A Comparison of the Effects of Chilling on Leaf Gas Exchange in Pea (*Pisum sativum* L.) and Cucumber (*Cucumis sativus* L.)

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ABSTRACT

The effects of chilling on the photosynthesis of a chilling-resistant species, pea (*Pisum sativum* L. cv Alaska) and a chilling-sensitive species, cucumber (*Cucumis sativus* L. cv Ashley) were compared in order to determine the differences in the photosynthetic chilling sensitivity of these two species. For these experiments, plants were chilled (5°C) for different lengths of time in the dark or light. Following a 1 hour recovery period at 25°C, photosynthetic activity was measured by gas exchange (CO₂ uptake and H₂O release), quantum yield, and induced chlorophyll fluorescence. The results show that pea photosynthesis was largely unaffected by two consecutive nights of chilling in the dark, or by chilling during a complete light and dark cycle (15 hours/9 hours). Cucumber gas exchange was reduced by one night of chilling, but its quantum yield and variable fluorescence were unaffected by dark chilling. However, chilling cucumber in the light led to reduced CO₂ fixation, increased internal leaf CO₂ concentration, decreased quantum yield, and loss of variable fluorescence. These results indicate that chilling temperatures in conjunction with light damaged the light reactions of photosynthesis, while chilling in the dark did not.

Chilling-induced photosynthetic inhibition appears to be due to direct inhibition of the photosynthetic process in chilling-sensitive plants, and not simply to the limitation of CO₂ supply by chilling-decreased stomatal conductance (1, 12). Chilling stress in combination with light is more inhibitory to photosynthesis than chilling in the absence of light (8, 11, 15, 16, 19, 20). Further, oxygen is a contributory factor to chilling-induced photosynthetic inhibition in the light, which distinguishes it from the oxygen-independent photoinhibition observed at higher temperatures (14).

In this paper, the effects of chilling on net CO₂ uptake in pea and cucumber are reported. Although there is a substantial literature concerning the effects of chilling on net photosynthesis (6), very few reports make direct comparisons of the effects of whole-plant chilling on a chilling-resistant and a chilling-sensitive species. The goal of these experiments was to compare photosynthetic responses of pea (chilling-resistant) and cucumber (chilling-sensitive) to chilling stress in the light and dark. In addition to using net CO₂ uptake as a measure of photosynthesis, values for stomatal conductance to H₂O, photosynthetic quantum yield, and induced Chl fluorescence were obtained for the two species following chilling treatments, thus providing an in-depth analysis of the effects of chilling on various aspects of photosynthesis.

The results reported here show that cucumber photosynthesis was inhibited by the imposed chilling stress, while pea photosynthesis was unaffected, directly demonstrating the differential sensitivities of these two species to this stress. In addition, our results confirm reports in the literature suggesting that the combination of chilling and light reduce photosynthesis more than dark chilling alone. The results also provide the groundwork at the whole-plant level for further research into the nature of photosynthetic chilling injury (13).

MATERIALS AND METHODS

Pea (*Pisum sativum* L. cv Alaska) and cucumber (*Cucumis sativus* L. cv Ashley) plants were grown from seed in the air-conditioned greenhouses of the Duke University Phytotron (4). Seedlings 10 to 14 d old were placed under control conditions (26°C day/18°C night, 15 h/9 h light/dark regime) in a growth chamber for several days prior to a chilling treatment. Irradiation was provided by a combination of fluorescent and incandescent lamps and was 350 μmol m⁻²s⁻¹ at the sample leaf level in the chamber. Plants were watered twice daily throughout the experiment, in the morning with deionized water and in the afternoon with half-strength Hoagland's solution.

The dark chilling treatment involved dropping the chamber temperature to 5°C during the 9 h dark period. Light and chilling conditions were imposed by maintaining the temperature at 5°C day and night. The chilling treatment was initiated between 1 and 2 h into the light period. Leaf temperature was approximately 10°C in the light when the chamber air temperature was 5°C. Chilled plants were allowed to recover for 1 h at 25°C in the light before gas exchange, quantum yield or induced fluorescence measurements were made. Therefore, the results reported here are concerned with the inhibition of photosynthesis following a chilling treatment. Control measurements were made during the 26°C day/18°C night cycle prior to the onset of the chilling treatments.

Gas exchange and stomatal conductance measurements were made with a Photosynthetic Analysis and Control System 9900 (Data Design Group, PO Box 3318, La Jolla, CA 92038). All measurements were made at 25°C, 350 μL/L CO₂ and 45% relative humidity. Relative humidity was low because the leaf being measured provides all of the humidity in the cuvette in this system. Chilling-stressed cucumber leaves, with reduced stomatal conductance, provided only 45% relative humidity. Light for the measurements was 1000 μmol m⁻²s⁻¹, and found to be saturating. The most recently fully expanded leaf was used for gas exchange measurements. The leaf area sampled was approximately 20 cm² for cucumber and 10 cm² for pea. Leaf internal CO₂ concentrations (Cᵢ) during chilling in both the light and dark were calculated from the gas exchange data (18). The results presented for net photosynthesis and gas exchange are

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3. Abbreviation: Fᵥ, variable fluorescence.
averages of measurements on at least three individual plants. Error bars represent ± one standard error.

A Hansatech oxygen electrode/fluorometer (2, 3) was used to determine apparent quantum yields and induced Chl fluorescence (Decagon Devices, Inc., NW 800 Fisk, Pullman, WA, 99163). Leaf discs with an area of approximately 10 cm² were removed from treated leaves following the 1 h recovery period and placed in the oxygen electrode cuvette. Measurements were made according to the technique of Delieu and Walker (2, 3) and Walker and Osmond (21). Quantum yields were determined from the rates of oxygen evolution observed at the following irradiance levels; 11, 25, 51, 67, 100, and 630 μmol m⁻² s⁻¹. The slope of the line determined by the rates of oxygen evolution at the different irradiances is reported as the quantum yield. A red light emitting diode (Hansatech) was used to excite Chl fluorescence, following 15 to 20 min of dark adaptation.

RESULTS AND DISCUSSION

The purpose of this study was to directly compare the photosynthetic responses to chilling of two species of plants, one of which has been described as relatively chilling resistant (pea) and one which is considered relatively chilling sensitive (cucumber). While an extensive literature exists on the chilling sensitivity and resistance of a number of plant species (for example, see Roughan [17]), very few studies have involved side-by-side comparisons of the photosynthetic responses of whole plants chilled under identical conditions. In addition, the wide variety of chilling conditions that have been employed in other investigations makes comparisons of the relative chilling-sensitivities of plant species difficult.

Controls. The results shown in Figures 1 and 2 indicate that prior to chilling (control day), photosynthesis (a) and stomatal conductance (b) varied during the course of the light period. Values for photosynthesis and conductance were higher at midday than at the end of the control photoperiod in both pea and cucumber.

One possibility for this variation is that stomatal conductance changes may be limiting the supply of CO₂ to the chloroplast, and therefore causing photosynthetic reductions by substrate limitation. If the decrease in photosynthesis at the end of the control photoperiod had been caused solely by reduced stomatal conductance, the Cᵢ would be expected to decline, since the process of CO₂ fixation would decrease the concentration of the substrate until it became limiting. Calculated Cᵢ values show that internal CO₂ concentrations remained the same or increased near the end of the control photoperiod (Figs. 1d and 2d), indicating that stomatal conductance was not limiting the rate of photosynthesis.

In contrast to photosynthesis rates and stomatal conductances, the apparent quantum yield of pea and cucumber did not vary during the course of the control photoperiod. Quantum yield is measured by the amount of O₂ produced by PSII per quantum of light incident to the leaf. It is generally taken as an indicator of the relative efficiency of the light reactions of photosynthesis. Within the leaf chamber of the Hansatech oxygen electrode, the CO₂ concentration was saturating at 5% because of the presence of a bicarbonate buffer (2). Under 5% CO₂ conditions, stomatal conductance has no effect on the rate of photosynthesis, because the external CO₂ concentration is so great that even a very low stomatal conductance will provide sufficient CO₂ to saturate photosynthesis. Since the quantum yield values were stable throughout the photoperiod for control pea and cucumber plants, this indicated that the light reactions of photosynthesis were not being inhibited, and therefore were not responsible for the observed late afternoon decrease in gas exchange observed in both species.

Since the light phase of photosynthesis was functioning properly, as shown by the quantum yield results, and internal CO₂ concentrations were adequate for photosynthesis in our controls, some factor or factors involved in the activity of the Calvin cycle must have caused the observed decrease in photosynthesis. A possible explanation is that sucrose production (source) exceeded the sucrose export capacity (sink) of the leaf by the end of the light period, resulting in a shortage of Calvin cycle substrates within the chloroplast. This would cause CO₂ fixation to slow, resulting in an increased Cᵢ, which would induce the stomates to close, therefore reducing stomatal conductance to H₂O (9).

Chilling in Dark. Little difference was seen in the response of the two species after one night of chilling (Fig. 1). This treatment (9 h dark, 5°C) resulted in lowered midday photosynthetic rates during the following photoperiod (d 1) in both pea and cucumber; however, the photosynthetic rates near the end of the light period approximated end of the day rates in the controls (Fig. 1a). Photosynthetic rates and stomatal conductance (Fig. 1, a and b) were uniformly lower in cucumber than pea. Quantum yield was affected very little by the first night of chilling in either species (Fig. 1c).

After two nights of chilling, species differences in response to the stress were seen. Cucumber photosynthesis and stomatal conductance were both reduced more than after one night of chilling (d 2, Fig. 1, a and b), while pea photosynthetic rates had returned almost to prechilling control values. However, quantum
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Fig. 2. The effects of light and chilling (5°C) on photosynthesis of pea (○) and cucumber (•). (a) Net photosynthesis (Ps), in μmol CO₂ m⁻² s⁻¹, (b) stomatal conductance to H₂O (Cn), in mol m⁻² s⁻¹, (c) quantum yield, and (d) internal CO₂ concentration (Cᵢ), in μL/L.

Yields for both pea and cucumber still remained at control levels after the second night of dark chilling (Fig. 1c, two separate quantum yield determinations are shown for cucumber). This suggests that the light reactions of photosynthesis were not appreciably affected by dark chilling. Since no significant changes occurred in Cᵢ values during dark chilling in cucumber (Fig. 1d), decreased stomatal conductance does not appear to be the cause of the decrease in the rate of photosynthesis. As with the fluctuation in photosynthetic rate and stomatal conductance seen during the control day measurements, it seems likely that some factor or factors involved in the activity of the Calvin cycle must have caused the observed decrease in photosynthesis following chilling in the dark. Source-sink variations, as postulated for the control day results, may be responsible for the inhibition. Chilling temperatures have been shown to affect the levels of metabolites and activities of enzymes in the Calvin cycle (7). Martin and coworkers (10–12) have seen a similar depression of photosynthesis in chilling-sensitive tomato following chilling stress in the dark. Their results also indicate that the inhibition of photosynthesis is not due to decreased stomatal conductance or reduced rate of electron flow through the thylakoid membranes. They have so far been unable to establish the cause of the observed inhibition.

Chilling in Light. Photosynthetic gas exchange in pea was not markedly altered by the light and chilling treatment (Fig. 2). Net photosynthesis, stomatal conductance, quantum yield, and Cᵢ values were close to those of control plants, demonstrating the relative chilling resistance of the photosynthetic apparatus of this species.

In contrast, the photosynthetic gas exchange response by cucumber was affected much more by the light-chilling stress than dark chilling. Net photosynthesis was greatly reduced in comparison with the midday control values recorded the first day of chilling (d 1, Fig. 2a). Furthermore, after the initiation of chilling, conductance values remained steady, while net photosynthesis continued to decrease (Fig. 2b). The quantum yield for cucumber markedly decreased during the light and chilling treatment (Fig. 2c), contrasting sharply with the lack of change observed with dark chilling (Fig. 1c).

The effects of chilling in the light on Cᵢ in cucumber leaves (Fig. 2d) shows that Cᵢ increased during chilling in the light, indicating that the decrease in photosynthesis was not due to a decrease in available CO₂ caused by a decrease in stomatal conductance, but that the photosynthetic reactions were being inhibited by the stress treatment. In contrast to control and dark chilling conditions, the stomates did not respond to the increase in Cᵢ by reducing conductance to H₂O. Figure 2d shows that pea leaf Cᵢ values were relatively stable during chilling in the light. Although pea Cᵢ was higher than the control value at the end of the first day of chilling in the light, by the next morning it had returned to control levels.

Chl fluorescence measurements also indicated that cucumber was damaged by the light and chilling treatment (Fig. 3). Dark chilling did not affect F₅ in either pea or cucumber (results not shown). The variable component (Фᵥ) of the induced fluorescence curve was substantially reduced by 10 h of chilling in the light, and almost completely absent after 27 h of chilling (1 d and 1 night at 5°C). Pea showed essentially no effect of a light and chilling stress on the variable component during the same 27 h period (Fig. 3). A reduction in the level of Фᵥ is generally interpreted to mean that PSI has been damaged, and this view is supported by the quantum yield results. Such reductions also appear as a response to several other environmental stresses including water stress and photo-inhibition (5).

A recent report by Yakir et al. (22) showed a similar response to chilling in the light in tomato leaves. While photosynthetic rates were inhibited by chilling in the light and dark, both quantum yield and Fᵥ were decreased only by chilling in the light. These results suggest that chilling in the light may have a common effect in a number of chilling-sensitive species, and that chilling in the light is more damaging to components of PSII than chilling in the dark.

In summary, the results of the side-by-side comparison pre-
sented in this paper show that pea was photosynthetically resistant to the stress conditions utilized in this study, while cucumber photosynthesis was severely reduced by identical conditions. In addition, the combination of chilling and light caused greater photosynthetic damage in cucumber than chilling in the dark. This is in agreement with the results of Taylor and Rowley (19) who investigated other chilling-sensitive species. The photosynthetic loss caused by chilling in the light in the experiments reported here correlate with damage to the light reactions of PSI. It is therefore important to establish how selective damage occurs and how chilling-resistant species are protected from this sort of injury when exposed to chilling in the light.

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