slightly greater than the OX941 temperature. On day 5, after soil moisture had recovered to higher levels, OX941 canopy temperature again exceeded OX942 canopy temperature. Shortly after OX942 sap flow peaked each day, canopy temperature dropped to its lowest daytime levels. Throughout the daytime hours, canopy temperature was inversely related to sap flow rates. These trends were not as clear in the OX941 line.

DISCUSSION

The large differences between lines in numbers of stomata resulted in relatively small differences between lines in sap flow rates and in the response of the plants to stress. Higher leaf temperatures in lines with a low stomatal frequency were likely due to reduced evaporative cooling. The coolest daytime canopy temperatures were measured when sap flow, and therefore transpiration, was at a maximum and in general, the line experiencing the highest sap flow had the lowest canopy temperature at any given time. Temperature

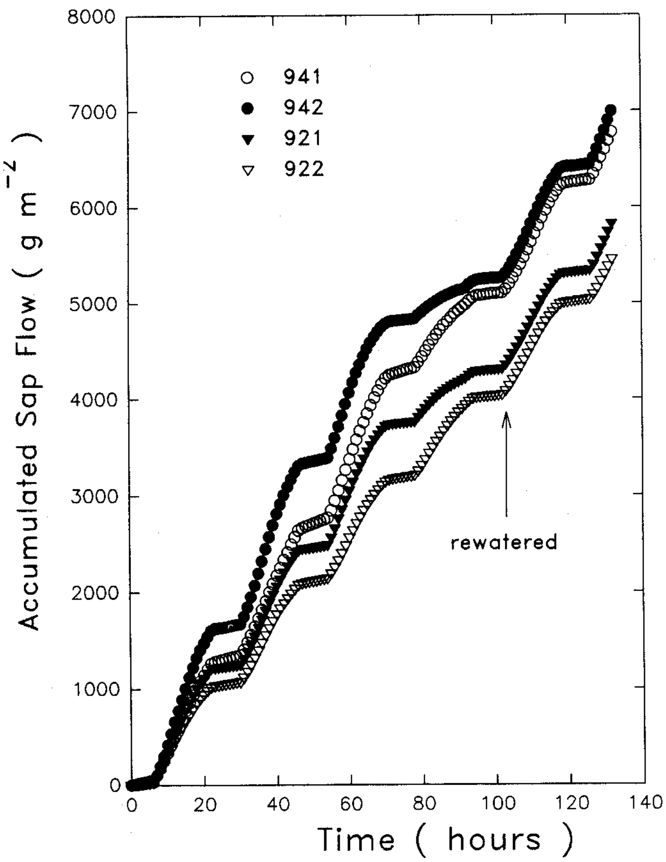

Fig. 3. The accumulated sap flow per unit leaf area (g m^{-2}) of two pairs of soybean isolines during a 4-d dry-down and rewater. OX921 and OX942 have high stomatal frequency: OX922 and OX941 have low stomatal frequency.

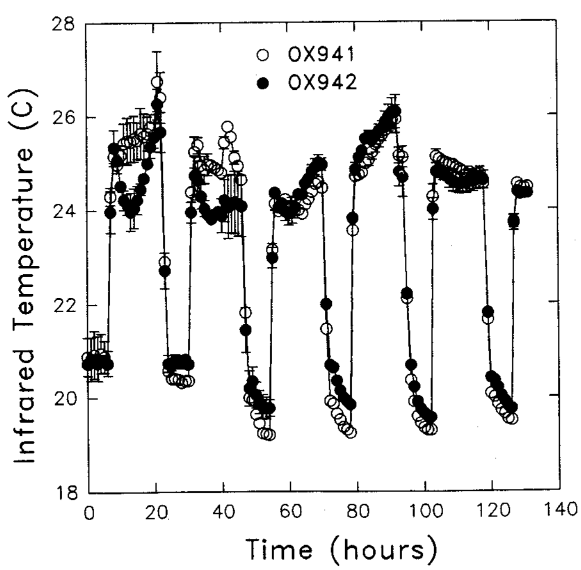

Fig. 4. The plant canopy temperature (°C) of one pair of soybean isolines [OX941 (low) vs. OX942 (high stomatal frequency)] during a 4-d dry-down and rewater. Vertical lines represent standard errors with six replications.

climbed for both pairs of soybean lines as soil moisture and sap flow dropped. Canopy temperature has been used as an indicator of water stress in crop plants (Jackson 1982; Keener and Kircher 1983; Tan 1993).

The daily sap flow rates (Table 1) and the hourly rates (Fig. 1) for each soybean line show differences in water use between the members of each isoline pair as soil moisture decreases. At high moisture levels, the high stomatal frequency lines transpired significantly more water than the low stomatal frequency lines, as indicated by the sap flow data.

The high stomatal frequency soybean lines had a higher stomatal conductance, as well as a higher photosynthetic rate, plant weight, and bean yield at high soil moisture levels than did the low stomatal frequency lines (Buttery et al. 1993; Buttery and Buzzell 1987). Our present results, however, show that at low soil moisture levels sap flow decreased greatly for the high stomatal frequency lines. Due to their high water requirements, they also used up the available soil water more quickly than their low stomatal frequency counterparts. OX942 soil moisture dropped the lowest of all lines. This water stress, well below the permanent wilting point, may have damaged the plant, as sap flow for this line did not recover as quickly after rewatering as the other lines. Severe wilting and leaf drop was noticed on OX942 plants during the water stress period. Under moisture stress conditions, the low stomatal frequency lines maintained sap flow rates closer to those measured under high moisture conditions partly because they required less water than the high stomatal frequency lines, thereby conserving the limited supply of water available. Buttery et al. (1993) found that moisture stress did not have a deleterious effect on the low stomatal frequency lines in terms of stomatal conductance, transpiration, and bean yield. However, the yield potential of these lines was well below that of the high stomatal frequency lines. This suggested that soybean lines with a high stomatal frequency were adapted to conditions of ample water thereby producing higher yields, but were not as well adapted to less than ideal moisture conditions. Low stomatal frequency lines, while withstanding water stress conditions without a loss in performance, did not show a yield increase under well-watered conditions. This suggests that in a sub-humid region, even when periodic droughts may be experienced, the high stomatal frequency lines would be more productive than the low stomatal frequency lines. However, where soybeans are grown in arid regions, the low stomatal frequency lines, with their lower water requirements and ability to withstand moisture stress, could be more productive.

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