Influence of Seismic Stress on Photosynthetic Productivity, Gas Exchange, and Leaf Diffusive Resistance of *Glycine max* (L.) Merrill cv Wells II

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**ABSTRACT**

Relative growth rate (RGR), leaf water potential (ψw), transpiration rate (Tr), photosynthetic rate (Pn), and stomatal and mesophyll resistances to CO₂ exchange were measured or calculated to determine how periodic seismic (shaking) stress decreased dry weight accumulation by soybean (*Glycine max* [L.] Merrill cv Wells II). Seismic stress was applied with a gyratory shaker at 240 to 280 revolutions per minute for 5 minutes three times daily at 0930, 1430, and 1930 hours. Fifteen days of treatment decreased stem length 21%, leaf area 17%, and plant dry weight 18% relative to undisturbed plants. Seismic stress also decreased RGR 4%, which was due entirely to decreased net assimilation rate. Transpiration decreased 17% and leaf ψw increased 39% 30 minutes after treatment. A reduction in Pn began within seconds after the onset of treatment and had declined 16% after 20 minutes, at which time gradual recovery began. Assimilation rate recovered fully before the next seismic treatment 5 hours later. Resistance analysis and calculation of leaf internal CO₂ levels indicated that the transient decrease in Pn caused by periodic seismic stress was due to increased stomatal resistance on the abaxial leaf surface.

Plants subjected to physical disturbance may respond by decreased stem length (21), leaf area, growth rate, fresh and dry weight (13–15, 24), and yield (1, 24, 27, 28) relative to undisturbed plants growing in an otherwise similar environment. Various methods have been used for administering mechanical stress treatments to plants, including forced-air currents or natural wind loads (24, 29, 31, 32), periodic shaking (4, 19), handling, flexing, or rubbing of individual plant parts (3, 15, 20). Seismic stress applied as periodic gyratory shaking of the entire plant affects growth in much the same way as exposure to natural wind loads (19, 25).

The inhibition of dry weight gain and yield caused by mechanical stress suggests that decreased photosynthetic productivity may be a stress response. To test this hypothesis, several growth dynamics parameters as well as whole plant transpiration rate, photosynthetic rate, leaf water potential, and stomatal and mesophyll resistances to leaf gas exchange by soybean were measured as a function of mechanical stress treatment or pretreatment.

**MATERIALS AND METHODS**

**Culture System and Mechanical Stress Treatments.** Seeds of *Glycine max* (L.) Merrill cv Wells II were sown four per pot into 12.7-cm-diameter plastic pots in a growth room. The growth medium consisted of 1:2 soil:2 perlite (by volume), adjusted to pH 6.2. Nutrients were incorporated at the following concentrations (g m⁻³) of the final mixture: trace element mix, 75; KNO₃, 597; MgSO₄, 597; and superphosphate (0-46-0, N:P:K), 896. Pots were watered as needed (without physically disturbing shoots) with tap water supplemented with 200 mg L⁻¹ K and N (as NO₃⁻) (pH 6.8). When primary leaves were completely unfolded, seedlings were thinned to one per pot and selected for uniformity of stem length and leaf area. Fluorescent and incandescent lamps supplied a PPFD² of 297 ± 26 μmol m⁻² s⁻¹ for 15 h daily (LI-COR LI-1776 Solar Monitor, quantum sensor Q 3870). Day/night temperatures were 32 ± 1°C/27 ± 1°C. RH was 45 ± 7% during the day.

To administer seismic treatment, pots were inserted into holes in a wooden platform mounted on a gyratory shaker and shaken in a horizontal plane with a circular radius of 1.3 cm. The shaker was located under the light bank in the growth room so that conditions of light and temperature were not changed while the shaking treatment was administered. Plants were shaken at 240 to 280 rpm three times daily (at 0930, 1430, and 1930 h) for 5 min each time. Control plants remained undisturbed at all times, and all plants were protected from extraneous air currents during the course of the experiment.

**Growth Dynamics Analysis.** Uniform seedlings were assigned randomly to undisturbed control or shaking treatments. Six plants per treatment were selected randomly for harvest 9, 12, 15, or 18 d after initiation of treatment. Leaf fresh weight and area (LI-COR model LI-3000 area meter) were determined at harvest, and dry weight of leaves, stems, and roots was obtained after drying for 3 d at 70°C in a forced-air oven. RGR was calculated by regressing ln plant dry weight with time of treatment. The slope of the first-order regression line represented mean RGR for days 9 through 18. LAR and NAR were calculated for days 9 through 18 (18, 26). A mean NAR and LAR value was obtained for each treatment by averaging the values calculated between each harvest period.

Growth data were subjected to analysis of variance and F test

**Abbreviations:** PPFD, photosynthetic photon flux density; RGR, relative growth rate; LAR, leaf area ratio; NAR, net assimilation rate; Tr, transpiration rate; Trn, boundary layer resistance to H₂O diffusion; Trs, stomatal resistance to H₂O diffusion; Trm, mesophyll resistance to CO₂ diffusion; C, internal CO₂ concentration; Pn, photosynthetic rate; ψw, leaf water potential.

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to determine differences between treatments at the 0.05 level of significance. RGR for control and shake treatments were tested for significant differences using a t test for comparison of regression coefficients (22).

Whole Plant Transpiration. Uniform seedlings were assigned randomly, six per treatment, either as undisturbed control or shake treatments. On day 9 of treatment and for five subsequent days, a plant from each treatment was prepared for Tr measurement by enclosing the pot in an opaque, white polyethylene bag sealed around the base of the stem with a twist-tie to minimize evaporation from soil. Each day at 1315 h, control and shaken plants were placed on separate top-loading balances, equilibrated for 30 min, and plant-plus-pot weight recorded at 5-min intervals. At 1430 h, the shake treatment was removed from the balance, shaken for 5 min, and then replaced on the balance. At the same time, the undisturbed control was gently lifted from the other balance, placed on a stationary table near the shaker for 5 min, and then returned to the balance. Weight measurements continued for 90 min following seismic treatment. Leaf area of the entire plant was measured immediately after H2O loss measurement. Data were subjected to analysis of variance and F test to determine differences between treatments at the 0.05 level of significance.

Leaf Water Potential. Eight uniform seedlings were assigned randomly as undisturbed or shake treatments. This procedure was repeated on three consecutive days with a group of eight plants, each of which had been planted 1 day later than the previous group. The experimental design thus included four groups of eight plants each, and data were analyzed statistically as four replications of each treatment by four blocks (days). After 12 d of seismic treatment, a 0.7-cm-diameter leaf disc was punched from the terminal leaflet of the third trifoliolate leaf of each plant 30 min after the 1430-h shake and immediately enclosed in the well of a thermocouple psychrometer (Wescor, Inc., C-52 sample chambers). An automated psychrometer system scanned and recorded data from eight samples per day. Data were taken after leaf discs had equilibrated for 2 h in the chambers. Microvolt readings were converted to water potential units using calibration curves developed for each thermocouple, and data were analyzed using statistical methods described in the preceding section.

Whole Plant Photosynthesis. Eleven uniform seedlings, including five control and six seismic-stressed plants, were treated for 10 or 12 d. Whole plant photosynthetic rates were monitored continuously on the final day of treatment, including the periods immediately before, during, and after the 0930, 1430, and 1930 h shake treatments. A transparent whole plant chamber (2) mounted on a gyratory shaker was incorporated into an open gas flow system to measure photosynthetic rates (16). Compressed air containing 330 μl/l CO2 was humidified by bubbling the airstream through water in a closed container. Humidity in the flowing atmosphere was maintained near that of the growth room, and was measured using a dew point hygrometer (EG&G, model 911). Humidified air flowed into the plant chamber at 6.2 L min⁻¹, and a variable-speed fan and baffles provided internal air mixing. Heat exchange coils near the base of the chamber provided an internal temperature of 29 ± 1°C by circulating coolant between the coils and a water bath. Outlet air was dehumidified using magnesium perchlorate, and CO2 concentration in the airstream was determined before or after the plant chamber using an IR CO2 gas analyzer (Infrared Industries model 705D).

Whole plant photosynthetic rates were determined for the 20-min period before each shake, and for up to 130 min after each shake treatment. Analysis of variance and F test at the 0.10 level were performed on Pn data for each 10-min interval to determine significant differences between treatments.

Leaf Stomatal and Mesophyll Resistance. Eight uniform seedlings per treatment either remained undisturbed or were shaken as described for 15 d, at which time Pn and Tr were measured for the terminal leaflet of the third trifoliolate leaf on each plant. Two separate experiments were done to collect gas exchange data, either just before or 25 min after the 1430 shake. To obtain these measurements, the gas flow system described was modified to accommodate a clamp-on leaf cuvette.
The leaf cuvette had a 3.9 × 3.9 × 2.0 cm Plexiglas base and domed top with a radius of 1.8 cm. Leaf chamber temperature was regulated by circulating water from a thermostatted bath through a Plexiglas jacket surrounding the surface of the cuvette. Upper and lower compartments had separate air inlet and outlet, with air mixing enhanced by a multiport inlet into each compartment directing seven small airstreams at various angles over the leaf surface. Flow rate over both leaf surfaces was 1.88 L min⁻¹.

Gas exchange measurements were performed under a saturating PPFD of 1100 μmol s⁻¹ m⁻² provided by a bank of nine 150-W incandescent dichroic reflector flood lights, with radiation filtered through 6 cm of water. Leaf temperature was measured as the average reading of two copper/constantan thermocouples pressed to the abaxial leaf surface. Diffusion coefficients of 0.258 and 0.165 cm² s⁻¹ were used for H2O and CO2, respectively, to calculate boundary layer (r ') and stomatal (r' s) resistances to CO2 diffusion (9). The ratio of these coefficients was raised to the two-thirds power when calculating r' s (16). Boundary layer resistance was estimated using H2O-saturated filter paper (8). A diffusion model was used which involved a parallel circuit consisting of r' s and r' s, in series for each leaf surface, with r' s in series with both parallel circuits (9, 17). Upper and lower compartments of the chamber created by leaflet placement permitted separate measurement of Pn and Tr for each leaf surface. Gas exchange measurements were made for each leaf surface without interrupting air flow through either compartment. Thus, r' s, r' s, r' s, and r' s were measured independently for upper (u) and lower (l) leaflet surfaces, namely, r' su, r' sl, r' lu, and r' ll, as well as r' su, r' sl, r' lu, and r' ll (23). To calculate r' s, CO2 concentration at the chloroplast was assumed to equal the CO2 compensation concentration (17, 18). Compensation concentrations for undisturbed control and shaken plants were determined under saturating PPFD (17).

Data from the pre- and postshake periods for Pn, Tr, r' su, r' sl, r' lu, and r' ll were subjected to analysis of variance and F test at the 0.05 level to determine significant differences between control and shake treatments.

RESULTS AND DISCUSSION

Periodic seismic stress decreased plant dry weight 22% and total leaf area 17% relative to undisturbed controls after 15 d of

<table>
<thead>
<tr>
<th>Growth Parameter</th>
<th>Control</th>
<th>Shake</th>
<th>Difference from Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant dry wt (g)</td>
<td>3.36a</td>
<td>2.62b</td>
<td>-22</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>525a</td>
<td>437b</td>
<td>-17</td>
</tr>
<tr>
<td>Stem length (cm)</td>
<td>20.2a</td>
<td>15.8b</td>
<td>-22</td>
</tr>
<tr>
<td>Specific leaf wt (g cm⁻² × 10³)</td>
<td>3.24a</td>
<td>3.22a</td>
<td>-1</td>
</tr>
<tr>
<td>Specific leaf water content (g H2O g⁻¹)</td>
<td>4.05a</td>
<td>4.55a</td>
<td>+12</td>
</tr>
</tbody>
</table>
FIG. 1. Undisturbed control versus seismic-stressed soybean (Glycine max [L.] Merrill cv. Wells II) after 15 d of treatment. The mechanical stress treatment consisted of 5 min of horizontal agitation on a gyratory shaker at 240 rpm three times daily.

Table II. Effects of Shaking on NAR, LAR, and RGR of ‘Wells II’ Soybean

Values represent NAR, LAR, and RGR over four harvest dates (9, 12, 15, and 18 d of treatment). Controls were undisturbed throughout the experiment while seismic-stressed plants were shaken as described in Table I.

<table>
<thead>
<tr>
<th>Growth Dynamics Parameter</th>
<th>Treatment</th>
<th>Difference from Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Shake</td>
</tr>
<tr>
<td>NAR (g cm⁻² d⁻¹ × 10⁵)</td>
<td>1.23</td>
<td>1.07</td>
</tr>
<tr>
<td>LAR (cm² g⁻¹ × 10⁻²)</td>
<td>1.47</td>
<td>1.62</td>
</tr>
<tr>
<td>RGR (d⁻¹ × 10)</td>
<td>1.80</td>
<td>1.73</td>
</tr>
</tbody>
</table>

treatment (Table I; Fig. 1). Stem length also was 22% less, but shaking did not affect specific leaf weight; there was, however, a trend toward greater specific leaf water content (Table I). Shaken plants also had 3.9% lower RGR, which was due entirely to decreased NAR (Table II). Shaking increased LAR relative to that of undisturbed plants, but increased LAR did not fully compensate for decreased NAR. In a greenhouse study conducted with soybean, seismic stress also decreased RGR, but in that case the effect was due to equal decreases in NAR and LAR (25). In a greenhouse study conducted with ‘Alaska’ pea, periodic seismic stress again reduced shoot RGR (1). As in the present study, this effect was due entirely to decreased NAR. Also, increasing wind speed caused a progressive decrease in RGR and NAR of rice seedlings, in spite of an increase in LAR (30). Thus, seismic stress decreases RGR, due in part or in total to decreased NAR (Table II) (1, 25, 32), which is a measure of photosynthetic efficiency (18).

Seismic stress significantly reduced soybean water loss rate 17 and 15% during the first and second 45-min periods following a 1430-h shake, respectively, relative to that from undisturbed controls (Fig. 2). Shaken plants also had significantly higher leaf water potential (−685 kPa) than did undisturbed control plants (−934 kPa) 30 min after treatment.

Seismic stress caused transitory decreases in net Pn of whole plants (Fig. 3). On day 15 of treatment, Pn started to drop within seconds after the 0930-h shake began, and 5 min after treatment ended Pn was 15% less than that of controls. Reduced Pn lasted for at least 60 min following the shake, at which time Pn averaged 10% less than controls. Subsequent treatments administered during afternoon and evening hours had similar effects on Pn, although degree of response was not as great as during mornings. Recovery to control rate also was more rapid for later treatments, being statistically indistinguishable from controls 50 min after treatment.

Decreases in Pn were investigated further to determine what components of leaf resistance might be affected by periodic shaking. Pn and Tr of upper and lower leaf surfaces were measuated...
ured for 5 min after clamping and equilibrating an attached leaf for 25 min. Although leaf gas exchange rates could not be measured sooner than 25 min after treatment due to the equilibration requirement, Pn of shaken plants remained significantly less than that of unshaken controls for at least 40 min after shaking (Fig. 3). Thus, leaf resistance was determined within the period of maximum plant response. Furthermore, no significant differences in Tr or Pn of upper and lower leaf surfaces between shaken and undisturbed plants carried over from 0930- to the 1430-h shake (Fig. 4). Leaf resistance calculations showed that r_u, r_u', and r_m of seismic-stressed and undisturbed plants were not significantly different just before the 1430-h shake (Table III). Complete recovery of gas exchange rates had occurred from the previous treatment, and even a trend toward lower leaf resistance was obtained for seismic-stressed plants relative to controls. However, 25 min after shaking, Pn of the lower leaf surface had decreased 9.7% (Fig. 5). Transpiration by the lower leaf surface also had decreased 18.7% at that time. More than 2-fold increases in r_u and r_u', of the lower leaf surface may have accounted for the decreased Tr and Pn of shaken plants (Table IV). Neither the 5.4% decrease in Pn nor the 8.3% decrease in

![Figure 4](image-url)  
**FIG. 4.** Effects of seismic stress on mean transpiration and photosynthetic rates of "Wells II" soybean (n = 8) under saturating PPFD (1100 μmol s⁻¹ m⁻²). Measurements were taken on day 15 of treatment 4 h after the 0930-h shake and immediately before the 1430-h shake. Treatments were as described for Figure 1. Bar pairs with different letters are different from each other according to F test at the 0.05 level of significance.

![Figure 5](image-url)  
**FIG. 5.** Effects of seismic stress on mean transpiration and photosynthetic rates of "Wells II" soybean (n = 8) under saturating PPFD (1100 μmol s⁻¹ m⁻²). Measurements were taken on day 15 of treatment 25 min after the 1430-h shake. Treatments were as described in Figure 1. Bar pairs with different letters are different from each other according to F test at the 0.05 level.

**Table IV. Leaf Resistances After Shaking**

Effect of shaking on stomatal resistance to water vapor and CO₂ diffusion by upper and lower leaf surfaces, as well as total leaf mesophyll resistance of "Wells II" soybean (n = 8). Measurements were taken under saturating PPFD (1100 μmol s⁻¹ m⁻²) on day 15 of treatment 25 min after the 1430-h shake treatment. The control treatment remained undisturbed throughout the experiment. Numbers within rows followed by different letters are different from each other according to F test at the 0.05 level of significance.

<table>
<thead>
<tr>
<th>Leaf Resistance Parameter</th>
<th>Treatment</th>
<th>s cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Shake</td>
</tr>
<tr>
<td>r_u</td>
<td>0.93a</td>
<td>1.24a</td>
</tr>
<tr>
<td>r_u'</td>
<td>0.30a</td>
<td>0.83b</td>
</tr>
<tr>
<td>r_m</td>
<td>1.52a</td>
<td>1.93a</td>
</tr>
<tr>
<td>r_u'</td>
<td>0.47a</td>
<td>1.30b</td>
</tr>
<tr>
<td>r_m (1 + u)</td>
<td>6.18a</td>
<td>6.55a</td>
</tr>
</tbody>
</table>

Tr of the upper leaf surface at 25 min were significantly different from controls. Although r_m of shaken plants averaged 6% more than that of controls 25 min after shaking, this trend also was not statistically significant.

Another way to assess whether a given perturbation in Pn is due to an accompanying increase in r_u', is to calculate leaf Ci (5). If a reduction in stomatal aperture occurs with no change in efficiency of CO₂ assimilation, Ci would be expected to decrease as CO₂ continues to be fixed but reduced stomatal aperture allows less CO₂ diffusion into the leaf. However, if carboxylation resistance increases concomitant with stomatal closure, then either no change or an increase in Ci might occur as Pn declines.

In our study, since r_u' increased temporarily but r_m did not, one would not expect Ci to increase in response to an episode of shaking. Based upon calculation of Ci from gas exchange and leaf resistance data collected during this study, Ci of control and treated plants differed by no more than 1.4% immediately before shaking (1.48 × 10⁻² *versus* 1.50 × 10⁻² mol·m⁻², respectively). However, 25 min after seismic stress, an 8.7% drop in Ci had occurred relative to the pretreatment level (1.37 × 10⁻² *versus* 1.50 × 10⁻² mol·m⁻²). During the same period of time, Ci of undisturbed controls fluctuated no more than 0.7%. Thus,
change of Ci in the same direction, and to a similar extent, as the change in Pn for the lower leaf surface is consistent with a role for transitory stomatal aperture reduction as a response to periodic seismic stress. Stomatal transients have, in fact, been reported to be more limiting to CO₂ assimilation than steady state reductions in stomatal aperture (5).

Numerous studies have demonstrated effects of continuous wind on ψw, Tr, Pn, r’w, and r’m under well-watered conditions. Plants generally respond to shaking or wind with a decrease in growth and yield relative to undisturbed plants. In the present study, periodic shaking of soybean increased ψw and decreased Tr (Fig. 2), whereas continuous wind loading has been reported to have the opposite effects in a number of species (6, 7, 11, 12). It has been suggested that these responses occur because leaves of wind-loaded plants lose stomatal control as a result of wind-induced abrasion of the leaf cuticle (11, 12). The resulting water loss was suggested to cause a decline in turgor of epidermal cells, leading to displacement of the normal hydrostatic balance between stomata and surrounding cells. Furthermore, the decreased Pn of continuously wind-loaded Festuca was found to be due entirely to increased r’m, and r’a actually decreased (i.e. stomates opened wider) under continuous wind relative to wind-protected controls (10).

A decrease rather than an increase in Tr following seismic treatment suggests that cuticle abrasion was not a factor in our study. The repeated recovery patterns observed also suggest that stomatal control was not lost. For example, no leaf resistance parameter was still significantly different from control 5 h after the 0930-h shake (Table III), but r’a and r’w both were greater than for controls 25 min after the 1430-h shake (Table IV). Stomatal aperture reduction therefore appears to be an immediate but temporary response of chamber-grown soybean plants to periodic seismic stress.

Whether plants growing in a controlled environment harden off to seismic stress in terms of stomatal transients or decreased NAR or Pn if agitated more frequently than three times per day remains to be determined. Other differences besides intensity, frequency, and duration of seismic disturbance between outdoor and indoor plant growth environments (amount of UV radiation, PAR level, spectral distribution of radiation, temperature and humidity fluctuations, etc.) also may contribute to differences in plant response to periodic seismic treatment. Seismic stress in a greenhouse has been shown to reduce dry weight of soybeans to the same extent as when the plants were grown outdoors (25). On the other hand, controlled shaking of outdoor-grown plants had no additional growth-inhibiting effect. Thus, seismic stress may be a component of wind action, but plant response outdoors apparently hardens off to mild seismic stress because of the total dosage of wind received and/or the confounding action of other environmental factors.

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