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Daytime and Nighttime Carbon Balance and Assimilate Export in Soybean Leaves at Different Photon Flux Densities[1]

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ABSTRACT

To evaluate daytime and nighttime carbon balance and assimilate export in soybean (*Glycine max* [L.] Merrill) leaves at different photon flux densities, rates of CO_2 exchange, specific leaf weights, and concentrations of sucrose and starch were measured at intervals in leaves of pod-bearing 'Amsoy 71' and 'Wells II' plants grown in a controlled environment room. Assimilate export was estimated from CO_2 exchange and change in specific leaf weight. Total diurnal assimilate export was similar for both cultivars. Large cultivar differences existed, however, in the partitioning of carbon into starch reserves and the relative amounts of assimilate exported during the day and the night. Total amounts of both daytime and nighttime export increased with increasing photon flux density, as did sucrose and starch concentrations, specific leaf weight, and rate of respiratory carbohydrate loss at night. Cultivar differences in nighttime rate of export were more closely related to the differences in amount of assimilate available at the end of the day than to differences in daytime rate of net CO_2 assimilation. Daytime rates of export, however, were closely related to daytime rates of net CO_2 assimilation within each cultivar. The total amount of starch depleted during the 10-hour night increased as starch concentration at the beginning of the night increased.

In soybean (*Glycine max* [L.] Merrill), a substantial portion of carbon assimilated by the leaf is partitioned into starch during the day and exported during the night (4, 5, 9, 16, 20). The accumulation of starch in the leaves of many species, including soybean, has been thought to represent carbon in excess of the amount needed for plant growth. Chatterton and Silvius (4, 5), however, investigated photosynthate partitioning and translocation in soybean plants grown under different photosynthetic periods and reported that starch accumulation in the leaf is a programmed synthesis, possibly influenced by assimilate demand during the dark period. They noted that vegetative plants partitioned 60 and 90% of their daily foliar accumulation into starch when grown in 14-h and 7-h photoperiods, respectively. Gordon (10) has reported that approximately 20 to 50% of the photosynthetically fixed carbon is retained in the leaf for later export in several species.

The diurnal allocation of leaf assimilates has been studied in several species (3, 8, 11, 12, 15, 18, 20). In soybean, diurnal allocation of leaf assimilates has been reported only for plants in vegetative growth (15, 20). It is necessary, however, to elucidate the diurnal allocation of leaf assimilates during the seed filling period in order to define potential limitations in assimilate supply for seed growth.

The purpose of this study was to evaluate the diurnal allocation of leaf assimilates and the amounts of daytime and nighttime export from soybean leaves as influenced by PPFD.[3] Two soybean cultivars known to differ in leaf starch accumulation (7) and rate of nighttime assimilate export (16) were evaluated during the stage of rapid seed growth.

MATERIALS AND METHODS

Plant Culture. 'Amsoy 71' and 'Wells II' soybean cultivars were grown in a controlled environment room with a 14-h photoperiod, constant 25 ± 1°C temperature, and approximately 50% RH. A mixture of cool-white fluorescent and tungsten-filament incandescent lamps provided a PPFD of 400 μmol m^{-2} s^{-1} (400–700 nm) at the upper level of the canopy. Seeds were planted in 2-L plastic pots containing a fertile greenhouse soil mixture inoculated with *Rhizobium japonicum*. Plants were thinned to one per pot at approximately 2 weeks and watered once daily during vegetative growth and twice daily during reproductive growth with deionized water. Flower initiation at node 6 occurred at approximately 30 d after planting for both varieties. Each plant developed 15 to 20 pods. All plants were sampled during the linear phase of seed growth (57 ± 7 d after planting).

Leaf Dry Matter and Carbohydrate Analyses. At approximately 1400 to 1600 h during the day two mature trifoliolate leaves per plant, located between the 6th and 10th nodes, were selected randomly and tagged according to nodal position. The PPFD for each lateral leaflet to be sampled was carefully measured with a LI-COR model LI-190SB quantum sensor without disturbing leaflet orientation in the canopy. Leaf samples for determination of SLW and carbohydrate concentration were obtained by removing one randomly-selected lateral leaflet from each selected leaf immediately prior to initiation of the dark period (2200 h) and then removing the opposite lateral leaflet immediately prior to the end of the dark period (0800 h). Sampling during the dark period was done under dim, nonphotosynthetic, green light. Areas of lateral leaflets were determined using a leaf area meter (Hayashi Denko model AAM-5 or LI-COR model LI-3000). Leaflets were then quickly submerged in liquid N_2 and stored at −29°C until freeze-dried. After freeze-drying, leaflets were weighed and then ground to pass a 1-mm screen in a Tecator/Udy sample mill. A subsample from each of these individual ground samples was used to determine sucrose and starch concentrations. Sucrose and starch concentrations were determined as previously reported (16).

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[3] Abbreviations: PPFD, photosynthetic photon flux density; SLW, specific leaf weight (laminar weight per unit laminar area); CH_2O, carbohydrate; CPR, carbohydrate production rate.

Carbon Assimilation and Respiratory CO_2 Efflux Measurements. Carbohydrate production rates (net CO_2 exchange rate $\times$ 0.68) at various PPFD were obtained from a previous study (7) in which Amsoy 71 and Wells II plants were grown in the same growth room and in the same environmental conditions described above. Respiratory CO_2 efflux rates were measured throughout the 10-h night on randomly selected individual leaves located between the 6th and 10th nodes. These leaves each received one of several different PPFD levels during the day prior to measurement of CO_2 efflux at night. Levels of PPFD ranged from 50 to 600 μmol m^{-2} s^{-1}. Measurements of CO_2 efflux were made as previously reported (16) and recorded on an Omnidata Polycorder model 516 at 2- to 4-min intervals during the dark period.

Estimation of Assimilate Export Rate. Estimation of export rate was made using the method of Terry and Mortimer (19). The gain in SLW during the 14-h day was assumed to be equivalent to the loss in SLW during the 10-h night. Changes in SLW were assumed to be due to carbohydrate-type compounds. In calculating export rates, CO_2 assimilation and efflux rates were converted to equivalent CH_2O. This was done by multiplying the CO_2 exchange rate by 0.68, the molar ratio of the two forms of carbon.

Rate of daytime export at each PPFD was calculated as net CO_2 assimilation rate minus the rate of increase in SLW from 0800 to 2200 h. Rate of nighttime export at each PPFD was calculated as the rate of decrease in SLW from 2200 to 0800 h minus the mean rate of CO_2 efflux during the night. Rates of CO_2 efflux during the night were predicted from the multiple regression analysis of 150 to 350 values taken throughout the night for each of the 12 or 14 leaves per cultivar subjected to CO_2 efflux measurements. The total amounts of assimilate exported during the day and night were calculated from rates of daytime and nighttime export and lengths of day and night periods.

Statistical Analysis. A completely randomized design was used and analysis of the data was done using the least squares method of regression. Linear regression analyses were conducted for data on sucrose and starch concentrations and SLW to determine the best fitting linear response to PPFD. Multiple regression analyses were conducted on data for rates of carbohydrate production and respiratory CO_2 efflux. These analyses were accomplished by employing full least squares analyses using indicator variables on the combined data and testing cultivar differences in the individual β-coefficients in a stepwise regression procedure. Where cultivar differences were nonsignificant at $P < 0.05$, common beta coefficients were used. A significant difference in one or more of the β-coefficients in the regression model implies a significantly different response between the cultivars.

RESULTS AND DISCUSSION

Sucrose and starch concentrations and SLW at 2200 and 0800 h increased in a linear manner ($P < 0.05$) with increasing PPFD in leaves of both cultivars (Figs. 1, 2, and 3).The linear response of sucrose concentration to PPFD was similar between the cultivars (Fig. 1). Leaves of Wells II, however, had a significantly greater concentration of sucrose at both 2200 and 0800 h than did leaves of Amsoy 71. The increases in starch concentration per unit increase in PPFD at 2200 and 0800 h were significantly greater in leaves of Amsoy 71 than in leaves of Wells II (Fig. 2). At PPFD greater than approximately 200 μmol m^{-2} s^{-1}, leaves of Amsoy 71 had greater starch concentrations than did leaves of Wells II. Trends in SLW (Fig. 3) were similar to those of starch, since starch was the primary component of change in SLW.

Total change (decrease) in sucrose and starch concentrations and SLW over the 10-h night increased with increasing PPFD in leaves of both cultivars (Fig. 4). Therefore, the decrease in concentrations of sucrose and starch during the night increased as the concentrations of sucrose and starch at 2200 h increased (Figs. 1 and 2). This positive correlation existed even though increased PPFD led to increased sucrose and starch concentrations at both 2200 and 0800 h. This suggests that the overall rate at which

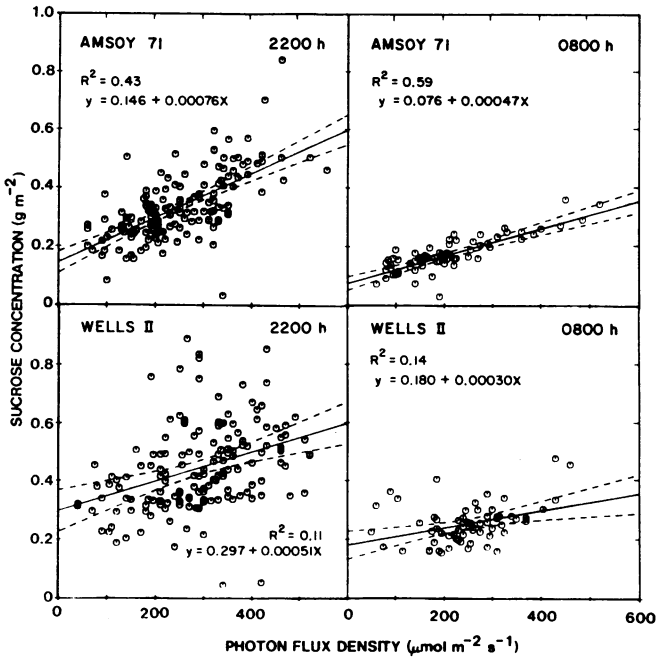

FIG. 1. Relationship between sucrose concentration and PPFD in Amsoy 71 and Wells II leaves; 2200 and 0800 h were the beginning and end of the 10-h night, respectively. Dashed lines indicate the 95% confidence interval for the regression line.

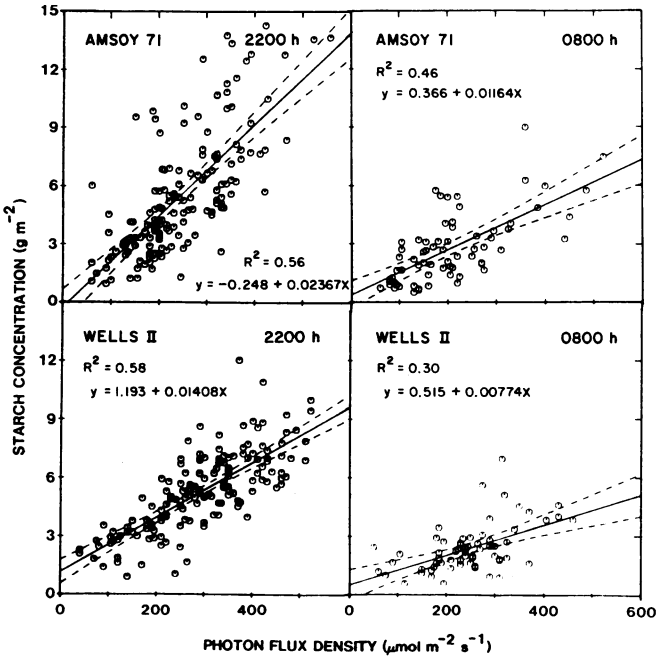

FIG. 2. Relationship between starch concentration and PPFD in Amsoy 71 and Wells II leaves. See Figure 1 for details.

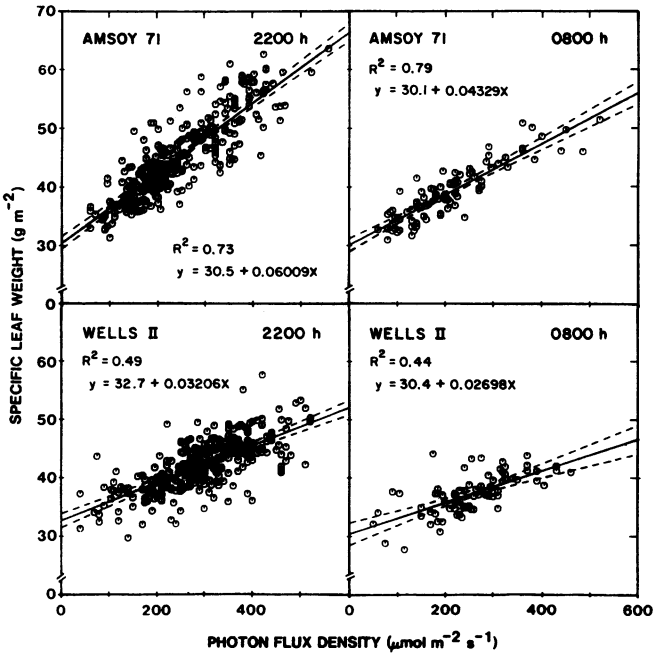

FIG. 3. Relationship between specific leaf weight and PPFD in Amsoy 71 and Wells II leaves. See Figure 1 for details.

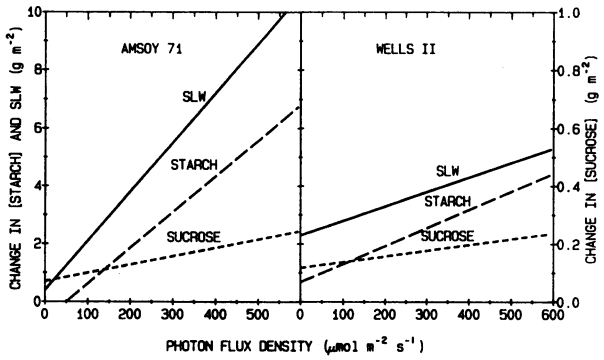

FIG. 4. Relationship between PPFD and the total change (decrease) in specific leaf weight and starch and sucrose concentrations during a 10-h night in Amsoy 71 and Wells II leaves.

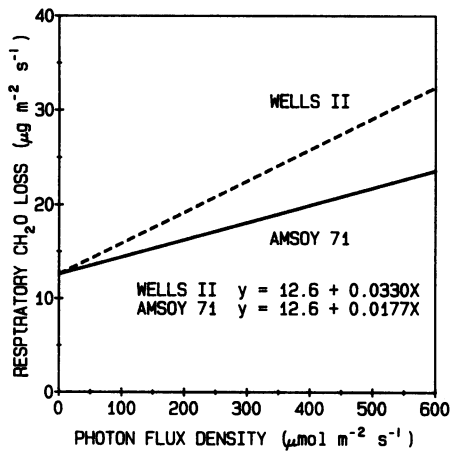

FIG. 5. Relationship between rate of respiratory carbohydrate loss and PPFD in Amsoy 71 and Wells II leaves. Rates of respiratory CH_2O loss were measured at various PPFD on a total of 12 or 14 leaves per cultivar. Measurements were taken at 2- to 4-min intervals throughout the night. The lines indicate the mean rates during the 10-h night predicted from the multiple regression analysis. R^2 value for the regression model was 0.39.

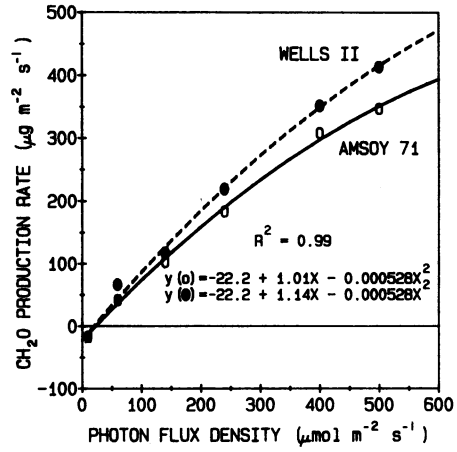

FIG. 6. Relationship between rate of carbohydrate production and PPFD in Amsoy 71 and Wells II leaves. Each value is the mean CPR of at least 20 leaves. R^2 value applies to both regression lines.

starch was mobilized during the night was partially associated with the amount of starch present. The observation that leaf starch concentration at 0800 h increased as PPFD increased suggests that the additional starch accumulated at higher PPFD was not completely degraded during the night.

Rate of respiratory CH_2O loss during the night increased in a linear manner in leaves of both cultivars as PPFD during the preceding day increased (Fig. 5). The higher rates of respiratory CH_2O loss in leaves of Wells II compared to leaves of Amsoy 71 probably were associated with the significantly greater sucrose concentrations in leaves of Wells II (Fig. 1). Positive correlations between carbohydrate concentration and respiration rate have been reported (1, 2, 6).

CH_2O production rate increased in a quadratic manner with increasing PPFD in both cultivars (Fig. 6). The increase in CPR per unit increase in PPFD was significantly greater in leaves of Wells II than in leaves of Amsoy 71. The smaller increase in CPR per unit increase in PPFD in leaves of Amsoy 71 indicates a greater CO_2 diffusion resistance. Stomatal diffusion resistance of Amsoy 71 has been reported to be greater than that of Wells (7). Cultivar differences in CPR could also be associated with differences in leaf starch concentration. High concentrations of leaf starch have been reported to be associated with increased mesophyll CO_2 diffusion resistance and, consequently, reduced net photosynthetic rates (17, 20).

Rate of daytime and nighttime assimilate export increased in a curvilinear and linear manner, respectively, with increasing PPFD in leaves of both cultivars (Fig. 7). The curvilinear trend for rate of export during the day with increasing PPFD (Fig. 7) is attributable to significant curvature in CPR with increasing PPFD (Fig. 6). Other studies (7, 13) have reported similar positive correlations between rate of daytime export and rate of carbon assimilation. The linear trend in rate of nighttime export with increasing PPFD (Fig. 7) is attributable to linear increases in SLW loss (Fig. 4) and rate of respiratory CO_2 efflux (Fig. 5) with increasing PPFD. The increase in rate of daytime export per unit increase in PPFD was greater for leaves of Wells II than for leaves of Amsoy 71. The increase in rate of nighttime export per unit increase in PPFD, however, was greater for leaves of Amsoy 71 than for leaves of Wells II.

Cultivar differences in rate of nighttime export were more closely related to differences in the amounts of assimilates available at 2200 h than to differences in CPR. A similar observation has been reported in mature leaves of tomato (14). Leaves of Amsoy 71 had a lower CPR than did leaves of Wells II (Fig. 6). Leaves of Amsoy 71, however, partitioned more fixed carbon into starch by 2200 h than did leaves of Wells II (Fig. 2).

The diurnal period when the rate of export was highest differed between the two cultivars. Leaves of Amsoy 71 had a higher rate of nighttime export than daytime export at all PPFD (Fig. 7). Leaves of Wells II, however, had a higher rate of daytime export than nighttime export at PPFD greater than approximately 140 μmol m^{-2} s^{-1} (Fig. 7). Below this PPFD, leaves of Wells II had a higher rate of nighttime export than daytime export.

The amount of photosynthetically fixed carbon immediately exported *versus* the amount retained in the leaf for nighttime export differed between cultivars (Fig. 7, inset). Leaves of Wells II tended to export a greater amount of newly fixed assimilates during the day than did leaves of Amsoy 71. Leaves of Amsoy 71, however, retained a greater amount of the newly fixed assimilates as starch for export at night than did leaves of Wells II. Overall, 40 to 80% of the assimilate produced from CO_2 assimilation was exported during the day in leaves of both cultivars. These percentages are similar to previously reported values (10).

Rates of daytime and nighttime export as a percentage of CPR changed with increasing PPFD (Fig. 7, inset). The percentage of CPR immediately exported increased continuously in leaves of Wells II but eventually decreased in leaves of Amsoy 71 with increasing PPFD.

The total amount of assimilate exported over a 14-h day was greater than that exported over a 10-h night (Fig. 8), except for Amsoy 71 leaves at PPFD approaching 600 μmol m^{-2} s^{-1}. The total amount of diurnal export increased in a curvilinear manner with increasing PPFD in leaves of both cultivars (Fig. 8). Leaves of Wells II exported approximately twice the amount of assim-

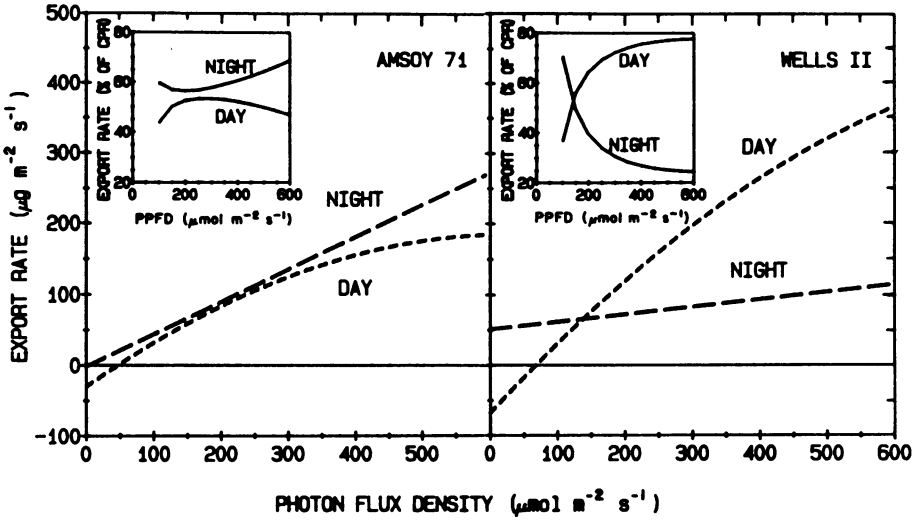

FIG. 7. Relationship between rates of daytime and nighttime assimilate export and PPFD in Amsoy 71 and Wells II leaves. Rates of export were estimated from the linear rate of change in SLW between 2200 and 0800 h and rates of CO_2 exchange. Inset: relationship between PPFD and rates of daytime and nighttime export as a percent of carbohydrate production rates.

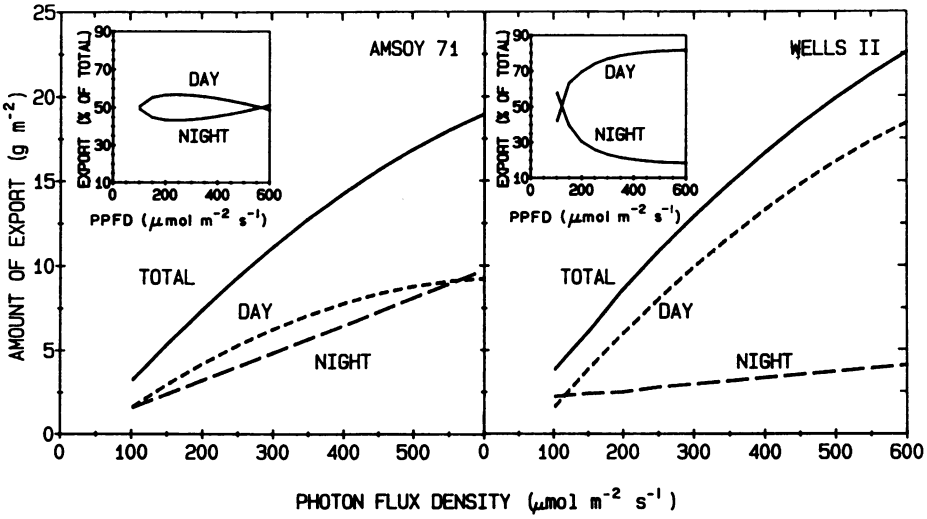

FIG. 8. Relationship between PPFD and the amounts of daytime, nighttime, and total diurnal assimilate export in leaves of Amsoy 71 and Wells II. Amounts of export were calculated from rates of export and length of the day or night period. Inset: relationship between PPFD and the amounts of daytime and nighttime export as a percent of total diurnal export.

ilates during the day than leaves of Amsoy 71 exported (Fig. 8). Leaves of Amsoy 71, however, exported approximately twice the amount of assimilates during the night than leaves of Wells II exported. Although large cultivar differences existed in the amounts of assimilates exported during the day and the night, the total amount of assimilates exported over a 24-h period was only slightly greater in leaves of Wells II than in leaves of Amsoy 71 (Fig. 8). Cultivar trends in total amount of export over a 24-h period (Fig. 8) were similar to trends in CPR (Fig. 6).

The relative contributions of daytime and nighttime export to total diurnal export changed with increasing PPFD (Fig. 8, inset). The contribution of daytime export increased continuously from 42% at 100 μmol m^{-2} s^{-1} to 82% at 600 μmol m^{-2} s^{-1} in leaves of Wells II. In leaves of Amsoy 71, the contribution of daytime export increased from 51% at 100 μmol m^{-2} s^{-1} to 57% at 250 μmol m^{-2} s^{-1}. Above 250 μmol m^{-2} s^{-1}, however, the contribution of daytime export decreased in leaves of Amsoy 71.

Although these two cultivars are agronomically similar, it is clear that their patterns of diurnal carbon allocation and export differed. Leaves of Amsoy 71 partitioned more assimilated carbon into starch reserves for later export and less into sucrose compared to leaves of Wells II. Consequently, leaves of Amsoy 71 had a lower daytime rate of export but a greater nighttime rate of export than did leaves of Wells II. Exploitation of genetic variation in carbon allocation to improve biological efficiency in soybean may be feasible.

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