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This is to certify that the

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WATER RELATIONS IN CUT ROSES (ROSA HYBRIDA L.)
MEASURED WITH IN SITU HYGROMETER AND
PRESSURE CHAMBER

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WATER RELATIONS IN CUT ROSES (*ROSA HYBRIDA* L.) MEASURED WITH *IN SITU* HYGROMETER AND PRESSURE CHAMBER

By

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ABSTRACT

WATER RELATIONS IN CUT ROSES (ROSA HYBRIDA L.)
MEASURED WITH IN SITU HYGROMETER AND
PRESSURE CHAMBER

I. Water Relations in Cut Roses: A Comparison of in situ Hygrometer and Pressure Chamber Water Potential Techniques

II. Water Relations in 'Samantha' and 'Golden Wave' Cut Roses

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Section I

Pressure chamber measurements of water potential of cut roses with leaves and/or flowers attached to the stem reflect stem xylem potentials. Pressure chamber and in situ dew point hygrometer measurements of leaf water potential are in close agreement. In situ hygrometers were used to measure rose petal potentials. SEM studies of leaf and petal surfaces revealed no damage to epidermal cells when abraded with carborundum to allow more rapid vapor equilibration in hygrometers.

Section II

Short-lived 'Golden Wave' and long-lived 'Samantha' leaf and petal water potential declined with time from cutting. 'Golden Wave' petal potential declined faster than 'Samantha' with 'Samantha'

petals lowest at senescence. Leaf potential of both cultivars declined at similar rates.

Both cultivars senesced when fresh weight declined below initial weight. Hydraulic conductivities decreased with time. 'Golden Wave' was more resistant to water flow. Differences in vase life may be due to larger water deficits of 'Golden Wave'.

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Readers:

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LITERATURE REVIEW

LITERATURE REVIEW

Introduction

Cut rose (Rosa hybrida L.) sales comprised 14.5% of the total floriculture sales in the United States in 1979 (3). The price per bloom is one of the highest of all cut flowers sold (3), yet cut roses have an average vase life of only 4-8 days (44) compared to 9-14 days for standard chrysanthemums (52) and carnations (28). Extending the vase life of cut roses is an important research area in floriculture.

The rapid loss of turgor pressure in cut rose petals is of major concern. The water balance of a cut rose is thought to be one of the main factors affecting longevity (1,18,19,56). Elucidating the water relations of cut roses depends on characterizing the physiological and physical processes which influence the flower's water balance, and the accurate measurement of the changing water status.

The water balance in general is determined by the difference between water loss and water uptake. A deficit develops when water loss exceeds uptake. Water loss is determined primarily by transpiration (45,49) so factors influencing stomatal movements under water stress are important. Water uptake rates in response to transpirational losses ultimately determine plant water status. A plant capable of replacing water lost through transpiration will not

develop water stress. If too great a resistance to water flow develops, transpirational water losses will result in a water deficit. The balance between transpiration and uptake changes throughout the life of a cut rose (43). The most accurate indicator of plant water status is tissue water potential (WP) (21,31). Two methods commonly used to measure plant water potentials are the pressure chamber and the psychrometer.

Transpirational Water Loss

Water is lost from plants by transpiration through both the cuticle and stomatal pores (45,49), with 97% of the water lost through the stomates (45). The amount of stomatal water loss is affected by the vapor pressure gradient between the stomatal chamber and the surrounding air, air movement around the leaf, and the degree of stomatal opening (45). Vapor pressure gradients change with air temperature and relative humidity.

In still air, as water diffuses into the atmosphere from a stomate, a layer of air at higher vapor pressure than the surrounding atmosphere develops around the leaf. This air layer decreases the vapor pressure deficit thereby reducing transpiration. This resistance to water diffusion is known as boundary layer resistance (45). Air moving across the leaf surface reduces the boundary air resistance, thus increasing stomatal evaporation.

The degree of stomatal opening is controlled by many factors such as: light, CO_2, hormones, and tissue WP (2,14,45,49). Stomatal

opening is thought to be mediated by potassium ion (K^+) flux. Organic acid production and dissociation may lead to a movement of K^+ through a hydrogen-K^+ exchange pump (49). When K^+ moves into the guard cells, the osmotic potential becomes more negative than the surrounding cells, causing water to diffuse into the guard cells. The increased turgor due to the diffusion of water causes the stomate to open. When K^+ leaves guard cells, stomates close (2,49).

Light

Stomates are photoreactive in that they usually close in the dark and open in the light. Two phases of stomatal response to light levels have been found in plants: first, a rapid increase in stomatal aperature under low flux densities, and second, a gradual increase with high flux densities (14). Moderate sunlight induces maximum stomatal opening in most temperate plant species (49). Light may influence stomates through organic acid synthesis (49).

Carbon Dioxide

Carbon dioxide levels are important in stomatal opening. Low CO_2 levels can induce stomatal opening even in the dark (49), while CO_2 levels above normal atmospheric levels induce stomatal closing (2,14,49). Maximal stomatal opening corresponds closely to the CO_2 compensation point (2,14). Evidence points to reduced CO_2 levels promoting stomatal opening through an influx of K^+ (2). It is unclear how this is brought about.

Plant Hormones

Plant hormones can influence stomatal opening (2,29,30,41) and, therefore, the water balance of a plant. The two plant hormones with some influence on stomates are abscisic acid (ABA) and cytokinins (29). Ethylene production due to water stress has been postulated (29,49).

Abscisic acid. Endogenous ABA levels rise with water stress (2,14,29), and exogenously applied ABA promotes stomatal closure (36). Therefore, ABA is thought to be important in regulation of water loss. In cut roses, ABA levels rise as senescence, with its concomitant water stress, progresses (40,42).

ABA applied exogenously to a cut rose at 1 ppm reduced water loss and water uptake (30). Since water loss is impeded more than water uptake, an improved water balance and increased vase life result (30). Studies on leafless roses have shown ABA application to accelerate senescence, increase respiration and reducing sugars, and decrease sucrose and protein content of petals (9). ABA application would not slow water loss from leafless flowers as rose flowers have functional stomates only on the leaves (18,41). Thus, ABA appears to have two different roles in cut rose longevity: first, to initiate stomatal closure, thereby slowing water loss, and second, to increase protein degradation in the absence of leaves. The vase life may be lengthened by stomatal closure since water stress increases RNAase activity and protein degradation (29,41). This may be offset in flowers with leaves due to ABA improving the water balance.

Cytokinins. Exogenously applied cytokinins can induce stomatal opening in some plants (29,36). They also increase water uptake in leafless roses which improves the water balance (41). Cytokinin levels decrease with water stress (29,36), which may be a factor in the decline in cytokinin levels in cut roses throughout vase life (42). Applied cytokinins delayed senescence in the short-lived cultivar 'Golden Wave'. The stomata of 'Golden Wave' do not close as completely in water stress situations as the longer-lived cultivar 'Baccara' (43) despite lower endogenous cytokinin levels (39). Thus, cytokinins' role in rose petal senescence may be due primarily to decreasing RNAase activity and protein degradation, and increasing petal growth (41), rather than to changes in stomatal opening.

Leaf Water Potential

Changes in leaf water potential control stomatal opening. A well-hydrated leaf has wider open stomata under constant light and CO_2 conditions than a water stressed leaf (45).

Water Uptake

The maintenance of adequate water uptake in cut roses is of prime importance. Senescence is correlated with a decrease in fresh weight due to water loss exceeding water uptake (1,18,37). There is a decreased hydraulic conductivity in cut rose stems with time after cutting (13,24,25,27,43,57). This is in contrast with the stem of a rose allowed to senesce on the plant which does not show a decrease

in water conductivity (25). Water uptake begins to decline 2-3 days after cutting (25) and is associated with lower transpiration rates and decreasing conductivity of the xylem elements.

Water moves through the stem of a rose in response to the WP gradients established when the leaves transpire. Water loss from the leaves causes the WP to decrease (become more negative) relative to the surrounding tissues. This establishes an energy gradient. The lower water potential is transmitted through the leaf to the stem and ultimately to the vase water as water moves down the energy gradient from tissues with higher water potentials to tissues with lower water potentials. Carpenter and Rasmussen (18) found the removal of leaves on 'Forever Yours' roses decreased water uptake by 78.5% with the rose flower accounting for another 20.4% of the water uptake. In this case, removal of leaves actually improved the water balance and consequently, the vase life due to the amount of water lost being less than the amount of water taken up by the flowers.

Sucrose

Sucrose, a common component in floral preservatives, is effective in prolonging the vase life of cut roses (19,37,38). Sucrose raises the respiration rate significantly (19) and assists in maintaining a good water balance. Sucrose closes stomates and promotes water retention (1,37,38). Due to increased water retention, sucrose treated flowers attain greater fresh weight than flowers held in water, and they maintain a positive water balance for a longer time after cutting (38).

Vascular Blockage

Decreasing conductivity of the xylem elements in cut roses cannot be attributed to a single factor. Rather, there appear to be numerous factors which may act singly or in combination to increase resistance to water flow. Micro-organisms, enzymatic degradation, and contaminants in the vase water all may contribute to loss of xylem element function.

Micro-organisms. Micro-organisms have been implicated in the blockage of xylem elements of cut roses (1,13,24). However, Marousky (37) found that 8-hydroxyquinoline citrate (8-HQC) in sterile conditions improved water conductivity over controls, indicating that vascular blockage by micro-organisms was not the sole reason for decreasing water conductivity. 8-HQC not only improves water balance by closing stomates (37), but it also lowers the pH to 4 (33). Aarts (1) demonstrated that low solution pH increased vase life and Marousky (38) showed that rose stems held in sterile solutions at pH 3 conducted 70% more water than stems held at pH 6 under sterile conditions. These data plus histochemical and microscopic examinations of aging rose stems (25,26,52) support the conclusion that micro-organisms are only one aspect of vascular blockage of cut roses.

Wound response. Physiological response to wounding may reduce hydraulic conductivity in rose stems (17). Wounding can initiate gum formation in xylem elements of woody stems (26), and gum-like substances have been found occluding rose xylem elements (35,50).

Low pH is postulated to decrease enzymatic activity responsible for vascular tissue breakdown and decrease subsequent production of occluding materials (37,38).

Water quality. Water quality affects cut flower longevity. Waters (65) found that increasing salt concentrations in vase water decreased cut flower water uptake. The amount of air dissolved in the vase water also affects longevity (23). Dissolved air blocks xylem elements, causing those elements to become nonfunctional, thereby impeding water uptake. Air also enters cut stems when held out of water. Recutting stems under water to eliminate trapped air may lengthen the vase life of cut flowers (34). Air embolisms do not show up in histochemical and microscopic studies, making it difficult to estimate the extent to which air blockages decrease water uptake.

Measurement of Water Potential

WP being a measurable thermodynamic property reflects the chemical energy of water in a system. WP gradients influence the direction of water flow to a great extent (12,64), and cellular chemical energy influences physiological reactions (12,21). The cellular water potential is determined by a combination of osmotic and matric potentials and turgor pressure. Increased solute concentrations, adsorption to and capillarity between cellular structures lower the free energy of water, thus lowering the WP. Increasing turgor pressure against cell walls increases the free energy of water.

Turgor pressure is most commonly a positive number while osmotic and matric potentials are negative numbers, usually expressed in units of pressure.

Different cellular processes can be influenced by one or more of the components of cellular WP (21), but it is difficult to ascribe specific processes to a specific component of WP (21). WP quantitatively reflects the sum of the chemical energy of plant cells and is a good measure of tissue water deficits (5). Relative water content (5,12,61), isopiestic techniques (5,12,61), measurement of leaf and stem thickness (5,61), pressure chambers (PC) (5,21,59,60,61), and psychrometric techniques (PT) (5,12,61,62) have been used to estimate plant WP. The PC and PT have proven to be useful and flexible methods of measuring WP and its components.

Pressure Chamber

The PC technique suggested by Dixon in 1914 (22), is a method of quantitating shoot water relations. He suggested that application of pressure to a leafy shoot enclosed in a chamber with only the cut end exposed to the atmosphere, would force water out of the cells and out of the cut surface when pressure was equal to the attraction of the water to the cells. Although his premise was sound, CO_2 toxicities at high pressures and two explosions kept Dixon from pursuing the technique. In 1964, Scholander et al. (60) developed a similar PC. Nitrogen gas was used to express xylem sap from leafy shoots. They used the PC to obtain balancing pressures to the pressures on the xylem sap as well as to determine osmotic potential

and turgor pressure (58,59). Tyree and Hammel (63) developed the theoretical basis for the PC further.

Other researchers have since adapted the technique for use on leaves as well as on leafy shoots. The interpretation of the pressures obtained is based on the relationship (11,61):

$$\Psi_w = P + \Psi_{s(xylem)}$$

where

Ψ_w = water potential of leaf cells

P = applied pressure

$\Psi_{s(xylem)}$ = osmotic potential of xylem sap

P and $\Psi_{s(xylem)}$ are the forces which remove water from the leaf cells while Ψ_w is the force of the attraction of the water to the cell. Sap will just begin to be expressed from the cut end of the shoot or leaf when $P = \Psi_w - \Psi_{s(xylem)}$. The osmotic potential of xylem is often ignored in PC determinations as it is usually higher (wetter) than -2 bars (11,12). The balancing pressure is taken to be equal but opposite to the WP.

The method is simple, and the equipment durable making it a suitable field technique. Modifications of this basic procedure have extended the use of the PC to osmotic potential and turgor pressure determinations (20,55,63). The PC technique does have some drawbacks: first, increased pressure and temperature changes within the chamber during pressurization may lyse cells making the technique destructive (51). Secondly, the pressure may force water into

intercellular spaces (11) resulting in spuriously high pressures. The anatomy of the plant part is important in determining the extent to which this occurs (11). Lastly, the PC measurements reflect only the highest potential in the shoot, rather than an average potential (46). It has become standard practice to compare PC with PT determinations on a plant species to evaluate the PC measurements (8,11,55).

Psychrometric Technique

The PT was first introduced by Spanner in 1951 (62). The technique is based on measuring the vapor pressure of an enclosed tissue sample. WP is related to vapor pressure in the following manner (64):

$$\Psi_w = \frac{RT}{\overline{V}} \ln \frac{e}{e_o}$$

where

Ψ_w = water potential

R = universal gas constant

T = temperature

$\overline{V}$ = partial molal volume of water

e = vapor pressure of water at T

e_o = vapor pressure of pure water at T

Thus, the vapor pressure above a solution or tissue is directly related to the WP.

The PT measures vapor pressure with a very fine thermocouple enclosed in a chamber with a plant tissue or solution equilibrated

with the chamber's atmosphere. The Peltier effect is used to cool the thermocouple below the dew point temperature which causes water to condense on the junction. The junction than acts as a very small "wet-bulb" thermometer to the dry thermocouple junction. The small size of the thermocouple causes as little temperature disturbance in the system as possible. This technique requires good temperature control of the system due to the sensitivity of vapor pressure to temperature changes (12). Richards and Ogata introduced a modification of Spanner's technique in 1958 (54). A sample chamber was constructed into which a drop of water was introduced manually, in order to eliminate the need for Peltier cooling. Boyer (10) took this one step further, by using isopiestic solutions in place of the water drop. All three approaches are accurate (5,12,61). The PT by Spanner (62) is in wide usage today and will be discussed in more detail.

In situ dew point hygrometers. The PT has undergone additional modifications in the past twenty years such as that of Neumann and Thurtell in 1972 (48). They adapted a Peltier cooled thermocouple to intact leaves. Previously, tissue discs were enclosed in sample chambers and the WP measured. Excision of tissue causes errors resulting from the solutes released by the cut cells being accumulated by the intact cells. This accumulation lowers the WP of the cells, causing released water to flow down the energy gradient into the intact cells. The WP of the intact cells is changed by the influx of solutes and water (6,7,47).

Other errors of the PT are avoided by using intact leaves. The effect of adsorption of water onto chamber walls is minimized with an intact leaf, as it has a continuous water source to supply water until equilibrium is achieved (8). Baughn and Tanner (8) also suggest that errors arising from heat of respiration (4) are eliminated because the intact leaf is heat-sinked, presumably by the aluminum block surrounding the thermocouple. Both methods are subject to problems of vapor equilibration with leaves whose cuticles effectively prevent water loss by the leaf (8,53). Leaf cuticles can be gently abraded to thin the cuticles (8).

Neumann and Thurtell modified the PT further when they developed the methods for measuring WP of intact leaves (48). Their technique involved measuring the dew point temperature of water vapor. Two thermocouples with a common measuring junction were used. Current passed through one thermocouple lowered the temperature below the dew point by the Peltier effect, and a drop of water condensed on the junction. The cooling current was then decreased until the temperature was reached at which point there was no net evaporation or condensation of water, i.e., the dew point. The temperature change was measured by the second thermocouple. This application of the PT is considered less sensitive to ambient temperature fluctuations than the PT (48) and avoids errors of vapor loss by the wet junction to the atmosphere, by adjusting the water potential of the droplet through temperature changes to that of the sample (48). Evaluation of the <u>in situ</u> dew point hygrometer has shown it to be

precise to 1 bar or better (16) and to agree well with the theory developed for the technique (15).

In situ dew point hygrometers are not destructive which makes them unique among most of the methods of measuring WP. Hygrometers have been kept on leaves for up to 10 days with no discernible injury (66). Disadvantages of the hygrometers compared with a PC include sensitivity to temperature gradients, relatively long equilibration times (up to 8 hours), and elaborate instrumentation. Comparison of the PC to a PT is common with the PT considered the more accurate measurement of tissue WP (8,12,55).

Cut Rose Water Relations

The only study to date which reported rose petal WP was by Mayak et al. (43) on cut and intact roses using an isopiestic method. They found that the WP of rose petals from intact plants did not decrease with flower senescence. In contrast, the WP of cut rose petals dropped significantly immediately before flower senescence. Zieslin et al. (67) demonstrated that water will move from cut rose petals into the stem under water stress conditions. Water stress increases during senescence (43). Water may preferentially move from the petals to the rest of the cut rose as water stress increases.

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SECTION I

WATER RELATIONS IN CUT ROSES: A COMPARISON OF *IN SITU* HYGROMETER AND PRESSURE CHAMBER WATER POTENTIAL TECHNIQUES

WATER RELATIONS IN CUT ROSES: A COMPARISON OF *IN SITU* HYGROMETER AND PRESSURE CHAMBER WATER POTENTIAL TECHNIQUES

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Abstract. Pressure chamber measurement of water potential of cut roses with leaves and/or flowers attached to the stem reflect primarily stem xylem water potentials. However, pressure chamber determinations of leaf water potential were very similar to leaf potentials obtained with *in situ* dew point hygrometers. At water potentials higher than -11.5 bars, the pressure chamber measurements were lower (-1.5 bar maximum) than a hygrometer on the same leaf, and below -11.5 bars the pressure chamber measurements were higher (-1.0 bar maximum) than a hygrometer. Both techniques gave similar results for water potentials of cut rose leaves over time. However, the pressure chamber required hundreds of flowers compared to 8 flowers with *in situ* hygrometers to determine the water status during the vase life of the rose.

In situ hygrometers were used to follow the changes in water potential of rose petals during senescence, and were successful in detecting differences in the water relations of senescing short-lived cv. Golden Wave and long-lived cv. Samantha.

Scanning electron microscope studies of upper leaf surfaces and lower petal surfaces revealed no damage to epidermal cells when gently abraded with carborundum to allow vapor equilibration in the dew point hygrometers. The cuticular waxes appeared to have been altered and redistributed by the abrasion process. The cellular water relations were presumed undisturbed by gentle abrasion.

The study of water relations in cut roses has gained interest in recent years since water stress is considered one of the primary factors leading to senescence in cut roses (1,9,18). Tissue water potential reflects the chemical energy status of water and is a useful

parameter as it affects many plant processes (3,21). A number of techniques have been developed to measure plant water potentials. Two commonly used techniques are the pressure chamber (PC) which uses pressure to express xylem sap (7,19) and the psychrometric technique (PT) which measures vapor pressure of air above an equilibrated sample (20). Vapor pressure is directly related to water potential (21). The PT commonly involves use of tissue discs in a small sample chamber, but may also be used for in situ measurements (17). In situ hygrometers are placed directly on a leaf and either measure temperature differences between wet and dry thermocouple junctions to quantitate water vapor (psychrometric determination) or determine the water vapor content by sensing the dew point of the water vapor (dew point determination). A hygrometer can be used in either a psychrometric or dew point mode (10).

The advantage of the PC is that it is quick and simple to use, durable, and well adapted to field work (6). The disadvantages of the PC are that it is not known how accurately the method reflects plant water potentials without calibration with the PT for a given plant species (6,8,12), and the PC is not suited to measure potentials in plant parts such as flower petals.

The techniques of sensing water vapor pressure in air equilibrated over a tissue are the most accurate methods in determining water potentials (3,8). The disadvantages are that equilibration times are long (up to 8 hrs), and the instruments are sensitive to temperature gradients (3,8) making the PT primarily a laboratory

technique. Maintenance and setup are also more time consuming than the PC. The PT using tissue discs has errors arising from cutting tissue (4,5,16), heat of respiration (2), vapor accumulation on the chamber walls (8), and it is destructive. *In situ* hygrometers avoid these drawbacks by using intact tissues, the leaf being heat-sinked by the aluminum block surrounding the hygrometer (6), and minimizing the effects of water adsorption to the chamber since the continuous water supply to the leaf allows it to lose or gain water until vapor equilibrium is reached (6). The surface area covered by the hygrometer is small, and it is assumed that although the stomates close, the tissue water potential is adequately reflected (8).

This study investigated the validity of using a pressure chamber to measure water potentials of rose leaves and shoots, compared the water potentials of rose leaves determined with *in situ* hygrometers and a pressure chamber, and developed procedures for *in situ* hygrometer measurement of water potential of cut rose leaves and petals.

Materials and Methods

Plant Material

Rosa hybrida L. cvs. Golden Wave and Samantha were grown in the Michigan State University Plant Science Greenhouses under standard cultural practices (14). Roses were harvested between 0800 and 1000 hrs, placed directly in opaque plastic bags, and stored at 3±0.5°C until the following morning. This treatment prevented the

roses from developing bent necks (13). Roses were graded for uniformity and stripped of all leaves except the first five leaflet leaf from the flower. The stems were recut under 18 megohm water to a length of 40 cm and held in continuous light of 300 μE (GE cool white flourescent bulbs and 75 watt incandescent bulbs) at 23±1°C at a relative humidity of 50±5%. Vase life was considered terminated when the rose petals were no longer turgid and felt limp to the touch.

Pressure Chamber Technique

PC determinations were made using a chamber similar to that of Scholander et al. (19). The bottom of the chamber was covered with moist cheesecloth to minimize water loss by the tissue. When only leaves were tested, a small plastic bag filled with more moist cheesecloth was attached to the top of the PC to decrease volume to which water could be lost by the leaf. Rose shoots were cut with a razor blade to 35 cm from the receptacle immediately prior to pressurization. Any cut surface where leaves or flowers had been removed was covered with petroleum jelly to prevent the entry of gas during pressurization. Pressure was applied slowly and the first appearance of xylem sap was considered the end point. PC measurements were taken the same time each day.

Repeated Pressure Chamber Measurements

To verify that repeated PC measurements of the same shoot were destructive, five shoots of each cultivar were tested. A rose

shoot consisted of the stem, the first five leaflet leaf from the flower, and the flower. The water potential of each shoot was determined with the PC 24 hrs after harvest. After the PC measurement, the stems were recut under water and held in continuous light. The same flowers were pressurized daily until each senesced.

Pressure Chamber Measurement of Different Plant Parts

The influence of leaves and flowers on PC water potential measurements of rose shoots was examined. Rose shoots comprised of the stem, the first five leaflet leaf, and the flower were held under the previously described conditions. Daily, some of the rose shoots were pressurized, after which the rose shoots were discarded. Thus, each measurement for a particular combination of flower parts and length of time from cutting was taken on a shoot which had never been pressurized. PC measurements were made on the following combination of plant parts: stem; stem and first five leaflet leaf; stem and flower; stem, flower, and leaf; and leaf. Plant parts were excised immediately prior to pressurization and the nonessential cut surfaces covered with petroleum jelly. Measurement of each shoot was taken daily for 11 days after harvest. Measurements continued on shoots with senesced flowers. Four shoots were tested for each plant part combination each day after cutting.

In Situ Hygrometer Technique

All hygrometer measurements were made using L-51 leaf hygrometers (Wescor, Inc.) on a HR-33T Dew Point Microvoltmeter (Wescor, Inc.).

Readings were taken using the dew point mode (17). The hygrometers were calibrated with NaCl solutions of known water potential, and the cooling coefficient values were corrected for temperature at each reading (17).

The surface of the leaf and petal had to be abraded before the hygrometers would reflect true changes in the internal water status of the tissue. The cuticle was abraded with a cotton swab moistened in a solution of 2% Tween 80 in water, then dipped in 600 grit carborundum. The tissue was gently rubbed in a circular motion with this mixture and washed with deionized water. The top surface of 'Golden Wave' leaves were rubbed 10 times versus 7 times for 'Samantha' leaves due to the heavier cuticle on 'Golden Wave'. The lower surface of the petals of both cultivars was rubbed 4 times. Hygrometers were sealed with petroleum jelly to the upper surface of the terminal leaflet of the first five leaflet leaf or to an outer petal over the abraded portion. The hygrometers were equilibrated for 24 hrs before the first measurement was taken.

One reading was taken daily at a fixed time on any given leaf or petal. Readings were taken for 11 days after harvest with the exception of 'Golden Wave' petals which often had water potentials too negative to be measured 2-3 days after flower sensecence. Dew point readings were discontinued at that time. Hygrometers were removed from the petals or leaves every 4-5 days for cleaning. Cleaning consisted of steaming the thermocouple junction with water vapor for 20 min, then drying with air. The hygrometers were reattached to

newly abraded sections of leaves and petals in positions immediately adjacent to their previous locations. At no time did the leaves appear yellow nor did the petals exhibit cork formation under the hygrometers after 4-5 days.

Comparison of Techniques

PT readings were taken on a 'Samantha' leaf after which the leaf was excised and a PC measurement was taken. This was repeated on 'Samantha' leaves with varying water potentials by removal of the stem from water for varying periods of time or using different aged stems with different water potentials.

Evaluation of Abrasion Damage to the Cuticle

The extent and nature of the damage to the leaf and petal cuticles by abrasion were determined with a scanning electron microscope (Joel JSM-35C). Leaf and petal samples from both cultivars were abraded in the manner described above, immediately quench frozen in liquid nitrogen and freeze dried for two days. The tissue was sputter-coated with a layer of gold 75-100nm thick, mounted, and examined in the microscope. Abraded sections were compared to adjacent nonabraded portions of the same leaf or petal.

Results and Discussion

Repeated pressurization of the same shoot significantly shortened the vase life of both 'Golden Wave' and 'Samantha' flowers (Table 1). Almost all shoots measured repeatedly exhibited neck

TABLE 1. Vase life of flower shoots with a stem, leaf, and flower after daily pressure chamber measurements taken on same shoot, and vase life of roses never pressurized.

Cultivar	Vase Life	
	Consecutive Measurements (days)	No Measurements (days)
'Golden Wave'	2.4	4.1
'Samantha'	3.0	8.3

bending or loss of turgor in the peduncle. In all PT experiments during which hundreds of roses were measured, not one rose exhibited bent neck. In addition, the water potential of roses repeatedly pressurized became much lower (drier) at a faster rate than the water potential of roses pressurized only once. These data reinforce the conclusion that the PC is a destructive technique for measuring water potential, and thereby necessitates a large amount of plant material if water potential changes are to be examined over time.

The results of PC measurements on different combinations of plant parts are given in Figures 1 and 2. The vase life of 'Golden Wave' flowers was 4.1 days while the vase life of 'Samantha' flowers was 8.3 days. (Table 1). PC measurements were taken on both cultivars after their respective vase lives had been reached. Since the determination of vase life was based on loss of petal turgor, measurements of the stem plus flower and stem plus leaf plus flower potentials may be influenced by the senescent flower after the end of vase life. Leaves of both cultivars appeared viable through 11 days.

The influence of the stem on water potential can be seen by comparing the leaf water potentials to the stem plus leaf water potential for both cultivars (Figures 1C,E and 2C,E). The leaf water potential is much lower (drier) than the stem plus leaf potential. In both cultivars, the stem plus leaf potentials closely follow the curves for the stem potentials (Figures 1C,D and 2C,D). This is in agreement with Meiri et al. (15) who concluded that the PC measures the highest water potential in the system, not the average potential of the tissues. The leaf appears to exert a small influence on the

Figure 1. Water potentials of 'Golden Wave' cut rose shoots with increasing time after harvest. Determined with a pressure chamber. Arrows indicate end of vase life. Best fit curves drawn through data points using spline approximation. Each data point is the average of 4 measurements.

A. Stem, leaf, and flower on shoot.

B. Stem and flower on shoot. Leaf excised immediately prior to pressurization.

C. Stem and leaf on shoot. Flower excised immediately prior to pressurization.

D. Stem. FLower and leaf excised immediately prior to pressurization.

E. Leaf. Leaf excised from rose shoot immediately prior to pressurization.

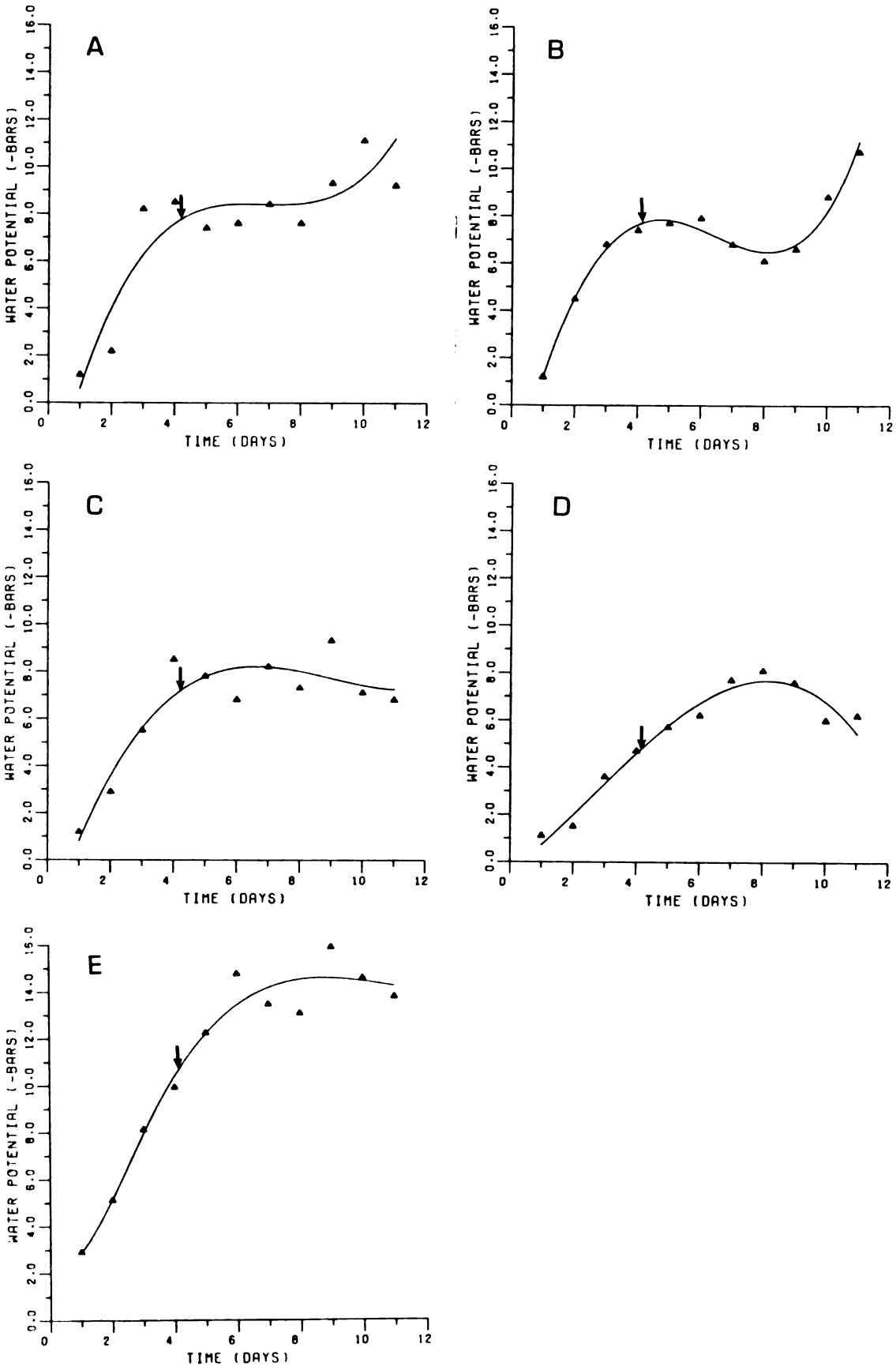

A
B
C
D
E
WATER POTENTIAL (-BARS)
TIME (DAYS)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
0
2
4
6
8
10
12

Figure 2. Water potentials of 'Samantha' cut rose shoots with increasing time after harvest. Determined with a pressure chamber. Arrows indicate end of vase life. Best fit curves drawn through data points using spline approximation. Each data point is the average of 4 measurements.

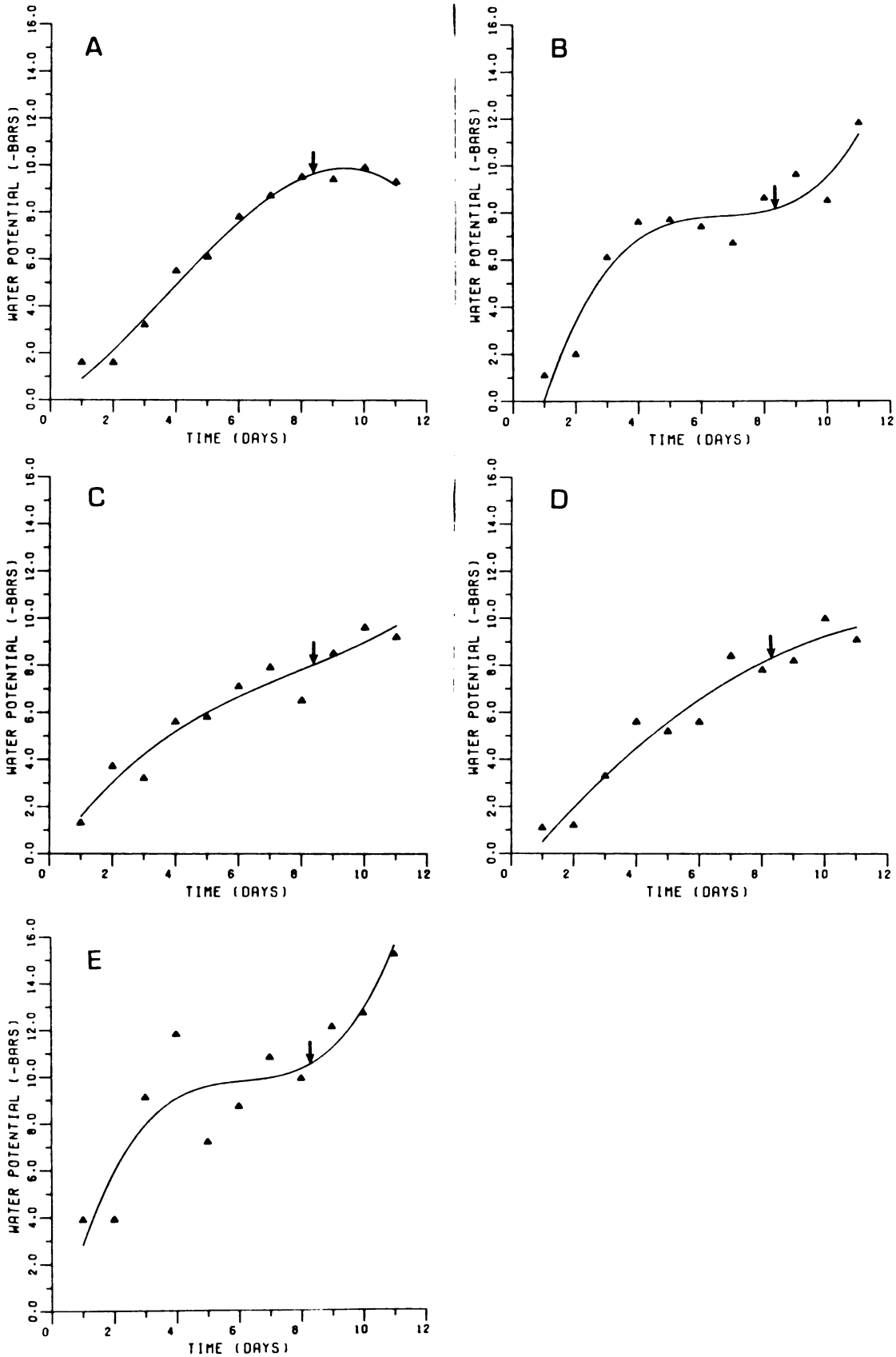
A. Stem, leaf, and flower on shoot.

B. Stem and flower on shoot. Leaf excised immediately prior to pressurization.

C. Stem and leaf on shoot. Flower excised immediately prior to pressurization.

D. Stem. Flower and leaf excised immediately prior to pressurization.

E. Leaf. Leaf excised from rose shoot immediately prior to pressurization.


A
B
C
D
E
WATER POTENTIAL (-BARS)
TIME (DAYS)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
0
2
4
6
8
10
12

stem water potential by lowering it slightly (less than -1.5 bars), but the water potential of the stem and leaf reflects the stem potential much more than the leaf potential. It is also apparent from Figures 1 and 2 that the high stem water potential produces a similar effect when the stem is attached to a flower or leaf and flower until several days after the flower senesces. Thus, the PC has limited usefulness when the water potentials of plant parts such as flowers and petals, which are not readily detached from the stem, are to be measured. When whole shoot PC determinations are made, one can only draw conclusions about changes in the stem water potential, not about the total shoot potential.

The in situ hygrometer technique has the obvious advantage that leaf and petal potentials can be measured directly without the complicating effects of other plant parts, and the same leaf or petal can be monitored throughout the entire vase life. When the technique was first applied to nonabraded rose petals and leaves, the hygrometers did not reflect tissue potential changes. This was shown by small differences in hygrometer readings between hygrometers equilibrated over turgid tissues and readings taken on the same tissue after water had been removed from the vase for a number of hours. Often the leaf or petal was visibly flacid, yet the hygrometer reading was the same as when the tissue was turgid. PC measurements on leaves given identical treatment showed the water potential decreasing significantly as time from water removal increased. The data indicated the cuticle was preventing vapor equilibration between the internal water of the leaf or petal and the hygrometer.

Cuticles were abraded with the carborundum-surfactant mixture, and the minimum amount of abrasion needed to ensure equilibration was determined. With abrasion, water potential changes with dehydration were reflected by the PT, and the leaf potential changes closely paralleled changes measured with the PC (Figure 3). It was of concern that the abrasion process did not damage epidermal cells and disrupt cellular water relations. A scanning electron microscope was used to determine the effect of abrasion on the leaf and petal surfaces.

The irregular wax distribution on the upper surface of a 'Golden Wave' leaf (Figure 4A) is in contrast to the smooth surface of an abraded portion of the same leaf (Figure 4B). The remnants of irregularities in the surface waxes can be seen in the upper right corner of Figure 4B. The rest of Figure 4B shows smoothing or removal of cuticular waxes from the surface of the epidermal cells. On smooth areas, it is impossible to determine if the surface waxes have been entirely removed, or if heat of friction during abrasion melted or deformed the waxes and eliminated the irregular wax distribution. If melting occurred, the result would be a more uniform, thinner coating of wax on the cell surfaces. Figure 4C demonstrates that surface waxes are physically moved by abrasion, that a smooth epidermal surface underlies the waxes, and that the movement of the waxes does not damage the cells.

Scanning electron micrographs of 'Samantha' leaves (Figure 5A,B,C) provide evidence for both wax movement and wax melting.

Figure 3. Comparison of *in situ* hygrometer water potential with the pressure chamber potential for 'Samantha' rose leaves. Pressure chamber values were not corrected for xylem sap potential. r = .9449. Slashed line represents equipotential values.

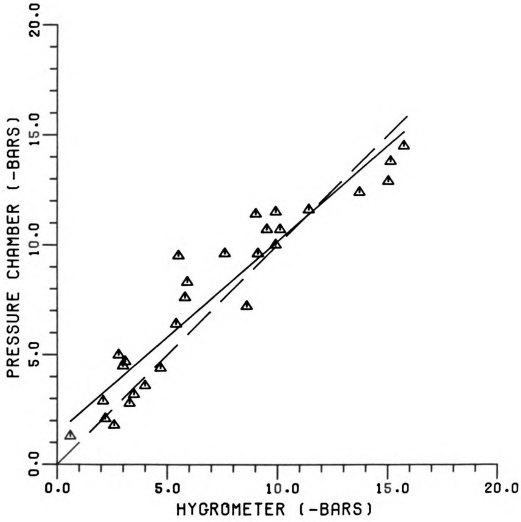

PRESSURE CHAMBER (-BARS)
HYGROMETER (-BARS)
0.0
5.0
10.0
15.0
20.0

Figure 4. Scanning electron micrographs of freeze dried 'Golden Wave' rose leaf, top surface.

A. Nonabraded surface, X2400. Bar equals 10μ.

B. Abraded surface, X2400. Bar equals 10μ.

C. Cuticular wax movement and distortion due to abrasion, X240. Bar equals 100μ.

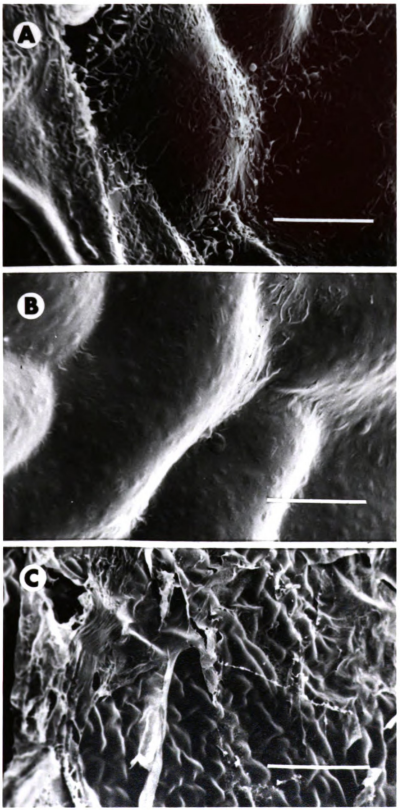

A
B
C

Figure 5. Scanning electron micrographs of freeze dried 'Samantha' rose leaf, top surface.

A. Nonabraded surface, X240. Bar equals 100μ.

B. Cuticular wax removal and displacement due to abrasion, X240. Bar equals 100μ.

C. Melted or deformed cuticular wax on abraded leaf, X2400. Bar equals 10μ.

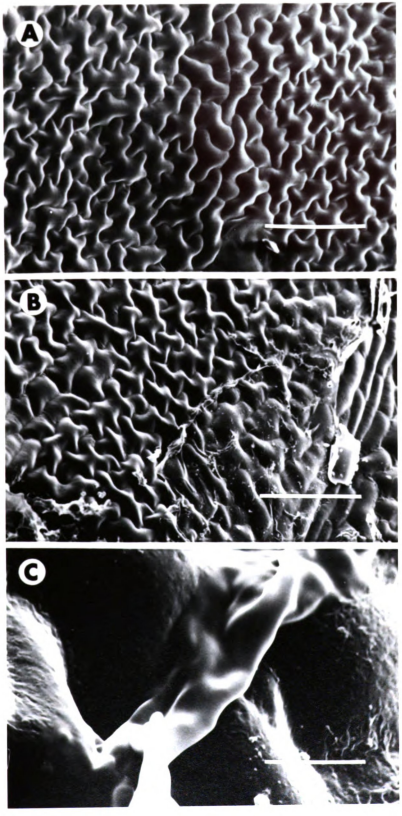

A
B
C

Figure 5A shows the regular surface of nonabraded 'Samantha' leaves. Abrasion resulted in removal and displacement of the thin layer of surface waxes (Figure 5B). Again, the underlying cells appear undamaged by abrasion. Good evidence for abrasion creating enough heat of friction to melt or deform surface waxes appears in Figure 5C. The wax appears to have melted and to have continuity with the remaining cuticlar waxes. Note that some of the original wax deposition in the lower right corner remains undisturbed, which is further support for the nondestructiveness of the technique to epidermal cells.

Originally, the upper surface of the rose petals was abraded in preparation for the hygrometers. However, after viewing the cellular collapse caused by abrasion (Figures 6A,B) the smoother lower petal surface was abraded and examined. The lower petal surface of 'Golden Wave' and 'Samantha' showed no evidence of cellular disruption by abrasion (Figures 7B,8B,9C).

Note the smoother, more even appearance in the surface waxes of an abraded 'Golden Wave' petal in Figure 7B compared to the same petal in a nonabraded section (Figure 7B). The waxes appeared to have been melted or deformed by abrasion. Figure 8A illustrates the general lower surface morphology of a nonabraded 'Golden Wave' petal. Figure 8B from the same petal shows the same general wax structure, but it also shows a portion which appears to be melted, and an accumulation of wax. "Melted" sections and wax accumulations of the type in Figure 8B were not found on nonabraded petals.

Figure 6. Scanning electron micrographs of freeze dried rose petals, top surface.

A. Cellular collapse due to gentle abrasion of 'Golden Wave' petal, X1000. Bar equals 10μ.

B. Cellular collapse due to gentle abrasion of 'Samantha' petal, X1000. Bar equals 10μ.

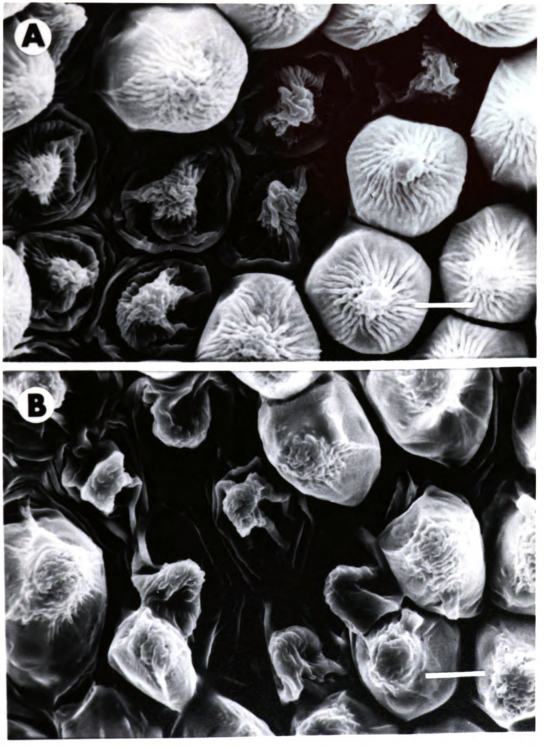

A
B

Figure 7. Scanning electron micrographs of freeze dried 'Golden Wave' rose petal, lower surface.

A. Nonabraded surface, X600. Bar equals 1μ.

B. "Melted" cuticular waxes due to abrasion, X600. Bar equals 1μ.

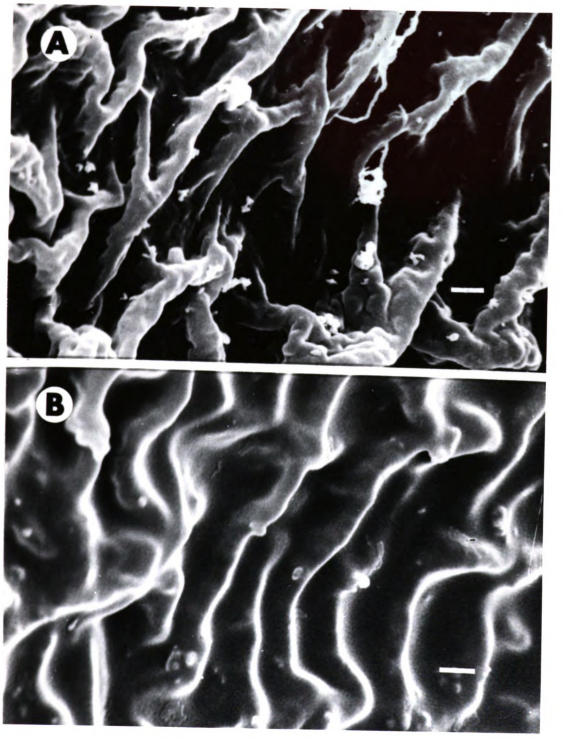
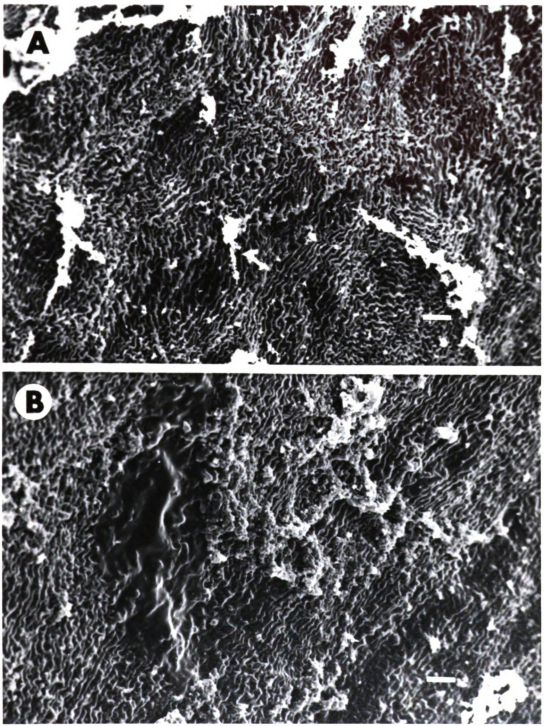

A
B

Figure 8. Scanning electron micrographs of freeze dried 'Golden Wave' rose petal, lower surface.

A. Nonabraded surface, X480. Bar equals 10μ.

B. Localized melting due to abrasion, X480. Bar equals 10μ.


A
B

Abrasion of 'Samantha' petals produced similar effects. Figure 9A shows cuticular waxes on a nonabraded lower petal surface, and Figure 9B shows cuticular waxes from an abraded portion of the same petal. Some melting of wax due to abrasion can be postulated from the continuous extensions on the wax surface of Figure 9B. The underlying wax seems to be essentially undisturbed by abrasion in this case. Figure 9C illustrates accumulated wax whose lower periphery appears to be melted onto the surface wax. The particle in the center also seems to be embedded in melted wax. The particle in Figure 9D is clearly embedded in melted wax (right side) while the underlying surface is undisturbed.

Gentle abrasion of the upper leaf surface and lower petal surface of 'Golden Wave' and 'Samantha' roses appeared to do little damage to epidermal cells, while thinning cuticular waxes sufficiently to allow vapor equibration with hygrometers. Abrasion of both leaves and petals appears to thin the cuticular waxes in two ways. First, surface waxes may be moved on the epidermal surface, exposing epidermal cells without cuticular waxes in some areas, and secondly, abrasion appears to thin the waxes by melting or deforming them, causing a more even wax distribution. The occasional holes in the melted wax provide additional pathways for vapor movement.

The PC and PT gave similar results when used on the same leaves (Figure 3). The PC measured lower water potentials than the leaf hygrometer until -11.5 bars was reached. At water potentials lower than -11.5 bars, the hygrometers measured water potentials

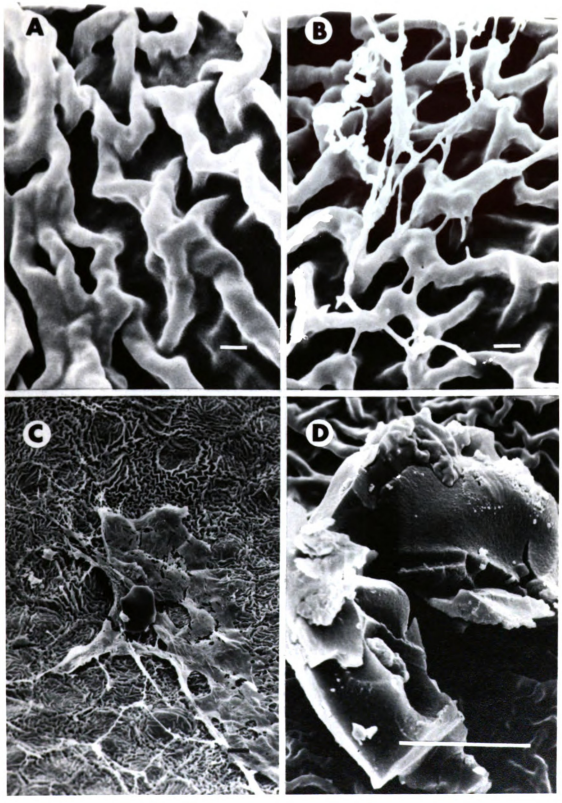
Figure 9. Scanning electron micrographs of freeze dried 'Samantha' rose petal, lower surface.

A. Nonabraded surface, X6000. Bar equals 1μ.

B. Cuticular wax melting or deformation and redistribution due to abrasion, X6000. Bar equals 1μ.

C. Cuticular wax redistribution, X480. Bar equals 10μ.

D. Particle embedded in melted wax on abraded surface, X3000. Bar equals 10μ.


A
B
C
D

lower than the pressure chamber. At the higher potentials, the difference between the two techniques was a maximum of -1.5 bars, and at the lower potentials, the difference was a maximum of -1.0 bar. Similar agreement between PC and PT was found by Baughn and Tanner (6) using 5 herbaceous species. The similarity between two approaches for measuring water potential, which contain entirely different sources of error, leads to the conclusion that both techniques adequately measure leaf potentials.

Additional evidence that the two techniques are monitoring the same potentials is seen in Table 2. Leaf water potentials of 'Golden Wave' and 'Samantha' were determined independently using the PC and the PT. The water potentials of the leaves using the two methods were very similar and were not statistically significant at the 10% level.

From the comparisons of the PC with the PT, it was concluded that the hygrometers adequately reflected changes in the water status of cut rose leaves. Hygrometers were then used to monitor the changes in water status of outer rose petals concurrent with the monitoring of leaf water changes (11, Section II). Differences between the water potentials of leaves and petals were clearly seen, as were marked differences between the water potentials of the petals of the two cultivars. Differences in petal potentials were suspected based on the cultivars' vase lives, and the hygrometers confirmed the existence of differences. Thus, the <u>in situ</u> hygrometers can be used successfully on both leaves and petals of cut roses.

TABLE 2. Water potential of rose leaves determined with pressure chamber and *in situ* dew point hygrometers. Different leaves were used for each pressure chamber measurement. The same four leaves were used for dew point measurements. Pressure chamber and hygrometer determinations were not significantly different at the 10% level.

Cultivar	Time after Harvest (Days)	Water Potential (-bars)[x]	
		Pressure Chamber	Hygrometer
'Golden Wave'	1	2.9	1.2
	5	12.2	12.6
	10	14.5	14.7
'Samantha'	1	3.9	3.4
	5	7.2	10.1
	10	12.7	11.5

[x]Average of 4 water potential determinations.

This study has shown that the PC technique adequately reflects the water potentials of rose leaves, but only reflects stem xylem potentials when used to evaluate water potential changes of leaves or flowers attached to the stem. In situ hygrometers used in the dew point mode also reflect internal water changes of rose leaves and petals. Abrasion of the cuticle of leaves and petals appears to melt or deform and redistribute cuticular waxes with little damage to underlying cells.

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LITERATURE CITED

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SECTION II

WATER RELATIONS IN 'SAMANTHA' AND 'GOLDEN WAVE' CUT ROSES

WATER RELATIONS OF 'SAMANTHA' AND 'GOLDEN WAVE' CUT ROSES

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Abstract. Short-lived 'Golden Wave' (4 days) and long-lived 'Samantha' (8 days) cut roses senesced when fresh weight declined below initial weight. Due to 81% greater leaf areas 'Golden Wave' leaves lost more water per day despite more tightly closed stomates. Hydraulic conductivities of both cultivars decreased with time, but 'Golden Wave' had slightly more resistance to water flow than 'Samantha' resulting in a larger water deficit in 'Golden Wave' flowers.

Leaf and petal water potentials in both cultivars declined with time from cutting. 'Golden Wave' petal potential declined more rapidly than 'Samantha'. At senescence, 'Samantha' petals had a lower water potential, indicating different mechanisms in the cultivars for maintianing turgor. Petal potentials were higher than leaf potentials until senescence. Leaf potentials in both cultivars declined at a similar rate and then plateaued. The differences in vase life between cultivars appeared due to the larger water deficit of cut 'Golden Wave' flowers.

The water balance of cut roses is a primary factor determining flower longevity (1,2,16). An increase in stem resistance to water flow in cut roses has been reported (2,5,6). Micro-organisms (1,10), gum formation (10,15), and unknown physiological processes (5,6) have been postulated to increase stem resistance. Increased resistance to water flow reduces water uptake in response to transpirational water losses, and induces water stress in the cut rose. Water stress has been associated with hormonal changes (7,11), protein degradation (7,11,14) and decreased cellular RNA levels (7,11,14). These factors may be associated with rose senescence.

Water potential is a measure of the energy status of water and has been used to quantify physiological responses to water stress. Water energy status can have a direct influence on cellular processes (7). Different species and cultivars respond differently to water deficits (8,17). Some stress resistant species such as sorghum undergo an active lowering of osmotic potential in response to water stress which lowers tissue water potential (8). Other species such as corn have rigid cell walls which cause low potentials to be reached in response to small water losses (17). Thus, mechanisms exist in plants to adjust cellular water potentials for more favorable competition for available water.

The objective of this investigation was to study the water potential changes of rose petals and leaves of a short-lived and a long-lived rose cultivar in relation to water uptake, loss, and conductivity during senescence. In situ dew point hygrometers were used to monitor water potential of the same cut roses throughout senescence.

Materials and Methods

Plant Material

Plant material, cultural practices, harvesting procedures, and postharvest conditions were as described previously (4, Section I). Vase life was considered terminated when the rose petals were no longer turgid and felt limp to the touch.

Water Uptake and Loss

'Golden Wave' and 'Samantha' flowers were placed singly in sterilized tubes with 18 megohm water and the tubes sealed with parafilm to prevent evaporation. The weight of the tube, water, and flower was taken daily as well as the weight of the tube and water. Water uptake was determined by subtracting the weight of the tube and water from a similar determination the previous day. Daily water loss was determined by subtracting the weight of the tube, water, and flower from a similar determination the previous day. Water loss should approximate transpiration (13). Daily fresh weight of the flower was obtained by subtracting the weight of the tube and water from the weight of the tube, water, and flower. Water uptake, loss, and flower fresh weight were determined on 6 flowers of each cultivar with 2 replications for a total of 12 flowers.

Diffusive Resistance

The diffusive resistance of the terminal leaflet of the first five leaflet leaf was measured daily with a diffusive resistance meter (Li-cor Lamda Model LI-60). The resistance to vapor diffusion was timed and corrected for temperature differences from 25°C. Calibration curves were determined using a calibration plate with known diffusive resistances (9). Diffusive resistance was taken for 11 days after the flowers were brought to the laboratory. Six flowers per cultivar were used in each of 2 replications.

Leaf Area and Stomatal Density

The average leaf area was determined using 13 first five leaflet leaves from the flower of each cultivar. The leaves were Xeroxed and the copy cut out and weighed to determine leaf area.

Stomatal densities and lengths were determined on the lower surface of 'Golden Wave' and 'Samantha' leaves with a microprobe (Applied Research Laboratories) using the secondary electron detector. Samples were quench frozen in liquid nitrogen, freeze dried, sputter-coated with gold (75-100nm thick), and observed in the microprobe.

Hydraulic Conductivity

Hydraulic conductivity of cut roses was calculated in the manner of van den Honert (18). Hydraulic conductivity is the reciprocal of hydraulic resistance which is calculated as follows under steady-state conditions:

$$T = \frac{-(\text{leaf-soil})}{r_p}$$

where

T = transpiration rate

leaf, soil = water potential of leaf and soil, respectively

r_p = plant hydraulic resistance

Hydraulic conductivity (L_p) then becomes:

$$L_p = -\frac{T}{\text{leaf-soil}}$$

Transpiration was calculated as:

$$T = \frac{\text{water loss/unit time}}{\text{leaf area}}$$

Leaf water potential values were the average of leaf potentials determined with a pressure chamber and leaf hygrometers as the two techniques did not give significantly different results (4, Section I). Soil water potential corresponds to the potential of the vase water which was assumed to be 0.0 bars since 18 megohm water was used. The roses were determined to be under steady state transpirational conditions when transpiration and water potential were measured.

Water Potential

Water potential was determined on 'Golden Wave' and 'Samantha' outer petals by *in situ* dew point hygrometers and on terminal leaflets of first five leaflet leaves by *in situ* dew point hygrometers and a pressure chamber. Both techniques were used as previously described (4, Section I).

Results

Water Uptake and Loss

'Golden Wave' and 'Samantha' had similar patterns of water uptake and loss (Figures 1 and 2). Both cultivars had maximum water uptake and losses on the second day on the laboratory bench. After the second day, rates of water uptake and loss steadily decreased. Water loss exceeded uptake in both cultivars on the third day of

Figure 1. Water uptake on a gram/day basis of 'Golden Wave' and 'Samantha' rose flower shoots. Vase life 'Golden Wave' 4.1 days, 'Samantha' 8.4 days. Each data point is an average of 12 measurements.

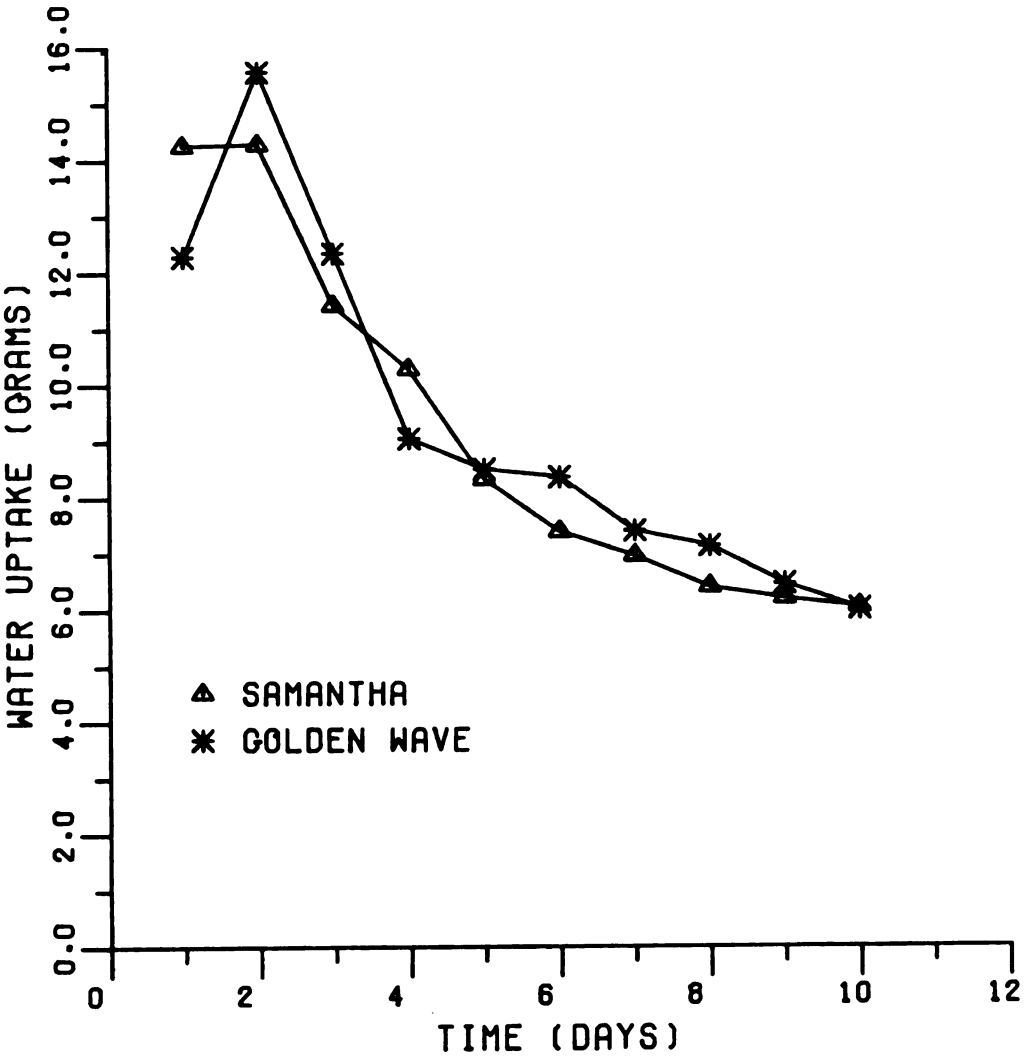
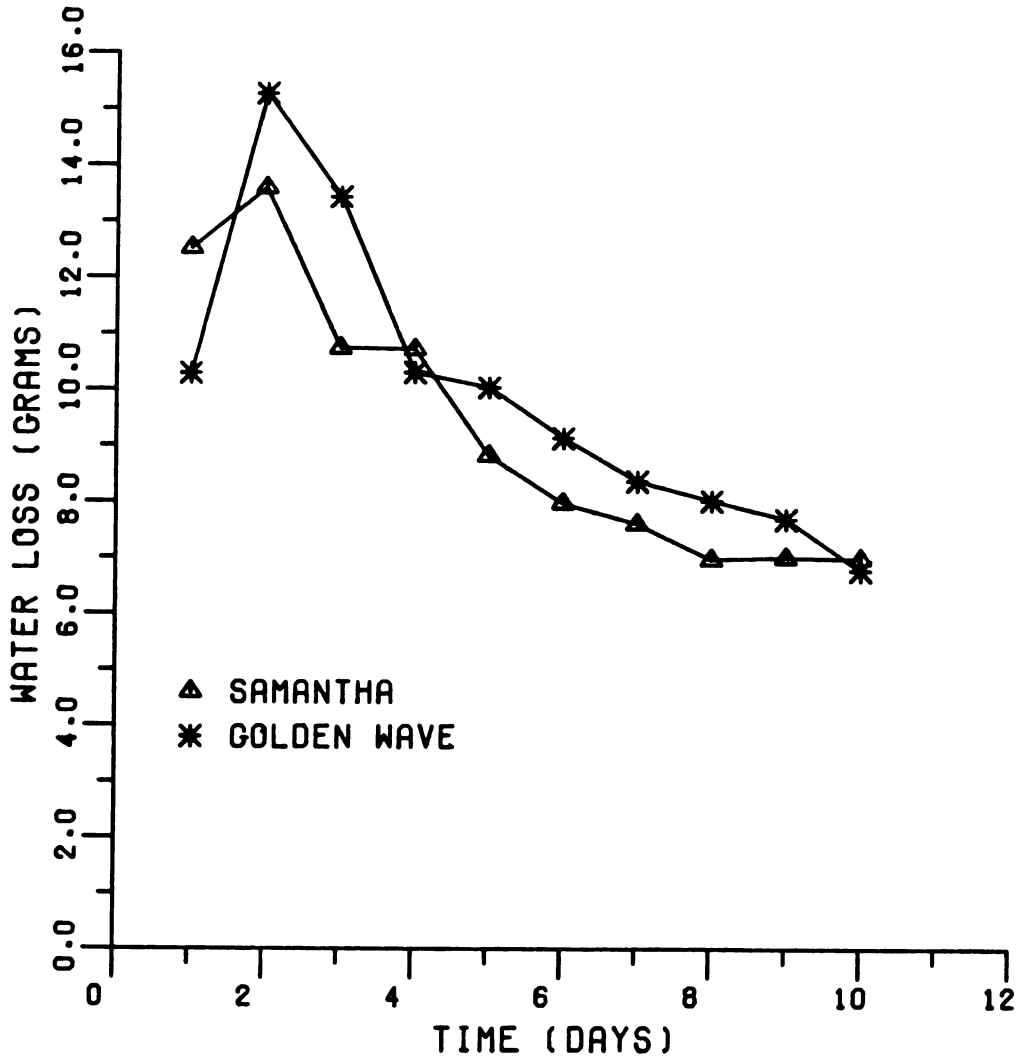

WATER UPTAKE (GRAMS)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
0
2
4
6
8
10
12
TIME (DAYS)
SAMANTHA
GOLDEN WAVE

Figure 2. Water loss on a gram/day basis of 'Golden Wave' and 'Samantha' rose flower shoots. Vase life 'Golden Wave' 4.1 days, 'Samantha' 8.4 days. Each data point is an average of 12 measurements.


WATER LOSS (GRAMS)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
0
2
4
6
8
10
12
TIME (DAYS)
SAMANTHA
GOLDEN WAVE

vase life and continued to exceed daily uptake for the duration of vase life. 'Golden Wave' flowers had slightly higher water losses than 'Samantha' flowers throughout the experiments. 'Golden Wave' flowers lost more weight than 'Samantha' flowers on a day-to-day basis after day 2 (Figure 3).

Diffusive Resistance

'Golden Wave' and 'Samantha' leaves exhibited different diffusive resistance patterns over time (Figure 4). The cultivar interaction with time in vase was significant at the 10% level. 'Golden Wave' leaves had higher diffusive resistance than 'Samantha' leaves until day 6, after which 'Golden Wave' resistance leveled off while 'Samantha' continued to increase. Diffusive resistance plotted against leaf water potential (Figure 5) show 'Samantha' resistance increased with decreasing water potential, while in 'Golden Wave', diffusive resistance increased until water potential reached -9.5 bars but did not increase further despite lower water potentials. 'Golden Wave' resistance was higher than 'Samantha' at water potentials higher (wetter) than -11.0 bars. The stomates of both 'Golden Wave' and 'Samantha' closed in response to increasing water stress during vase life.

Leaf Area

Leaf area and stomatal densities are given in Table 1. 'Golden Wave' leaves were 81% larger than 'Samantha' leaves. There were more stomates per unit area on 'Golden Wave' leaves, but they were shorter than 'Samantha' stomates.

Figure 3. Fresh weight of 'Golden Wave' and 'Samantha' rose flower shoots. Arrows indicate end of vase life, and slashed line indicates initial fresh weight. Each data point is an average of 12 measurements.

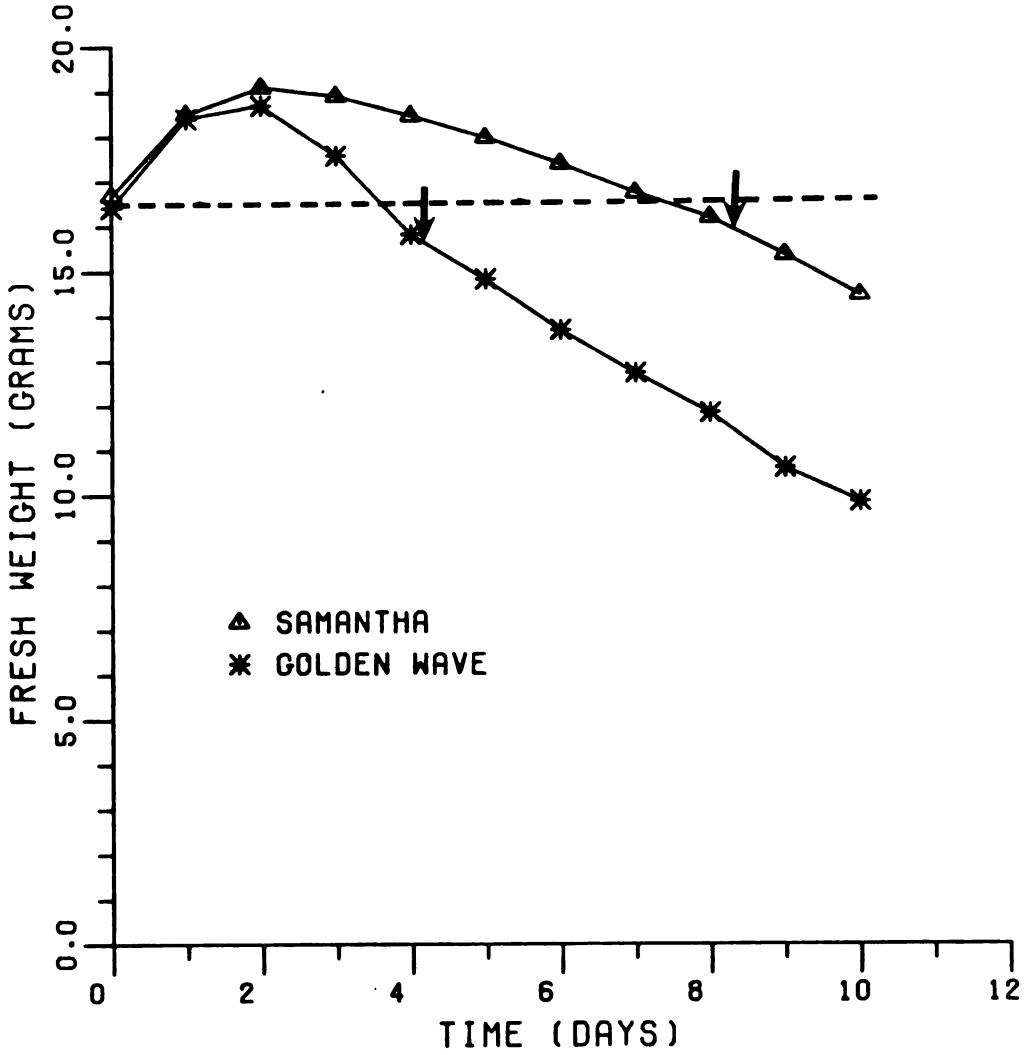

FRESH WEIGHT (GRAMS)
20.0
15.0
10.0
5.0
0.0
0
2
4
6
8
10
12
TIME (DAYS)
SAMANTHA
GOLDEN WAVE

Figure 4. Diffusive resistance of 'Golden Wave' and 'Samantha' rose terminal leaflets of the first five leaflet leaf from the flower. Arrows indicate end of vase life. Best fit curves drawn using spline approximation. Each data point is an average of 12 measurements.

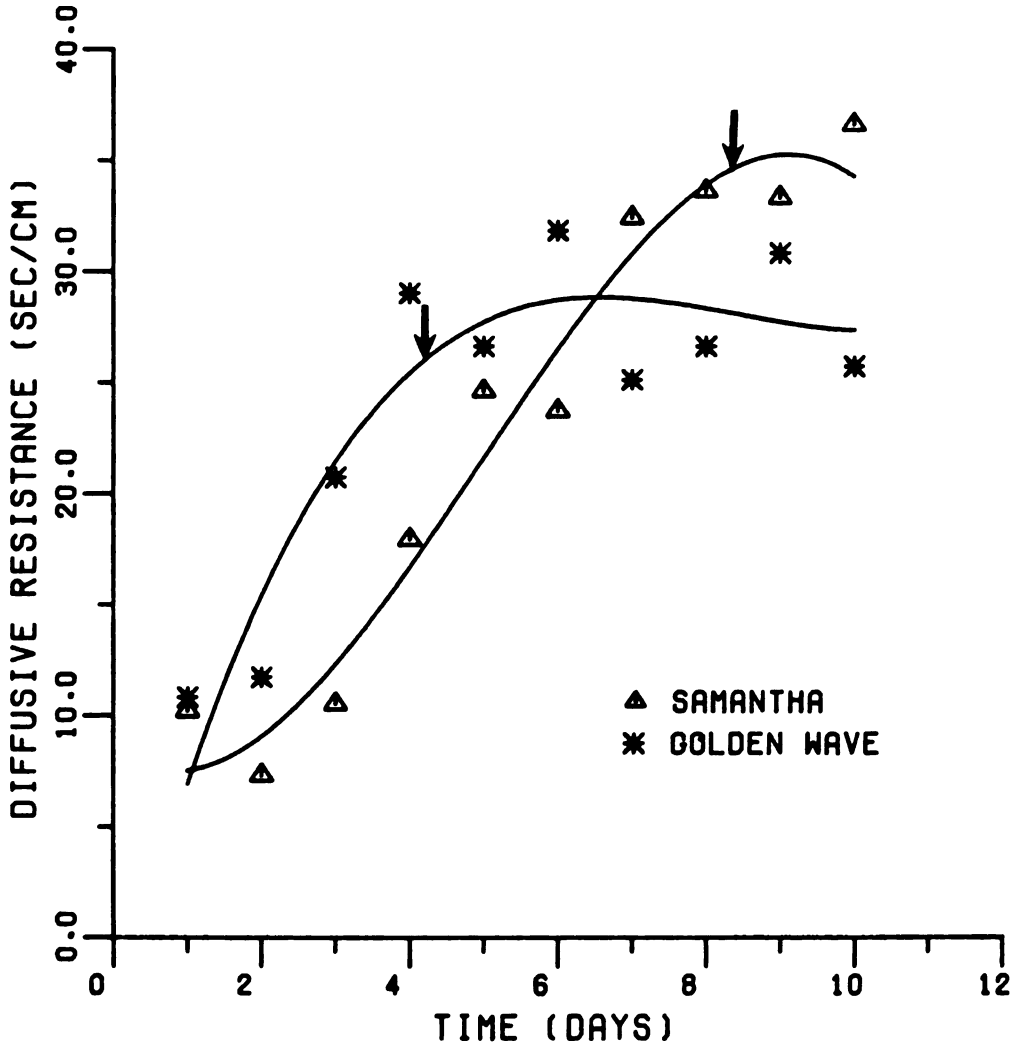

DIFFUSIVE RESISTANCE (SEC/CM)
40.0
30.0
20.0
10.0
0.0
0
2
4
6
8
10
12
TIME (DAYS)
SAMANTHA
GOLDEN WAVE

Figure 5. Diffusive resistance of 'Golden Wave' and 'Samantha' rose terminal leaflets of the first five leaflet leaf plotted against water potential of the first five leaflet leaf. Water potentials determined with pressure chamber and *in situ* hygrometer (4). Best fit curves drawn using spline approximation.

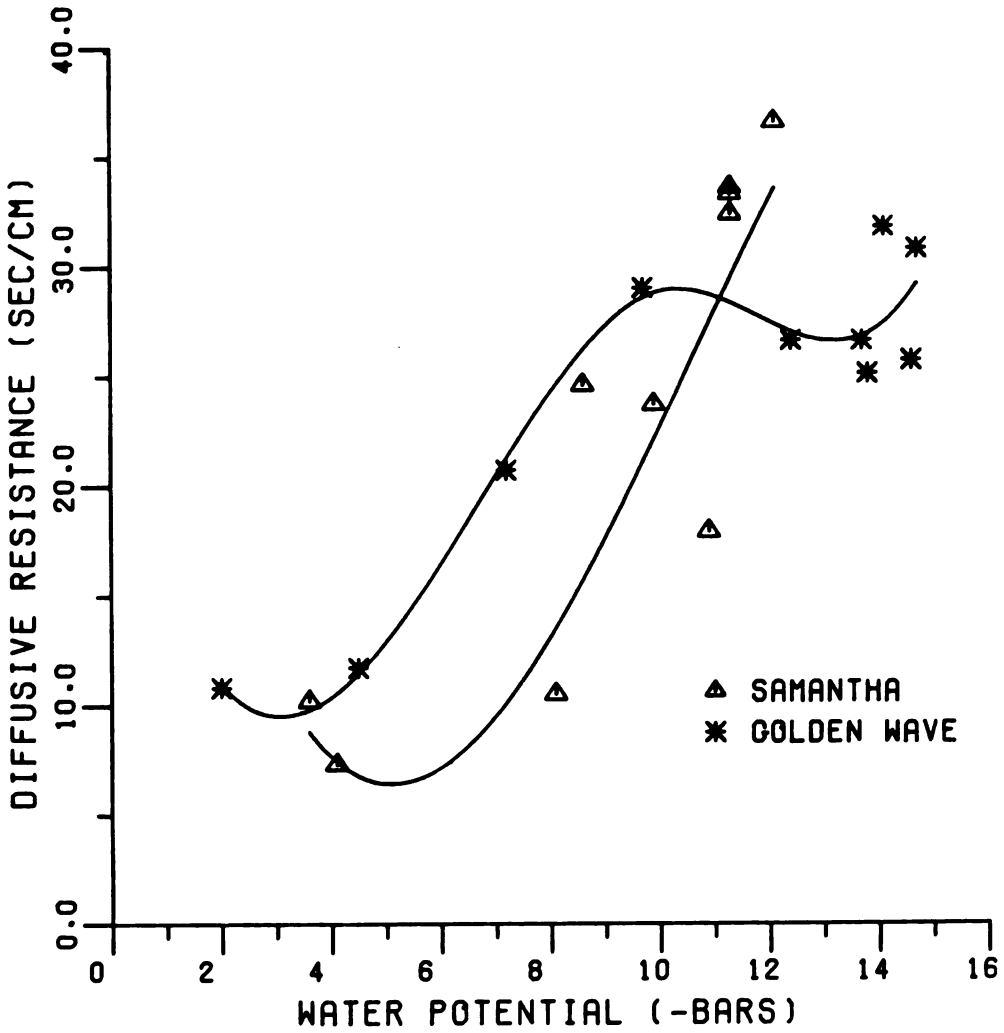

DIFFUSIVE RESISTANCE (SEC/CM)
40.0
30.0
20.0
10.0
0.0
0
2
4
6
8
10
12
14
16
WATER POTENTIAL (-BARS)
SAMANTHA
GOLDEN WAVE

Table 1. Leaf area, number of stomates, and stomatal length of 'Golden Wave' and 'Samantha' rose first five leaflet leaves.

	Leaf Area (cm^2)	No. Stomates per mm^2	Stomatal Length (μ)
'Golden Wave'	124.8	81	20
'Samantha'	69.0	67	25

Hydraulic Conductivity

The hydraulic conductivity of 'Golden Wave' and 'Samantha' flowers (Figure 6) decreased with time. The conductivity of 'Samantha' was higher than 'Golden Wave' at all times.

Water Potential

Water potential of cut rose leaves and petals decreased with time after harvest (Figures 7A,B). 'Golden Wave' and 'Samantha' petal potentials were similar through day 3. Four days was the average vase life of 'Golden Wave', and on day 4, 'Golden Wave' petal potential was lower (drier) than 'Samantha'. 'Samantha' flowers maintained a higher water potential one day longer than 'Golden Wave', and senesced at a lower potential.

Petals did not compete for available water as effectively as leaves as water stress increased. 'Golden Wave' had a larger fresh weight decline than 'Samantha' by day 4, yet leaf potentials were similar while 'Golden Wave' petal potential declined more than 'Samantha' (Figures 7A,B). 'Samantha' petal potential decreased as more fresh weight was lost, while leaf potential plateaued after 6 days (Figure 7B) despite further fresh weight losses.

Leaf water potentials decreased with time in both cultivars. Both began to plateau on day 6, but 'Golden Wave' leaves were at a lower potential than 'Samantha' leaves. This difference is consistent with less water available in 'Golden Wave' flowers. The differences in potentials were significant at the 5% level.

Figure 6. Calculated hydraulic conductivity of 'Golden Wave' and 'Samantha' rose flower shoots. Arrows indicate end of vase life.

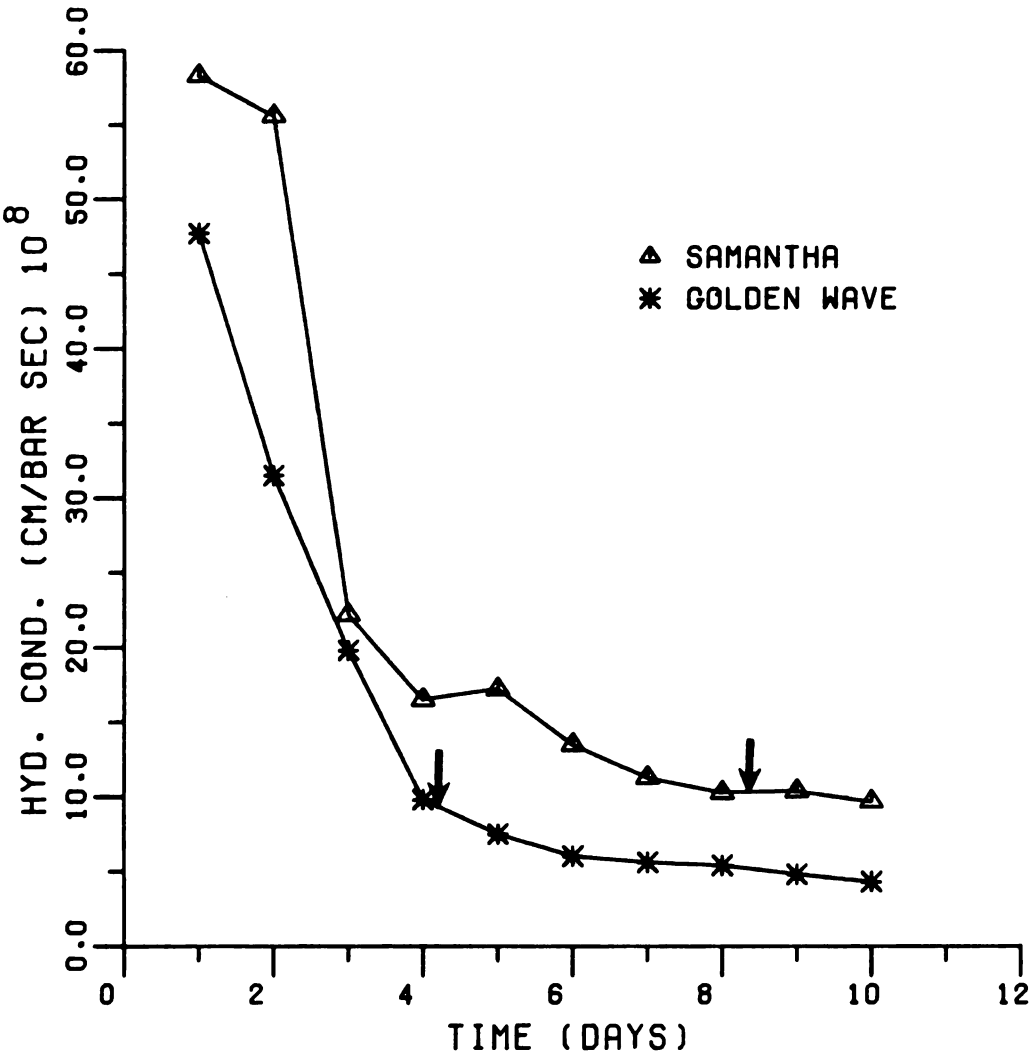
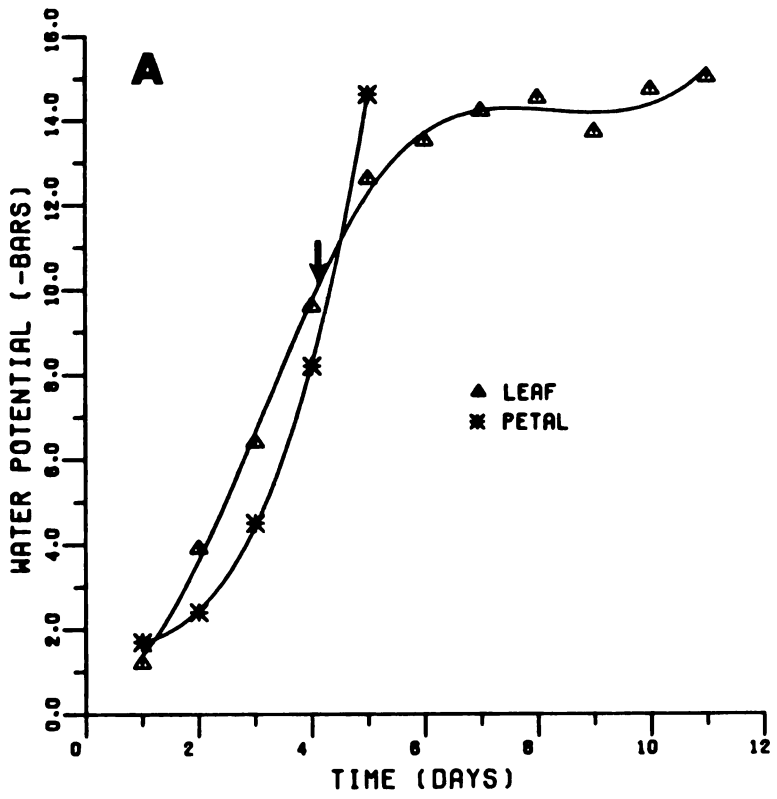
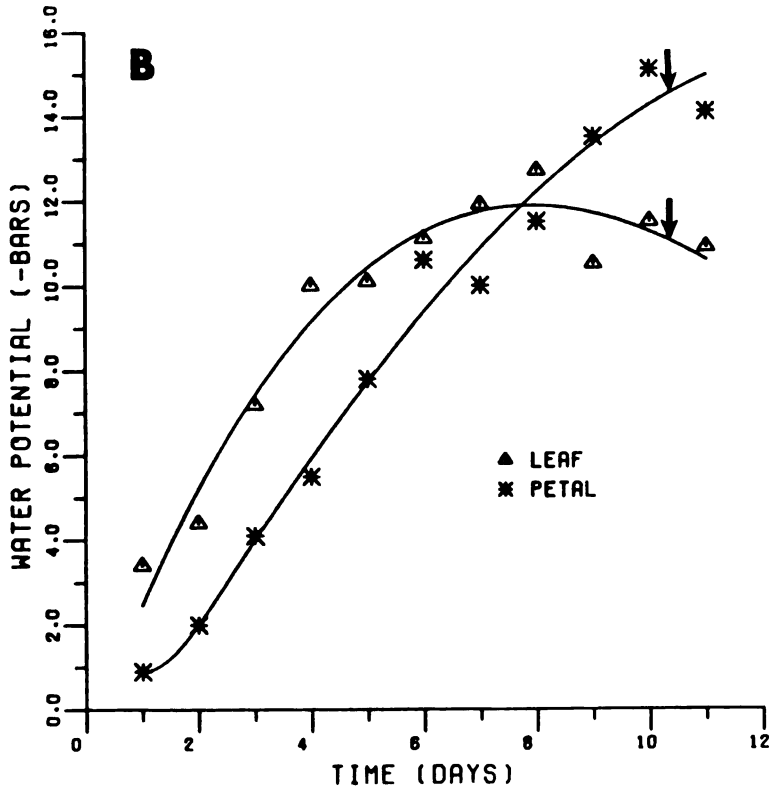

HYD. COND. (CM/BAR SEC) 10^8
0.0
10.0
20.0
30.0
40.0
50.0
60.0
0
2
4
6
8
10
12
TIME (DAYS)
SAMANTHA
GOLDEN WAVE

Figure 7. Water potential of rose leaves and petals determined with *in situ* dew point hygrometers. Measurements were taken on the same four flowers daily. Arrows indicate end of vase life. Best fit curves drawn using spline approximation.

A. 'Golden Wave'.

B. 'Samantha'. Vase life was two days longer than 'Samantha' control flowers, because flowers with no damage to the leaves or petals had to be selected in order to use the hygrometers.


A
WATER POTENTIAL (-BARS)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
0
2
4
6
8
10
12
TIME (DAYS)
LEAF
PETAL


B
WATER POTENTIAL (-BARS)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
0
2
4
6
8
10
12
TIME (DAYS)
LEAF
PETAL

Discussion

Short-lived 'Golden Wave' and long-lived 'Samantha' cut roses undergo decreased hydraulic conductivities after harvest. The inability to replace transpirational water loss causes changes in rose water relations during senescence. Similar changes were seen in both cultivars during senescence; however, the slightly larger water loss (Figure 2) and hydraulic resistance (Figure 6) of 'Golden Wave' led to a more rapid development of fresh weight deficits and decreased tissue water potentials.

Both cultivars appeared senesced when fresh weight dropped below initial fresh weight (Figure 3). This agrees with earlier work that roses senesce when fresh weight drops 5-10% below the original weight (3). Other researchers have found a similar correlation between fresh weight maintenance and flower longevity (1,12).

Stomatal closure minimized water loss as water stress increased during the vase life of both cultivars. Thus, differences in stomatal response were not the basis of differences in vase life. Stomates of 'Golden Wave' closed more tightly in response to a given water potential than 'Samantha'. The increased diffusive resistance of 'Golden Wave' was offset by an 81% larger leaf area. As a result, total water losses per flower were similar between cultivars.

As water stress increased, water potential decreased in leaves and petals of both cultivars (Figure 7A,B). The roses senesced at approximately the same leaf potentials. Leaf potentials of both cultivars became relatively constant after 6 days due to

essentially closed stomates (Figure 4) and a leveling off in hydraulic conductivities (Figure 6). Before day 5 or 6, hydraulic conductivities decreased very dramatically, and the stomates were open, thereby causing increasing water deficits and decreasing tissue water potentials.

When water was limiting in the cut roses, the petals did not compete successfully with the leaves despite lower petal potential than leaf potential in 'Samantha' one day prior to senescence. Hydraulic conductivity calculations reflect the sum of the resistances between the water supply and the leaf, and do not indicate the magnitude of conductivities in the plant parts. It is possible that conductivity to water flow decreased more in the vascular system to the flower than to the leaf. There are no reports in the literature on receptacle conductivity changes in cut roses. Stem conductivity is known to decrease with time from harvest (2,5,6,10,13). However, conductivity might be influenced by the location in the stem. There are no conductivity studies reported in terms of differences in hydraulic conductivity in stem segments between the water supply and the leaf and segments between the leaf and the flower. Decreased hydraulic conductivity due to tissue degeneration or gum formation in the stem section immediately below the flower, receptable, or petal tissues would result in decreased water flow to the petals. Maintenance of cellular integrity in the stem, receptacle, or petal may be genetically controlled.

'Samantha' petals senesced at lower water potentials than 'Golden Wave'. 'Samantha' petals may maintain turgor at a lower

water potential than 'Golden Wave' through accumulation of solutes which lowers the osmotic potential causing an increase in the water potential gradient between the vase water and the petal while still maintaining the same turgor. Since water in plant systems flows from high water potential to low water potential, an increase in potential gradient would make the petal more competitive for available water. Differences in the cell wall elasticity of 'Golden Wave' and 'Samantha' petals could exist which would influence the magnitude of turgor pressure and the water potential change as water is lost from a cell.

The difference in vase life between cut 'Golden Wave' and 'Samantha' roses seems to be based on maintenance of a positive water balance. Both cultivars senesced when fresh weight declined below the initial fresh weight, but 'Golden Wave' flowers declined to that level in 4 days versus 8 days for 'Samantha'. Lower hydraulic conductivity of 'Golden Wave' contributed to the development of a water deficit. The water potential of petals and leaves declined with time in vase, with 'Golden Wave' petals senescing at a higher potential than 'Samantha' petals. Petal potential in both cultivars was higher than leaf potential until the petals began to senesce. Increased hydraulic resistance to water flow to the petals is postulated as a contributing reason for petal water deficit.

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APPENDIX

APPENDIX

A PRACTICAL GUIDE TO THE USE OF IN SITU DEW POINT HYGROMETERS

APPENDIX

A PRACTICAL GUIDE TO THE USE OF *IN SITU* DEW POINT HYGROMETERS

This guideline was written due to the paucity of practical information on the use of *in situ* dew point hygrometers. It was intended to help the researcher with no experience with hygrometers, specifically leaf hygrometers. The care and use of L-51 leaf hygrometers and HR-33T microvoltmeters (Wescor, Inc.) will be elaborated. This is a supplmenet for the HR-33T instruction manual (1), and is not a replacement for it. The theory of dew point water potential measurements may be found in the manual (1).

Π_v Determination

Follow the procedure in manual (1) for determining the cooling coefficient (Π_v) of each hygrometer. Note the temperature of which Π_v is determined. Correct Π_v for operational temperatures which deviate from the temperature at which Π_v was determined. Use the following formula (1) to correct Π_v.

$$\Pi_v \text{ at } T_1 = 0.7 \text{ μvolts } (T_1-T_0) + \Pi_v \text{ at } T_0$$

where

T_0 = temperature used for Π_v measurement

T_1 = new temperature

Π_v should be corrected for temperature at every reading.

Dew Point Measurement

To obtain hygrometer readings with the dew point mode, the following procedure should be followed:

1. Connect hygrometer to microvoltmeter.

2. Determine temperature at thermocouple junction. Use either temperature detector of hygrometer or an external thermometer close to hygrometer.

3. Correct Π_v for temperature deviations from the temperature at which Π_v was determined.

4. Set corrected Π_v on microvoltmeter.

5. Check for thermal gradients by switching between input short and read. Proceed with measurements if the readings do not differ by more than 3 μvolts, as excessive thermal gradients are not present.

6. Set range switch to the anticipated range.

7. Adjust meter reading to zero with zero offset control with function switch in read position.

8. Rotate function switch to cool to condense water on the thermocouple junction. Ten second cooling is long enough for water

potential in the range of 0 to -20 bars. Use longer cooling times for drier samples. Use same cooling time for measurements in a given water potential range, including calibration measurements.

9. Rotate function switch to dew point. Observe deflection of the meter needle (junction is warming) until a constant μvolt reading is reached (the junction is at the dew point temperature).

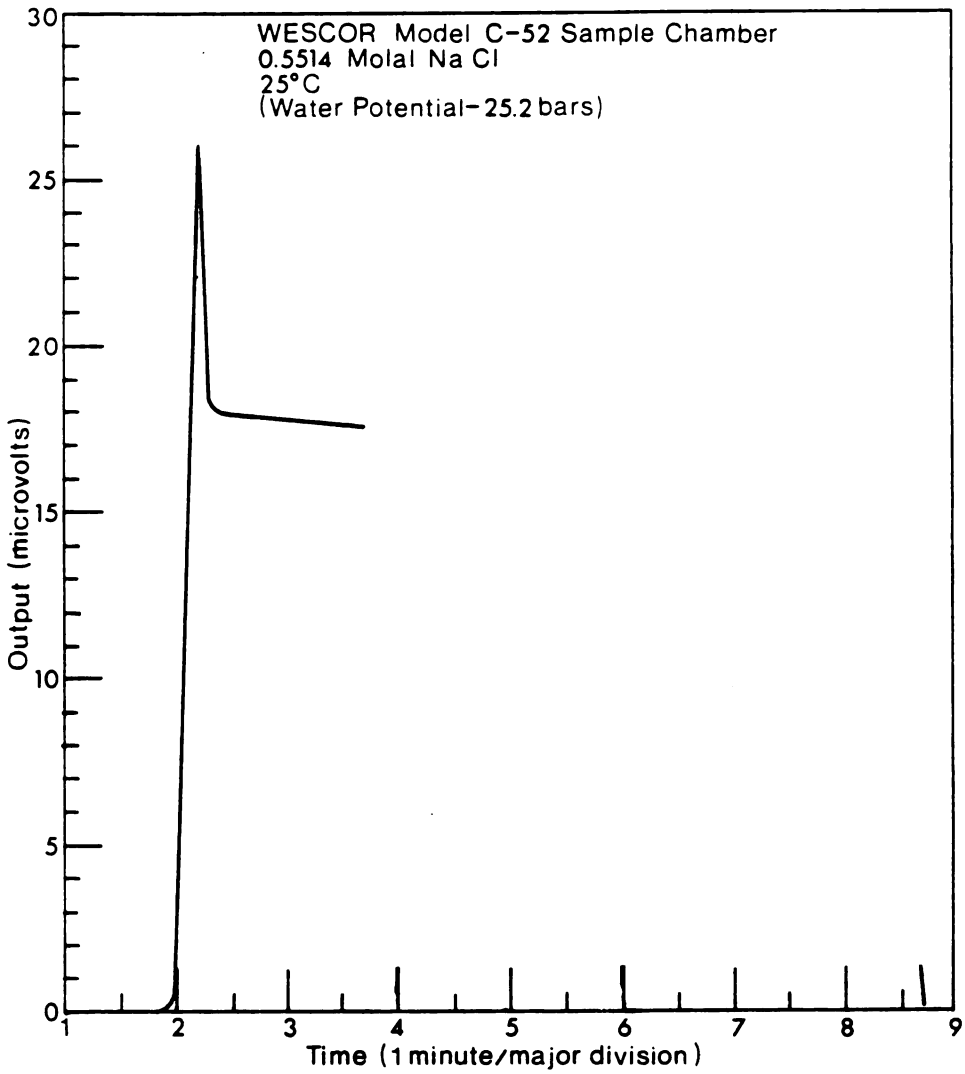
10. Convert the number of microvolts produced at the dew point temperature to water potential by referencing a calibration curve (μvolts plotted against water potentials of standard solutions, see Calibration section).

11. A strip chart recorder can be used to monitor dew point temperature deflections. A sample trace of a dew point measurement is shown in Figure 1. The number of μvolts at the plateau reflects the temperature of the junction at the dew point and is directly proportional to the water potential of a sample or solution.

12. As the water potential of a sample becomes more negative (drier) the dew point will be lower. More μvolts reflect a lower thermocouple junction temperature.

13. Do not take measurements with the same hygrometer more frequently than once every 45 min. The water condensed on the thermocouple junction during the cooling cycle must evaporate from the junction before another measurement can be taken.

Figure 1. Output of dew point mode on strip chart recorder (1).


WESCOR Model C-52 Sample Chamber
0.5514 Molal Na Cl
25°C
(Water Potential-25.2 bars)
Output (microvolts)
0
5
10
15
20
25
30
1
2
3
4
5
6
7
8
9
Time (1 minute/major division)

Calibration

L-51 leaf hygrometers can be calibrated by suspension in solutions of known water potentials.

1. Lower hygrometer into a solution with known water potential at a 90° angle to the solution surface to ensure trapping an air bubble around the thermocouple junction.
2. Equilibrate for 15-20 minutes.
3. Take dew point measurement.
4. Repeat using solutions of varying water potentials in the range of interest. Take a number of measurements in each solution.
5. Plot μvolts versus water potential to construct a calibration curve for each hygrometer.

Cleaning

The cleanliness of the thermocouple junction is of utmost importance. L-51 leaf hygrometers have no procedures to follow to check for cleanliness. From practical experience, the L-51 hygrometers need cleaning when the microvoltmeter needle continually drifts toward zero after the cooling cycle with no plateau being reached or plateaus at a much lower μvolt reading than is expected for the tissue. An effective cleaning technique follows.

1. Boil deionized (or purer) water in a flask connected with a hose to an empty flask with a pasteur pipette

protruding through a rubber stopper in the top. (The empty flask collects condensation from the steam.)

2. Clamp the dirty hygrometer to a ringstand with the thermocouple junction directly above the tip of the pipette. Take care not to touch the thermocouple junction with the pipette.
3. Steam the hygrometer for 20 min and blow dry with dust-free air.
4. Store clean hygrometers tightened onto clean filter paper to prevent contamination.

Sample Preparation

Follow the steps enumerated below to prepare hygrometers and plant tissue for in situ hygrometer measurements.

1. Clean thermocouple junction.
2. Wash tissue surface with water. Dry thoroughly.
3. Place a small bead of petroleum jelly on the hygrometer where it will contact the tissue. Do not touch thermocouple junction with petroleum jelly.
4. Slide tissue into slit in hygrometer holder. Seal hygrometer to tissue with gentle pressure and a rotating motion.
5. Take periodic readings on the tissue (approximately one every 45 min). Equilibration between the tissue water potential and the hygrometer is achieved when a number of consecutive measurements are the same. Equilibration times differ between plant parts and plant species.

6. Determine if the cuticle is preventing vapor equilibration. Artificially induce water stress by removing the water source from the tissue. Monitor the water potential during dehydration. If water potential does not decrease with increasing water stress, the cuticle must be thinned. One can also compare water potential changes in dehydrating tissues measured with hygrometers with another method of water potential determination which is not dependent on vapor loss through the cuticle for accuracy.

7. Thin cuticle if necessary using a cotton swab moistened with 2% Tween 80 and water, dipped in 600 grit carborundum. Hold tissue against one's hand and gently rub area to be placed under the hygrometer in a circular motion with swab. Rinse abraded area with water and dry well. Determine minimum amount of abrasion necessary for vapor equilibration. Observe abraded area when hygrometer is removed from tissue after a few days for signs of tissue degeneration. Pencil erasers can also be used to abrade cuticles. The type of tissue will determine which technique is preferable.

8. <u>In situ</u> hygrometers can be left on plant tissues for a number of days. Preliminary studies should be conducted to determine the length of time hygrometers can be left in place without causing tissue degeneration. Hygrometers should be removed for cleaning when the thermocouple junction is contaminated (see Cleaning section).

LITERATURE CITED

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1. Anonymous. HR-33T instruction manual. Wescor Inc., U.S.A.