Communication

Ethylene Directly Inhibits Foliar Gas Exchange in Glycine max

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ABSTRACT

Gas exchange of individual attached leaves of soybean, Glycine max (L.) Merr cv Davis, was monitored during exposure to exogenous ethylene (C₂H₄) to test the hypothesis that the effects of C₂H₄ on net photosynthesis (Pₙ) and stomatal conductance to H₂O vapor (gₛ) are direct and not mediated by changes in leaf orientation to light. Leaflets were held perpendicular to incident light in a temperature-controlled cuvette throughout a 5.5 hour exposure to 10 microliters per liter C₂H₄. Declines in both Pₙ and gₛ were evident within 2 hours and became more pronounced throughout the exposure period. In C₂H₄ treated plants, Pₙ and gₛ decreased to 80 and 62%, respectively, of the rates in control plants. Because epinastic movement of the leaflets was prohibited by the cuvette, the observed declines in Pₙ and gₛ were a direct effect of C₂H₄ rather than the result of reduced light interception caused by changing leaf angle.

Several studies have examined the effects of C₂H₄ on foliar gas exchange, and decreases in Pₙ and/or gₛ have been reported in a number of species (2–5, 7, 9, 11, 12, 14). Other researchers have reported no change in gas exchange and have concluded that for some or all species, gas exchange is unresponsive (6, 16–18). Interspecific differences in responsiveness to C₂H₄ seem to explain the discrepancies in many cases; species differences have been reported in several studies (4, 5, 7, 9, 11), but differences in technique could also be a factor. For example, it has recently been proposed (16–18) that C₂H₄ has no direct effect on Pₙ or gₛ, but rather that it exerts its effect on gas exchange by causing leaf epinasty and reduced light interception by the leaf surface. For example, Woodrow et al. (17) found no changes in gₛ or Pₙ per unit leaf area in Xanthium strumarium (17) or Lycopersicon esculentum (16) that could not be reversed by returning the epinastic leaves to their original horizontal positions. They further suggest (16–18) that reports of direct effects on gas exchange such as we have reported in soybean (3, 11, 12) have been biased by nonsteady-state conditions and leaf angle changes. They have concluded that reduced light interception and lower photosynthetic induction states of epinastic leaves account for any reported reductions in gas exchange in response to C₂H₄.

It is our hypothesis, however, that C₂H₄ can cause reductions in both Pₙ and gₛ independent of leaf angle changes, at least in those species sensitive to C₂H₄. However, because some previous studies were conducted using whole plant cuvettes (7, 9) or a single leaf cuvette, either applied at intervals (3, 11, 12) or enclosing a compound leaf (7), reduced light interception during measurement or acclimation periods could not be excluded as a factor.

The present study has been designed to reevaluate our previous conclusions on the response of gas exchange in soybean based on these considerations. We used a continuously monitored, steady-state leaf gas exchange system with the measured leaflet held perpendicular to incident light throughout the C₂H₄ exposure. Glycine max (L.) Merr cv Davis was chosen to test the hypothesis because Pₙ and gₛ in this species are sensitive to C₂H₄ (11). Although G. max does respond epinastically to C₂H₄ at concentrations greater than 0.5 μL/L (our personal observation), experimental conditions in the present study precluded such leaf movement.

MATERIALS AND METHODS

Plant Material

Plants of Glycine max (L.) Merr cv Davis (soybean) were grown from seed in 1-L pots in a greenhouse as described elsewhere (12), with maximum PPFD of 600 μmol m⁻² s⁻¹. Studies were conducted 2 to 3 weeks after germination, when the first trifoliate leaves were fully expanded. Seedlings were moved to the exposure system 2 h before initiation of C₂H₄ exposures.

Exposure System

C₂H₄ exposures were conducted in a single pass controlled exposure system for whole plants (11) equipped with ceiling mounted turbulator blades and a 1000 W High Intensity Discharge multivapor lamp (Sylvania Metalarc, M1000). Air entering the chamber passed through charcoal and particle
filters, and humidity was increased with a cool-mist impeller humidifier. Ethylene was supplied from a cylinder (1.5% v/v \( \text{C}_2\text{H}_4 \) in \( \text{N}_2 \), Matheson Gas Products) and injected continuously into the inlet airstream during the exposure. The concentration of \( \text{C}_2\text{H}_4 \) in the chamber was monitored by flame ionization detection (model 400 Hydrocarbon Analyzer, Beckman Instruments). The concentration in the chamber was maintained at 10 \( \mu \text{L} \)/L, which is 10 times that needed to saturate the gas exchange response (3), thereby assuring that the concentration in the leaf cuvette would be well above saturation levels.

Gas Exchange and Exposure Methods

Steady-state gas exchange was measured in an open flow gas exchange system (Walz, Effelterich, FRG) with a temperature-controlled cuvette placed inside the controlled exposure chamber. The distal half of the terminal leaflet of the first trifoliate leaf was clamped gently into the cuvette where it was held perpendicular to incident light throughout the exposure. Leaflet gas exchange was allowed to reach steady state (approximately 40 min) before the initial measurement was recorded. Air for the cuvette was pulled from inside the exposure chamber through a 2-L buffer vessel so that the enclosed leaflet and the whole plant were exposed to the same airstream. Cuvette conditions (mean ± SD) were as follows: PPFD 456 ± 27 \( \mu \text{mol} \) m\(^{-2}\) s\(^{-1}\), air temperature 25.0 ± 0.01°C, leaf temperatures 25.1 ± 0.02°C, and leaf-to-air vapor pressure deficit 1.2 ± 0.3 kPa.

\( \text{C}_2\text{H}_4 \) injection began after the initial gas exchange measurement, and exposure of the whole plant (and the leaflet inside the cuvette) continued uninterrupted for the next 5.5 h. Steady-state \( P_n \) and \( g_s \) were calculated from data collected every 30 min, using \( \text{CO}_2 \) and \( \text{H}_2\text{O} \) differentials, flow rate, leaf and air temperatures, humidity, and projected leaf area (15).

The exposures were replicated with six different plants at each concentration following the same protocol for control (0 \( \mu \text{L}/\text{L} \)) and \( \text{C}_2\text{H}_4 \)-treated (10 \( \mu \text{L}/\text{L} \)). Gas exchange in each plant was expressed relative to its initial rate to normalize for plant-to-plant differences in intrinsic gas exchange. Differences between mean values at each 30 min interval were evaluated with \( t \)-tests. Where variances were unequal, the approximate \( t \) and Satterthwaite’s approximation for degrees of freedom were used to compute the significance probability (10). Significant differences between the two treatment groups (\( P \leq 0.1 \)) are indicated in the figures.

RESULTS

Initial (pretreatment) steady-state gas exchange rates for the two groups of plants did not differ significantly (Figs. 1 and 2). In control plants, \( P_n \) and \( g_s \) either remained constant or rose gradually during the exposure period. In \( \text{C}_2\text{H}_4 \)-treated plants, however, \( P_n \) and \( g_s \) began to diverge from rates in control plants after 2 to 2.5 h and eventually declined substantially (apparent at 2.5–3.5 h in most plants). Gas exchange in each plant was expressed relative to its initial rate; the mean at each 30 min time point is shown in Figures 1 (\( P_n \)) and 2 (\( g_s \)). Significant differences between gas exchange rates in control and \( \text{C}_2\text{H}_4 \)-treated plants (\( t \)-test) were apparent at all time intervals \( \geq 3.5 \) h for \( g_s \) and 4 h for \( P_n \) (Figs. 1 and 2). Rates continued to diverge with increasing exposure time. At 5.5 h, \( P_n \) in \( \text{C}_2\text{H}_4 \)-treated plants was 80% of that in control conditions.
plants and \( g_s \) had declined to 62% of the control rate, indicating that the effect of \( \text{C}_2\text{H}_4 \) on \( g_s \) was more pronounced than that on \( P_n \).

**DISCUSSION**

In the present study, \( P_n \) and \( g_s \) of individual leaflets responded to \( \text{C}_2\text{H}_4 \) exposure while the leaflet was held in place in the cuvette, perpendicular to incident light. These data support the hypothesis that \( \text{C}_2\text{H}_4 \) exerts its effects on gas exchange directly. Although *Glycine max* does respond to exogenous \( \text{C}_2\text{H}_4 \) with epinastic changes in leaf angle, the hypothesis (17) that these changes in leaf angle account entirely for the reductions in \( P_n \) is not supported by these studies. In fact, the magnitude of the reduction in gas exchange reported here is comparable to that reported previously for *G. max* in nonsteady-state single leaf cuvette studies (\( P_n \) reduced to 69–75% of control, \( g_s \) reduced to 32–52% of control; 3, 11, 12). This suggests that most if not all of the response of gas exchange in those studies was also a direct \( \text{C}_2\text{H}_4 \) effect, with variability due to leaf developmental stage or possibly to reduced light interception during the acclimation period (17). By contrast, \( P_n \) in *G. max* measured in a whole-plant cuvette (9) decreased to 38% of control \( P_n \). In such an experimental system, both the range of leaf developmental stages present and a decrease in whole-plant interception of light could have contributed to the greater decline in \( P_n \).

Although a direct effect of \( \text{C}_2\text{H}_4 \) on gas exchange has been demonstrated in *G. max*, the response in other species remains to be resolved. There are a number of reasons which could explain why some other studies have reported no direct effects of \( \text{C}_2\text{H}_4 \) on foliar gas exchange parameters. A primary factor may simply be interspecific variation. For example, *Zea mays* and *Pisum sativum* have been repeatedly found to be unresponsive in a variety of exposure regimes with widely dissimilar experimental objectives (4, 6, 7, 9, 11), which suggests that these two species are \( \text{C}_2\text{H}_4 \)-insensitive. Similarly, contrasting observations at the intraspecific level could reflect differences between cultivars or leaf ages. As in other hormonally mediated processes, changes in tissue sensitivity that take place during development would be imposed upon the intrinsic responsiveness of a species (1, 13).

Differences in methods could also account for contradictory results. An important factor is the timing of the measurement relative to exposure initiation; a 1 to 2.5 h lag in \( \text{C}_2\text{H}_4 \) response has been consistently reported (2, 4, 7, 11, 12). Measurements in 20 min steps up to a maximum of 2.6 h (6) might not detect an effect, although such data do indicate that \( \text{C}_2\text{H}_4 \) does not close stomata by the same mechanism as does \( \text{CO}_2 \) (6). A delay of 24 h (16, 18) or 36 h (17) before taking a single steady-state gas exchange measurement could also lead to a conclusion of unresponsiveness, if gas exchange recovers or if response varies diurnally. The method of \( \text{C}_2\text{H}_4 \) application (gaseous fumigation versus ethephon spray) or the \( \text{C}_2\text{H}_4 \) concentration could also influence results, although reductions in gas exchange have been demonstrated at concentrations as low as 0.1 \( \mu \text{L/L} \) (3), and in response to an ethephon spray (14). The mechanism underlying \( \text{C}_2\text{H}_4 \) action on \( P_n \) and \( g_s \) has not been determined, nor has it been for other \( \text{C}_2\text{H}_4 \)-mediated responses (8). Although gas exchange in some species appears to be insensitive to \( \text{C}_2\text{H}_4 \), this study and previous work indicate that, at least in *G. max*, there is a biochemical basis for the response of gas exchange. Responses of both \( P_n \) and \( g_s \) are ultrasensitive within the range of effective concentrations (3), consistent with a mechanism involving binding to specific receptors. Furthermore, although the decline in \( g_s \) is more pronounced than that in \( P_n \), analyses of light- and \( \text{CO}_2 \) response curves in a previous study showed a loss of intrinsic photosynthetic function apart from the decline in \( g_s \) (12). Although the details of the response mechanism remain unresolved, this study demonstrates that, in responsive species, the effects are not mediated solely through leaf epinasty.

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