Comparing the Accuracy and Efficiency of Leaf Gas Exchange Measurements of Excised and Nonexcised Strawberry (*Fragaria ×ananassa* Duch. 'Camino Real') Leaves

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Keywords. leaf gas exchange measurement time, net leaf photosynthetic rate, stomatal conductance

Abstract. **Acquiring leaf gas exchange (LGE) data from field experiments is critical for numerous research endeavors. However, because of the extended time needed to perform measurements and the necessity for moving equipment, the number of leaf samples collected is often limited. To address this concern, two studies were conducted to evaluate the accuracy and efficiency of LGE measurements of excised and nonexcised strawberry leaves. Research was conducted in experimental field plots located in Lubbock, TX, USA, where 'Camino Real' strawberry leaves was sampled for LGE measurements, during the 2021 growing season. Using an auto program mode, two LI-6400/XT portable machines simultaneously measured the LGE of nearby selected leaves from the same plant. Measurements were recorded every 30 seconds. After 90 seconds, the petiole of one leaf was cut (excised leaf treatment), and the auto program continued for an additional 480 seconds. The results indicated that although the LGE for nonexcised leaves remained stable, excised leaf LGE changed after excision. Within 30 seconds, the postexcision stomatal conductance to water vapor (g_{sw}) and net leaf photosynthetic rate (A) exhibited nearly 97% and 99% accuracy, respectively, of nonexcised leaf g_{sw} and A. However, the excised LGE decrease accelerated over time, with g_{sw} continuing to decrease by more than 5% after 43 seconds, indicating ≤95% accuracy of the g_{sw} results. The data suggested that strawberry LGE may be measured accurately within 30 to 40 seconds after leaf excision. During a separate experiment, the mean time to complete each individual strawberry LGE measurement was 68.4 seconds (±0.9 seconds) for nonexcised leaves. Conversely, the mean time to complete each LGE measurement of excised leaves was 42.2 seconds (±0.2 seconds). Therefore, leaf excision appears to be a viable method of maintaining accuracy and increasing the sample size while collecting strawberry LGE data.**

Leaf gas exchange (LGE) measurements are a valuable tool for assessing and improving plant health, productivity, and adaptation strategies. Therefore, a fundamental aspect of plant physiological research is performing LGE measurements (Missik et al. 2021; Tominaga and Kawamitsu 2024). The LGE may estimate the rate at which plants absorb carbon dioxide and release oxygen, thus providing a direct assessment of the plant net leaf photosynthetic rate (A) efficiency. This process is crucial for plant growth and development because an efficient A underpins plant productivity and biomass accumulation (Ariza et al. 2021; Tominaga and Kawamitsu 2024). The LGE may also estimate the postexcision stomatal conductance to water vapor (g_{sw}), which appraises the stomatal function. By estimating A and g_{sw}, LGE measurements may determine the water use efficiency of a plant. Additionally, analyzing the plant LGE provides insights into the resilience of a plant, can assist researchers with the development of strategies to enhance plant stress tolerance, and ultimately aide in more effective management of soil nutrient and water supplies (Ariza et al. 2021; Ibrahim et al. 2022; Missik et al. 2021; Tominaga and Kawamitsu 2024).

Recent advancements in commercial portable LGE systems have expanded the options available to researchers and enhanced the capability of scientists to study the plant physiological status in both controlled and field environments (Missik et al. 2021). However, because of the prolonged duration necessary to acquire each LGE measurement, researchers frequently encounter restrictions in the quantity of samples collected during each experiment (Kar et al. 2021a). For example, Montague and McKenny (2016, 2018) reported logistical challenges when measuring the LGE of field-grown ornamental tree species and indicated that researchers were required to move and reposition the portable LGE system for each individual LGE measurement (g_{sw}, A, and leaf transpiration rate). The necessity to move the LGE system extended the time required to complete each measurement (approximately 108 s/sample or approximately 33 samples/h) and limited the total number of samples collected each day. Similarly, Kar et al. (2021b) reported a single LGE measurement of field-grown winegrape (*Vitis vinifera*) vines required approximately 87 s/sample (allowing for approximately 41 samples/h). An optimal sample size is vital to the accuracy of the experimental design and facilitating an accurate interpretation of observed data (Freiman et al. 1978; Hernandez et al. 2006; Morellato et al. 2009; Paine et al. 2012). Furthermore, researchers who collect LGE data require methods that may increase the number of samples collected during each experimental measurement period but still provide accurate and precise information. Kar et al. (2021a, 2021b) indicated that leaf excision techniques may increase the measurement speed and sample size without compromising the accuracy of LGE data collected from ornamental tree and winegrape cultivars; furthermore, they suggested a specific time "window" during which LGE readings from excised and nonexcised leaves exhibited no difference. This technique facilitates faster data collection and an increased sample size (Kar et al. 2021a, 2021b).

Because of its high market value (Kouloumprouka et al. 2024), taste (Ibrahim et al. 2022), and nutritional components (antioxidant and phenolic compounds) (Giampieri et al. 2012; Hernández-Martínez et al. 2023), both the global production and US production of strawberry have significantly increased over the past 50 years (Food and Agriculture Organization of the United Nations 2023). Similarly, over the past 7 to 10 years, strawberry production in Texas has dramatically increased. The current strawberry production area within Texas is approximately

202 ha (Russ Wallace, personal communication). Strawberry production systems within Texas typically use an annual hill system, drip irrigation, and plastic mulch (Samtani et al. 2019; Wallace and Webb 2013); furthermore, on the Texas High Plains, low tunnels are required to protect strawberry plants from winter damage (Wallace and Webb 2013). Because of diverse climates and soils found within Texas (Samtani et al. 2019; Wallace and Webb 2013), recent research has focused on investigating production methods and finding adapted cultivars to enhance the yields and profitability of Texas strawberry producers (Wallace et al. 2022). Although not conducted within Texas, previous research has used LGE estimates to investigate the strawberry response to leaf temperature (Jun et al. 2017), water stress (Ariza et al. 2021; Jensen et al. 2009; Klamkowski and Treder 2008), salinity (Malekzadeh et al. 2023), and light (Jun et al. 2017; Kanno et al. 2022; Mochizuki et al. 2019) and to explore genotypic characteristics (Kanno et al. 2022). Because LGE measurements are crucial for optimizing resource management and investigating the plant stress response across differing environment and soil conditions, LGE measurements are essential when selecting appropriate production practices and cultivars for strawberry production systems in Texas. To measure the LGE parameters of strawberry, researchers typically conduct measurements using nonexcised leaves (Ariza et al. 2021; Ibrahim et al. 2022; Jun et al. 2017; Mochizuki et al. 2019). However, when measuring the LGE of strawberry with portable systems, researchers face the challenge of moving the plant to the gas exchange system (containerized plants) or moving the gas exchange system to the plant (containerized or field-grown plants). Both methods are often difficult and time-consuming. Currently, information regarding the use of leaf excision when measuring the LGE of strawberry plants in field settings is lacking. Therefore, the first objective of this research was to conduct field trials to determine optimum timing and whether measuring the strawberry LGE on excised leaves provides an accurate measure of LGE parameters. The second objectives were to compare the time between consecutive strawberry LGE measurements of excised and nonexcised leaves and determine whether leaf excision reduces the time to complete LGE measurements and, therefore, increase the LGE sample size.

Received for publication 6 Aug 2024. Accepted for publication 16 Sep 2024.

Published online 22 Oct 2024.

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This research was funded in part by the Texas Department of Agriculture and the USDA Specialty Crop Block Grant program (grant number SC-2021-38). The APC was funded by Texas Tech University.

We acknowledge the USDA Specialty Crop Block Grant program, the Texas Department of Agriculture, Texas A&M AgriLife Research and Extension, and Texas Tech University.

Conceptualization, T.M.; methodology, T.M.; validation, T.M., R.W., A.V.M. and M.A.R.K.; formal analysis, A.V.M. and M.A.R.K.; investigation, T.M., R.W.; resources, T.M., R.W.; data curation, T.M., A.V.M. and M.A.R.K.; writing—original draft preparation, T.M.; writing—review and editing, T.M., R.W., A.V.M., S.P. and M.A.R.K.; supervision, T.M.; project administration, T.M. and R.W.; funding acquisition, R.W. and T.M. All authors have read and agreed to the published version of the manuscript.

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https://doi.org/10.21273/HORTTECH05516-24

Materials and methods

Experimental site and cultivation procedures. A study that compared the accuracy and efficiency of excised and nonexcised strawberry LGE measurements was conducted during the 2021 strawberry growing season at the Texas A&M AgriLife Research and Extension Center, Lubbock, TX, USA (lat. 33°41′23.8″ N, long. 101°49′11.6″ W, elevation 984 m). Before strawberry planting, the experimental field was disked and rototilled, and three beds (rows) were shaped (beds were oriented north–south) using tractor-driven implements. Each bed had a height of approximately 0.2 m (aboveground level), width of 0.6 m, and length of 30.6 m, and beds were spaced 0.9 m apart. Preplant granular ammonium sulfate fertilizer (21N–0P–0K; American Plant Food, Galena Park, TX, USA) was applied by hand over the top of each bed (0.0067 $kg \cdot m^{-2}$ N). Additionally, sulfentrazone pre-emergent herbicide (Spartan 4F; FMC Corporation, Philadelphia, PA, USA) was applied (0.6 $mL \cdot m^{-2}$) to the soil surface using a tractor-mounted six-nozzle sprayer. Following application of granular fertilizer and pre-emergent herbicide, a single drip irrigation tape (Netafim Streamline X-638; Agricultural Drip Tape; Netafim Ltd., Tel Aviv, Israel) was installed at the center of each bed. Emitters within the irrigation tape were spaced 0.3 m apart; the 69.8-kPa pressure flow rate for each emitter equalled 875 $mL \cdot h^{-1}$. Black polyethylene mulch film [0.0254 mm (thickness) × 1.2 m (width); Berry Global, Inc., Evansville, IN, USA] was installed simultaneously with irrigation lines such that soil from between beds was used to cover edges and hold polyethylene mulch in place.

In association with an ongoing strawberry experiment (Texas Specialty Crop Block Grant #SC-2021-38), bareroot 'Camino Real' transplants (Lassen Canyon Nursery, Redding, CA, USA) were planted 22 Oct 2020. Each bed was divided into nine subplots. Each subplot had length of 3 m, and a 0.45-m alley divided each subplot. For the current experiment, transplants within subplots of each bed were planted with spacing of 0.3 m along each side of the drip tape. Therefore, within each subplot, there were 20 total plants. Based on grower expertise, weather data, and Texas strawberry production guidelines (Production Guide for Texas-Grown Strawberries 2014), each bed received similar irrigation throughout the growing season. To protect from adverse winter weather conditions, following planting, each bed was covered with clear plastic [0.1524 mm (thickness) × 1.8 m (width); Sun Master Cut and Pull Greenhouse Film; FarmTek, Dyersville, IA, USA]. Clear plastic was held approximately 0.5 m above the strawberry plants using small-gauge wire supports (product number 7213; Johnny's Selected Seeds, Winslow, MA, USA) spaced every 1.5 m within each row. Additionally, hand pegs (product number 9814; Johnny's Selected Seeds) were placed every 2 m along edges of each row to secure clear plastic and polyethylene mulch to the soil. During plant establishment and throughout the growing season, between-row

weed control was completed as needed by hand or with spot treatments of glyphosate (Bayer Crop Sciences, Research Triangle Park, NC, USA) using a single-nozzle hand-held knapsack sprayer (model #425; Solo, Newport News, VA, USA). In Jan 2021, applications of soluble Agrolution (20.0N–8.3P–16.6K fertilizer; ICL Specialty Fertilizers, Summerville, SC, USA) were applied weekly at a rate of 0.00056 $kg{\cdot}m^{-2}$ through the drip system. The total amount of N applied during the growing season was 0.0202 $kg{\cdot}m^{-2}$. After the chance of frost had passed (15 Apr 2021), clear plastic mulch was removed from each plot. Although strawberry harvest data are not discussed in this work, the berry harvest for 2021 began 7 Apr and concluded 10 Jun.

Throughout the experiment, weather data were collected on-site using a weather data acquisition system (Campbell Scientific Inc., Logan, UT, USA). Each sensor within the weather station logged data points at 1-min intervals for air temperature (°C; T_{air}), relative humidity (%; *Rh*), *PAR* ($W{\cdot}m^{-2}$), and wind speed ($m{\cdot}s^{-1}$). Hourly means were computed and stored in a data logger (model CR800; Campbell Scientific Inc.). Additionally, each hour, the mean vapor pressure (*VP*) deficit was computed using the saturated *VP* and ambient *VP* of the hourly average T_{air} and sample *Rh* (Jones 2013). Furthermore, one soil moisture sensor (model CS655; Campbell Scientific Inc.) was installed within each bed. Soil moisture sensors were installed below polyethylene mulch and placed horizontally into the side of the bed approximately 0.15 m below the soil surface. Once installed, polyethylene mulch was placed back over each soil moisture sensor. Soil moisture sensors were linked to a datalogger (model CR1000X; Campbell Scientific Inc.), and soil moisture data were collected and stored in a manner similar to that used for the weather station measurements.

Data collection procedure performed to evaluate LGE measurements. On six cloudless dates in 2021 (11, 15, 18, and 25 Jun and 7 and 9 Jul), mid-day (1100 to 1400 HR) LGE excision experiments were conducted. During experimental measurements, the age of the strawberry plants was between 8 and 9 months. To ensure consistency across treatments, the same plants were used for both excised and nonexcised LGE measurements. This approach was implemented to maintain uniformity in plant canopy and sunlight exposure. To control external variables and isolate effects of the leaf excision treatment, leaves from the same plants that were similar in size, maturity, and exposure to direct sunlight were selected. Daily weather parameters and mid-day mean soil moisture data for each date are presented in Supplemental Table 1. The LGE was estimated using two LI-6400/XT portable photosynthesis systems (LI-COR Environmental, Lincoln, NE, USA). Each machine used a standard chamber attachment and was outfitted with a 6400-02B red/blue external light-emitting diode lighting system and a CO_2 blending unit. To provide light-saturated photosynthetic rates within the chamber cuvette for all measurements, the chamber photon flux density (400–700 nm) was fixed at 1800 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ (Jun et al. 2017; Kanno et al. 2022). In addition, the leaf chamber CO_2 concentration was set at 400 $\mu mol{\cdot}mol^{-1}$, and the air flow rate was sustained at 500 $\mu mol{\cdot}s^{-1}$ for each measurement. Before and at various intervals during each day's measurements, the cuvette's water vapor (mmol H_2O mol/air) was adjusted to ambient conditions by clamping the cuvette to an adjacent leaf not involved in sampling. Water vapor inside each cuvette was adjusted to match the specified environmental conditions (Kar et al. 2021a, 2021b). Strawberry LGE parameters were measured on the center leaflet of full-sun, recently matured, fully expanded leaves (Kanno et al. 2022; Malekzadeh et al. 2023). On each measurement day, the data collection process followed a randomized complete block (matched pairs) design for leaf excision treatments with repeated measures. With this design, the treatment factor involved leaf excision comprising two levels (excised and nonexcised), whereas the blocking factor consisted of individual plants. Paired measurements of LGE using the two LI-6400/XT systems were obtained for excised and nonexcised leaves from an individual strawberry plant. Nine plants (blocks) were measured on one date, and six plants (blocks) were measured each of the other dates. The time of measurement was the repeated measures factor, with levels represented by each consecutive 30-s measurement period during individual LGE measurements. Before commencing LGE measurements each day, a row (bed) of plants was randomly selected; within this row, a subplot was selected at random. To initiate measurements, a plant (block) was randomly selected within the chosen subplot. The LGE of two healthy leaves exposed to direct sunlight and located nearby on the same individual plant was measured using each LI-6400/XT system (Supplemental Fig. 1). At the beginning of each day's data collection, leaf chamber conditions of each LGE system were adjusted to match previously described settings. Leaf chambers were simultaneously secured onto individual strawberry leaves and allowed to stabilize in each cuvette. Once each cuvette reached equilibrium [coefficient of variation ($CV < 10\%$)], an automated program recorded LGE readings every 30 s and continued for a duration of 600 s (Kar et al. 2021a, 2021b). After 90 s, a randomly chosen experimental leaf was selected and cut near the base of the leaf using a sharp blade (model number RA-60229; Dorco USA Inc., CA, USA). Approximately 15 cm of the leaf petiole remained attached to the leaf (excised leaf treatment). Once the auto program was complete, each LI-6400/XT system was relocated to another randomly selected subplot within the same row. A plant within the next subplot was randomly selected, and the process was repeated.

Comparison of the time required to measure LGE data of excised and nonexcised leaves. An additional experiment was conducted to explore the time required to estimate LGE measurements of excised and nonexcised field-grown strawberries. For this study, data were collected from previously described experimental plots. The time required for nonexcised LGE measurements was collected on 10 measurement dates and resulted in 467 individual leaf measurements. The measurement protocol for estimating the time of nonexcised strawberry LGE measurements consisted of randomly selecting a row of plants and locating a suitable leaf (as described previously) from a randomly selected plant within an arbitrary subplot.

Then, an LI-6400/XT cuvette was affixed to a leaf, and the LGE was viewed until g_{sw} and A stabilized ($CV < 10\%$). Once cuvette measurements were stable, the operator logged the data, and the LI-6400/XT system was moved to the next plant within the subplot. This procedure was used to measure the LGE from three strawberry plants within each subplot. Then, the operator moved to a different subplot within the row and initiated LGE measurements on a randomly selected plant. This procedure was repeated until the LGE values of 27 plants within each row were measured. Then, the next row was randomly selected, and the procedure was repeated. On each measurement day, the procedure was repeated within each row of plants and each subplot within each row. The time required to estimate the LGE from excised leaves was determined in manner similar to that used for nonexcised LGE measurements, except without relocating the LI-6400/XT system following each LGE measurement. The system was stationed centrally among three or four subplots within a row, with the cuvette attached to a tripod. The operator randomly selected a plant and a random leaf from the plant. Then, the operator cut the leaf from the selected plant (ensuring that approximately 15 cm of the petiole remained attached to the leaf). The excised strawberry leaf was subsequently brought to the LI-6400/XT chamber, where LGE was measured following a protocol similar to that used for nonexcised leaves. The excised LGE time data were collected on 11 dates and resulted in 1045 individual measurements.

Statistical analysis. The GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used to determine the accuracy and precision of LGE data of the excised and nonexcised strawberry leaves. All excised and nonexcised LGE data were exposed to GLIMMIX, and all LGE parameters were analyzed separately by fitting a repeated measures linear mixed model, with the repeated measures factor being the time point of measurement. The Akaike information criterion corrected (AICc) produced the appropriate covariance structure for the repeated measures (Hurvich and Tsai 1989) from several alternative candidate models. In each case, the AICc preferred a compound symmetry covariance structure for repeated measurements (30 s, 60 s, … , 570 s). Main effect terms and the excision treatment (excised and nonexcised leaves) × time point of measurement interactions were included in the linear predictor for the mixed model as fixed effects. The measurement date within the growing season and corresponding blocks (plants) measured on each date were regarded as a random effect. The SAS LSMEANS statement (using the ADJ = TUKEY option to obtain a Tukey–Kramer multiple comparison adjustment of P values for differences of least squares means) was used as a comparison between means. In each case, significance was tested at $\alpha = 0.05$. The response of nonexcised and excised A (dependent variable) to g_{sw} (independent variable) were analyzed and compared by performing a regression analysis (SAS version 9.4). In addition, an analysis of variance was performed to determine differences between excised and nonexcised regression line slopes and y intercepts. Furthermore, percent g_{sw} and A decrease was calculated based upon differences of g_{sw} and A between 30 and 480 s post leaf excision, and for each 30 s time point results were normalized to the initial value at 30 s. In addition, to illustrate g_{sw} decrease 31 to 60 s post leaf excision, percent gsw decrease was calculated in a similar manner. To generate a heatmap analysis, the 'pheatmap' and 'gplots' packages in R version 4.1.3 were used (https://www.r-project.org/; accessed 5 Jun 2024).

To assess differences between excised and nonexcised strawberry LGE leaf measuring times, the measurement time was estimated using the original LI-6400/XT data sets. Using Microsoft Excel software (version 2016; Microsoft Corporation, Redmond, WA, USA), the LGE measurement time differences were calculated for consecutive LGE measurements on each measurement date. Outlying data points (measurement times deemed excessively fast or slow compared with the data set) were eliminated by arranging data in a scaler order and removing the slowest and fastest 5% of data from the data set (Kar et al. 2021b). Within this analysis, the "treatment" variable denoted the time interval between consecutive measurements, and the strawberry LGE measurement times of excised and nonexcised leaves were evaluated. To assess the mean time for consecutive measurements of excised and nonexcised treatments, independent unequal variance samples t test ($\alpha = 0.05$) methodology was executed using the TTEST procedure of SAS (version 9.4).

Results

LGE measurements of excised and nonexcised strawberry leaves. Depending on the time after leaf excision, LGE data indicated that excised and nonexcised strawberry leaves responded differently. Although this study primarily focused on excised and nonexcised responses of g_{sw} and A, all LGE parameters estimated followed similar trends (Fig. 1; Supplemental Tables 2 and 3). Before leaf excision (90 s into instrument auto program), the mean g_{sw} and A of excised and nonexcised strawberry leaves did not differ ($P > 0.4392$ and $P > 0.4641$, respectively) (Supplemental Table 3). In addition, the mean g_{sw} and A did not differ between excised and nonexcised leaves for a minimum of 90 s after leaf excision (Figs. 2 and 3). However, as the experiment concluded, the excised mean leaf g_{sw} and A differed from those of the nonexcised leaf counterpart. During measurement periods, the mean nonexcised leaf g_{sw} ranged from 0.144 $mol \cdot m^{-2} \cdot s^{-1}$ at time of excision (90 s into measurement time) to 0.128 $mol \cdot m^{-2} \cdot s^{-1}$ at the conclusion of the 570-s measurement period, representing a decrease of 10.7%. In contrast, the mean excised leaf g_{sw} decreased by 64.7% (0.152 $mol \cdot m^{-2} \cdot s^{-1}$ at the time of leaf excision compared with 0.054 $mol \cdot m^{-2} \cdot s^{-1}$ at the conclusion of measurements) (Fig. 2; Supplemental Tables 3 and 4). Likewise, the mean A of nonexcised strawberry leaves at the time of leaf excision was 8.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the concluding mean leaf A was 8.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, indicating a decrease of 1.16%. Conversely, the mean A for excised leaves decreased from 9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 4.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$, representing a decrease of 51.42% (Fig. 3; Supplemental Tables 3 and 4). However, the mean leaf g_{sw} only decreased 2.4% during the 30-s postexcision period (approximately 97% accuracy), and the mean leaf g_{sw} only decreased past 5% after 43 s postexcision (approximately 95% accuracy). Furthermore,

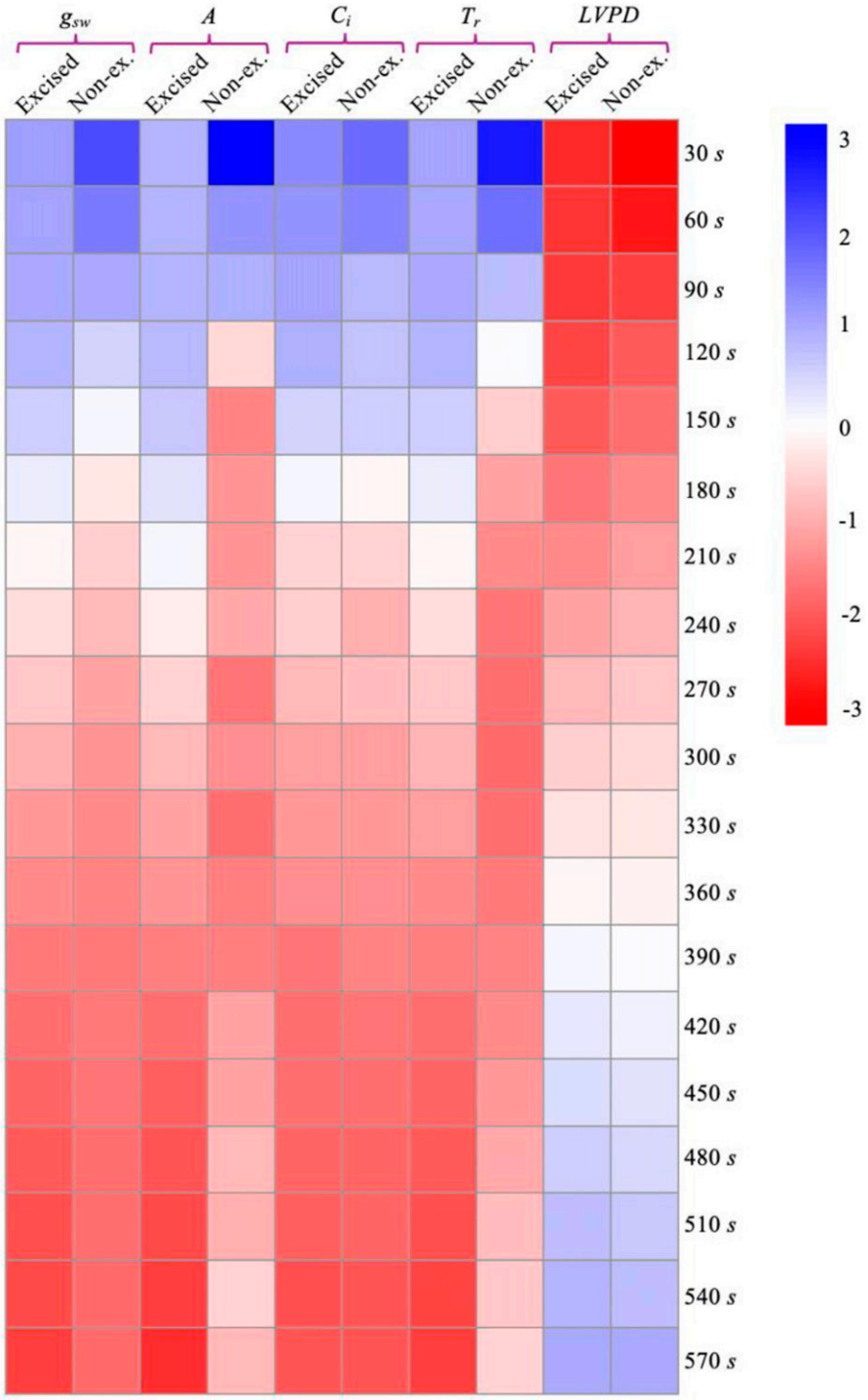

Fig. 1. Heatmap illustrating the effect of the measurement time on stomatal conductance to water vapor (g_{sw}), net leaf photosynthetic rate (A), intercellular CO_2 concentration (C_i), transpiration rate (T_r), and leaf to air vapor pressure deficit (LVPD) of excised and nonexcised strawberry (*Fragaria ×ananassa* 'Camino Real') leaves. The X-axis represents leaf excision treatments (excised and nonexcised), and the Y-axis depicts the time window of leaf excision (seconds). The heatmap of normalized mean values indicates the responses of each leaf gas exchange parameter under different measurement times.

the results indicated that the mean postexcision decrease of g_{sw} at 60 s postexcision was still approximately 8.1% (approximately 92% accuracy) (Supplemental Table 4). The excised mean leaf A followed a similar trend. At time of leaf excision, the mean A was 8.9 μmol·m^{-2}·s^{-1}. However, by termination of the experiment, the mean excised leaf A was 4.4 μmol·m^{-2}·s^{-1} (decrease of 51.4%) (Fig. 3; Supplemental Table 3). Although the data indicated that the g_{sw} and A of excised leaves did not differ from the g_{sw} and A of nonexcised leaves for a minimum of 150 s postexcision ($P \leq 0.05$), within this time "window," each measured excised LGE parameter decreased (Figs. 1–3; Supplemental Table 3).

Linear equations of the relationship between g_{sw} and A indicated that a reduced g_{sw} directly resulted in a lower A, and that the correlation between g_{sw} and A of excised (R^2 = 0.81) and nonexcised (R^2 = 0.69) strawberry leaves differed (Fig. 4). Slopes of excised and nonexcised leaf treatment equations did not differ (P = 0.73). However, the y-intercepts differed between leaf excision treatments ($P < 0.0001$) (Fig. 4). Therefore, the response of g_{sw} to A differed between leaf excision treatments.

Comparison of the time required to measure the LGE of excised and nonexcised strawberry leaves. Table 1 presents a comparison of time required for consecutive LGE measurements between excised and nonexcised leaves of field-grown strawberries. The 95% confidence interval of the difference in the mean time that transpired between two consecutive measurements of the excised and nonexcised treatments was between –28.1 and –24.2 s. The t test data revealed a difference in mean measurement intervals ($P < 0.0001$). Therefore, the mean measurement time of excised leaves (42.2 ± 0.2 s) was less than that of nonexcised leaves (68.4 ± 0.9 s) (Table 1).

Discussion

Moving portable LGE systems to measure LGE between different plants and even different leaves within the same plant is often a difficult and slows the process. In the current study, the precision and efficiency of estimating LGE measurements from excised and nonexcised strawberry leaves under field conditions were assessed. We found that excised and nonexcised leaf treatments influenced LGE parameters differentially over time (Figs. 1–3; Supplemental Tables 2–4). The LGE of the nonexcised leaf treatment remained stable over the time "window" (Supplemental Tables 3 and 4). However, for the

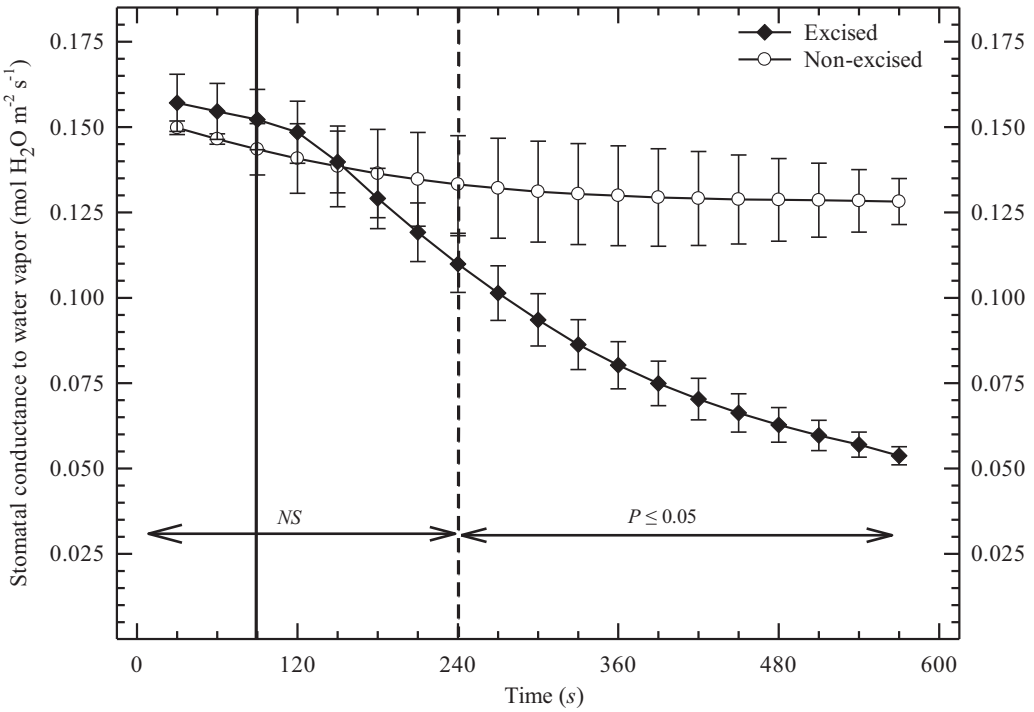

Fig. 2. Least squares means (±*SE*) for stomatal conductance to water vapor (g_{sw}) of excised and nonexcised strawberry (*Fragaria ×ananassa* 'Camino Real') leaves. Solid vertical line indicates the time (90 s postinitiation of leaf gas exchange measurements) when the petiole of the excised leaf was cut. The mean separation was performed using *P* values derived from the Tukey-Kramer multiple comparison technique ($\alpha = 0.05$). Dashed vertical line represents the time (120 s postexcision) when the g_{sw} of excised and nonexcised leaves differed. Therefore, the time before the dashed vertical line indicates the g_{sw} of nonexcised and excised leaves was similar [not significant (NS)], whereas the time to the right of the dashed vertical line indicates that the g_{sw} of nonexcised and excised leaves differed ($P \leqq 0.05$). Each symbol represents the mean of 39 measurements.

excised leaf treatment g_{sw} results suggested that even after 30 s, the postexcision mean g_{sw} of excised leaves exhibited 97% accuracy. Furthermore, the *A* results indicated 99% accuracy of excised leaves 30 s postexcision (Supplemental Table 4). In addition, after 43 s, the decrease in the mean excised g_{sw} was less than 5% of nonexcised leaf g_{sw} (≤95% accuracy), and the *A* results followed similar trends (Figs. 2 and 3; Supplemental Table 4). Kar et al. (2021b) reported similar results regarding LGE in winegrape cultivars. Comparable to the current study, leaves from five cultivars (Tempranillo, Grenache, Chardonnay, Cabernet Franc, and Cabernet Sauvignon) were excised, and the LGE measurements were compared with those of nonexcised leaves. The results indicated that although the time "window" between cultivars varied in terms of excised and nonexcised LGE, the minimum "window" of time in which LGE did not differ was 180 s. Similarly, the stability of LGE postexcision has been reported for several established ornamental tree species. Kar et al. (2021a) found that the time "window" of the leaf excision treatment of ornamental trees varied greatly by genotype. For example, the time "window" for English oak (*Quercus robur*) *A* and g_{sw} measurements was approximately 90 s postexcision, whereas there was no difference between excised and nonexcised LGE parameters (*A*, g_{sw}) for 450 s postexcision for Mexican redbud (*Cercis canadensis* var. *Mexicana*) (Kar et al. 2021a).

Relatively stable g_{sw} and *A* (Figs. 2 and 3) values observed in nonexcised strawberry leaves likely resulted from the well-maintained water status of the tissue. In contrast, the decline in g_{sw} and *A* following leaf excision for excised leaves indicated stomata closure, which is a response that occurs to prevent leaf tissue water loss (Barden et al. 1980; Clarke and McCaig 1982; Kar et al. 2021a, 2021b; Montague and Bates 2015; Xu et al. 2001). A reduction in g_{sw} could be attributed to declining leaf water potential in excised leaves, whereby water loss through transpiration is no longer offset by water uptake from the roots, leading to a more pronounced decrease in g_{sw} compared with that of nonexcised leaves. As stomata regulate both CO_2 diffusion into a leaf and H_2O diffusion out of the leaf (Chaves et al. 2002), plants reduce g_{sw} to mitigate rapid transpirational water loss. This reduction in g_{sw} following leaf excision results in a decrease in the CO_2 influx, consequently leading to reduced *A* (Chaves et al. 2003; Pinheiro and Chaves 2011). The significant decline in g_{sw} limits the availability of CO_2 for photosynthesis. This reaction is evidenced by decreasing *Ci* values (Fig. 1; Supplemental Table 3), which indicate that the reduction in *A* is primarily driven by stomatal limitations rather than metabolic impairment in the photosynthetic machinery. Our data suggest that nonexcised leaves maintained relatively stable g_{sw} and *A*, whereas g_{sw} and *A* of excised leaves sharply declined, thus emphasizing the importance of a continuous water supply to sustaining LGE. As the leaf water status declined, the excised leaf g_{sw} also decreased,

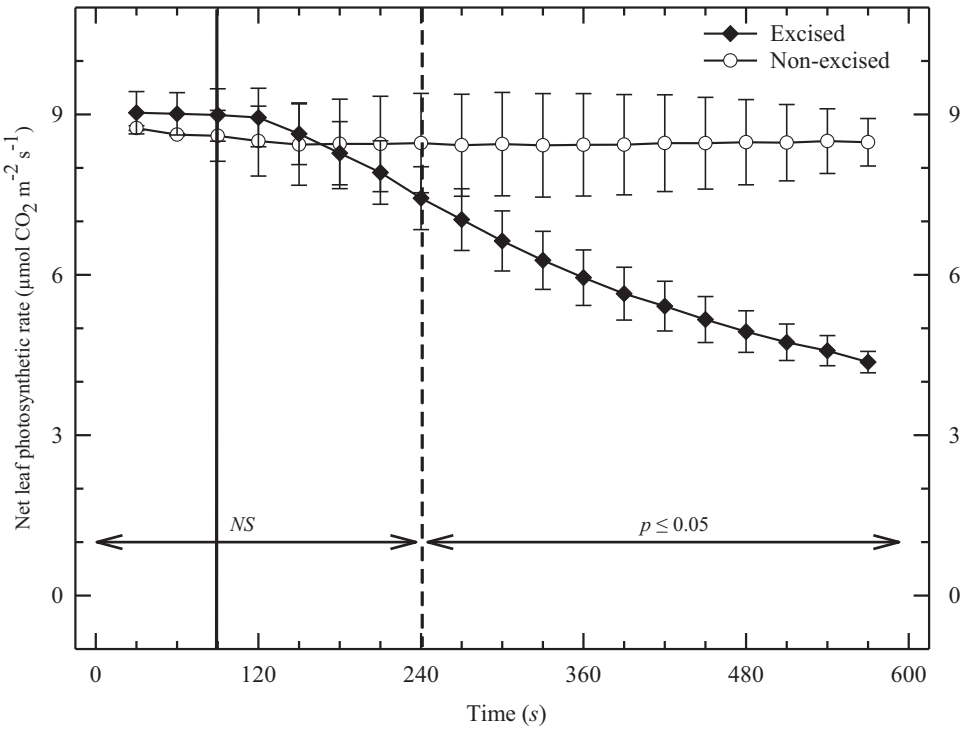

Fig. 3. Least squares means (±*SE*) net leaf photosynthetic rates (A) of excised and nonexcised strawberry (*Fragaria ×ananassa* 'Camino Real') leaves. Solid vertical line indicates time (90 s postinitiation of leaf gas exchange measurements) when the petiole of the excised leaf was cut. Mean separation was performed using *P* values derived from the Tukey-Kramer multiple comparison technique (α = 0.05). Dashed vertical line represents the time (150 s postexcision) when the *A* of excised and nonexcised leaves differed. Therefore, the time before the dashed vertical line indicates the *A* of nonexcised and excised leaves was similar [not significant (NS)], whereas the time to the right of dashed vertical line indicates that the *A* of nonexcised and excised leaves differed ($P \leq 0.05$). Each symbol represents the mean of 39 measurements.

thereby restricting CO_2 flow into leaf tissues and leading to reduced *A* (Fig. 4) (Buckley 2019; Chaves et al. 2002). However, as observed during the current study, the leaf CO_2 assimilation rate may persist if the tissue retains adequate water postexcision (Kar et al. 2021a, 2021b; Xu et al. 2001). The responses of other LGE parameters to leaf excision treatments showed similar physiological responses (Fig. 1; Supplemental Table 3).

For nonexcised leaves, the mean time observed for consecutive strawberry LGE measurements required 68.4 s (±0.9 s) between measurements, whereas the measurement mean time of excised leaves was 42.2 s (±0.2 s) (Table 1). Therefore, the time required for LGE measurements of excised leaves was approximately 26 s faster than the time required to perform LGE measurements of nonexcised strawberry leaves. Montague and McKenny (2016, 2018) reported that the time required to estimate consecutive excised LGE measurements of field-grown ornamental tree species was 60 s, whereas that for nonexised leaves was 109 s. Kar et al. (2021b) found that leaf excision reduced the LGE measurement time by approximately 30 s when compared with that of nonexcised leaves of grapevine cultivars. Our results indicated that on a typical day (±2 h of solar noon) of measuring strawberry LGE using the leaf excision technique, approximately 341 LGE measurements could be estimated. However, within the same time period, estimating strawberry LGE by applying the nonexcised LGE technique would yield a total of approximately 210 data points. Using the excised LGE techniques, strawberry researchers may potentially increase the frequency of LGE measurements obtained within a specified timeframe and, consequently, increase the data sample size. Furthermore, regarding cultivar performance field trials, the utilization of the leaf excision method may enable the assessment of a larger number of genotypes within a designated time frame. Time saved between strawberry LGE as described within the current experiment is likely situational and would depend on the plot-to-plot distance, expertise of individuals who conduct LGE measurements, experimental design, and other factors. However, the practice of leaf excision presents a viable choice under particular experimental circumstances, including field, greenhouse, and growth chamber studies that focus on strawberry LGE research. Because establishing a larger sample size is essential to confirming data trends (Morellato et al. 2009) and developing accurate explanatory models (Wisz et al. 2008), using the leaf excision technique when gathering strawberry LGE data would greatly assist scientists who conduct strawberry LGE research.

Conclusions

For numerous research activities, moving a portable LGE system between plants and even among different leaves within the same plant presents scientists with abundant challenges. When working in field situations, these challenges include physical difficulties such as setup time, weight of the LGE

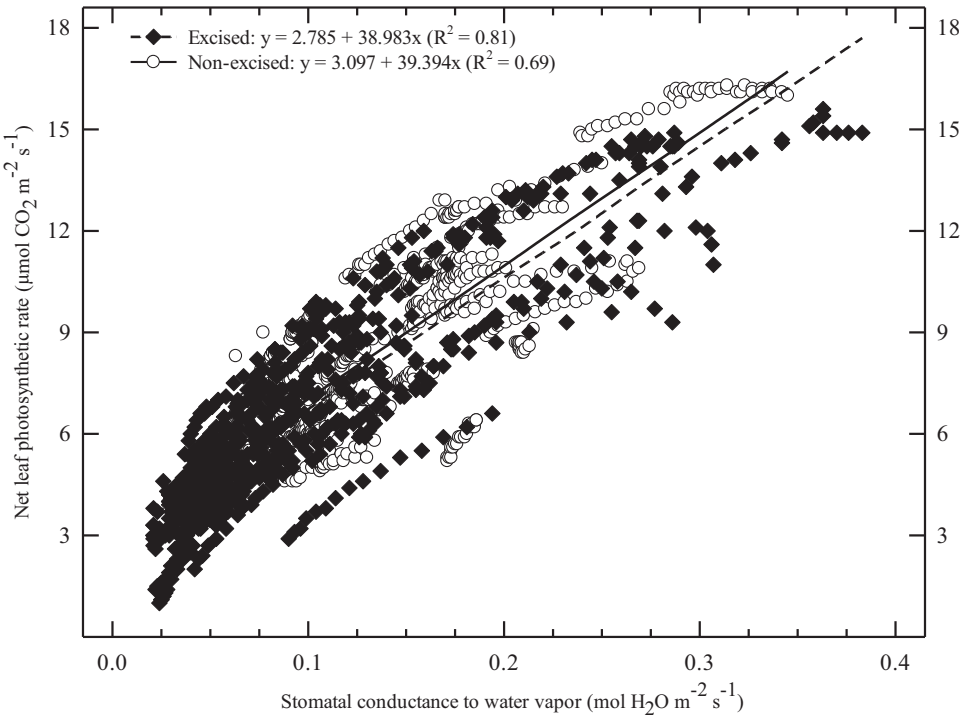

Fig. 4. Correlation between stomatal conductance to water vapor (g_{sw}) and net leaf photosynthetic rate (A) for excised (n = 732) and nonexcised (n = 740) strawberry (*Fragaria ×ananassa* 'Camino Real') leaves (equation for nonexcised and excised leaves is significant; $P < 0.0001$). Slopes of predicted linear regression equations do not differ ($P = 0.73$). However, the y intercepts of predicted linear regressions equations do differ ($P < 0.0001$). Therefore, the relationship of g_{sw} to A is presented separately for excised and nonexcised leaves.

system, adverse weather conditions (T_{air} ambient light, precipitation), distance between sample plants, and uneven terrain. Therefore, if strawberry researchers estimate LGE using the described techniques and methods, then gathering a sufficient number of samples within a limited period of time would be less challenging. The results indicated that the LGE of nonexcised leaves remained stable during each measurement period, whereas the LGE of excised leaves decreased postexcision. However, despite variable weather and soil moisture conditions 30 s postexcision, the excised leaf g_{sw} was measured at nearly 97% accuracy, whereas the excised leaf A data indicated 99% accuracy. However, 43 s postexcision, the g_{sw} continued to decrease to greater than 5%, indicating ≤95% accuracy of g_{sw} results, suggesting that an accurate LGE of excised strawberry leaves may be estimated within 30 to 40 s postexcision. In addition, the mean consecutive measurement time for nonexcised strawberry leaves was 68.4 ± 0.9 s, whereas the mean consecutive measurement time for excised leaves was 42.2 ± 0.2 s. Furthermore, it should be noted that the accuracy of techniques described may likely vary depending on the plant species, cultivars, experimental setup, environmental variables, leaf orientation, and individuals who perform the measurements. Moreover, it is crucial to note that the current results should not be considered the decisive standard for previous, ongoing, or future strawberry LGE measurements. Consequently, it is advisable to compare experimental outcomes exclusively with data obtained within circumstances of each specific experiment. Nevertheless, estimating strawberry LGE using the procedures described will provide researchers with the option of greater measurement speed and increased sample size, as well as precision and accuracy. Additionally, using the leaf excision method may prove to be a viable strategy within other particular experimental situations such as range or forest

Table 1. Comparison of time durations for consecutive leaf gas exchange (LGE) measurements of excised and nonexcised leaf treatments of field-grown strawberry (*Fragaria ×ananassa* 'Camino Real') plants during the 2021 growing season. For the excised leaf treatment, leaves were cut near the base of the petiole (leaving approximately 15 cm of leaf petiole) and carried to the LI-6400/XT leaf chamber. For the nonexcised treatment, the LI-6400/XT leaf chamber was moved to each plant, and the chamber was affixed to a leaf connected to the plant.

	Observations (n)	Duration between measurements (s)
Treatment		
Excised leaves	1,045	42.2[i] ± 0.2
Nonexcised leaves	467	68.4 ± 0.9
95% confidence interval for the mean difference between excised and nonexcised leaf treatments		−28.1, −24.2

[i] The time is expressed as the mean ± *SE*. The mean separation in the time duration between consecutive LGE measurements was evaluated using an independent t test with unequal variances ($\alpha = 0.05$). The mean time duration between measurement treatments differs by treatment ($P < 0.0001$).

ecology, greenhouses, orchards, growth chambers, and terrain, which may hinder the mobility of the portable LGE system. However, caution is necessary when using excised leaves to measure the LGE of plants with limited leaf area (young plants) or situations in which an accurate leaf area measurement is crucial. Moreover, moisture stress is known to restrict plant physiological processes. Therefore, using a portable system to estimate the LGE of excised strawberry leaves under water stress conditions would require additional caution and experimentation.

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