Temperature Dependence of Photosynthesis of Bean Plants 
as Affected by Decenylsuccinic Acid

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Photosynthesis of most plants decreases rapidly when the leaf temperature drops below 10°. In contrast, however, several frost resistant plant species still perform appreciable photosynthesis at temperatures below 5° (8).

Vetuhova (14, 15) demonstrated that photosynthesis at low temperature was related to winter hardiness. When leaves were cooled, she observed a smaller depression of photosynthesis in winter hardy wheat varieties than in less hardy varieties.

Since a solution of decenylsuccinic acid applied to the roots induces considerable frost resistance in young bean plants (7), I determined whether this compound could change the temperature dependence of photosynthesis and report the results here.

Materials and Methods

Young bush bean plants (Phaseolus vulgaris, var. Bountiful) were grown in vermiculite in the greenhouse. After germination the treated plants were irrigated daily with 100 ml of a 10⁻³ m solution of decenylsuccinic acid (CH₃(CH₂)₆•CH = CH•CH₂•CH(COOH) CH₂COOH). The chemical (Humphrey Chemical, Inc., North Haven, Conn.) was dissolved in a few drops of hot ethanol; then it was added to distilled water and the slightly cloudy liquid was used for irrigation. The untreated plants received an equal irrigation with water.

CO₂ fixation was measured by infrared gas analysis. The air flow through the leaf chamber was 3.25 liter per minute. The air was stirred inside the leaf chamber (5 × 22 × 30 cm inside dimensions). Experimental plants were used when only the first pair of leaves had developed and the rate of photosynthesis of these leaves was measured. A description of the apparatus has been given by Moss (9). Leaf temperature was measured with a thermocouple, stuck into one of the veins of the leaf. The leaf temperature was varied by changing the temperature of the water circulating through the double-walled chamber. The leaves were exposed to 350 ppm CO₂ in the incoming air. The CO₂ content of the outgoing air was at most 20 ppm lower. The radiant flux of 12 × 10⁴ erg • sec⁻¹ • cm⁻² in the range of 400 to 700 μm was produced by incandescent lamps with a water filter. Increased light would not increase photosynthesis.

Water uptake was measured with a potometer which contained the roots. Water uptake by the roots is the same as water loss from the leaves if leaf water content does not change.

Experimental Results and Discussion. Treatment with decenylsuccinic acid greatly inhibited expansion of the first pair of leaves, which were thick and dark green. When the treatment was discontinued, the first pair of leaves expanded slowly, and, after about 7 days, their area approached the area of the leaves of the untreated plants.

Five days after treatment began, some of the plants were exposed to a frost of −3° for 1 hour. The treated plants showed no frost damage, while untreated plants were killed. Evidently the chemical penetrated and protected most of the leaf cells.

Photosynthesis of plants treated for 8 days was hardly affected by temperature (fig 1). In some plants a small decline was observed below 10°, in others no effect at all.

Photosynthesis of untreated plants decreased rapidly as they were cooled below 20° and was very slow at 5°. A detailed description of experiments with untreated plants is given below. In some experiments photosynthesis of an untreated bean plant fluctuated for 30 minutes before a steady rate was reached. The curve in figure 1 demonstrates the effect of temperature on photosynthesis when the rate of such a plant remains steady.

In other experiments photosynthesis of untreated bean plants continued to fluctuate for more than 2 hours, confirming Howe's observations (5). Fluctuations were smaller at lower temperatures. Maxima and minima of photosynthesis of such a bean plant are also shown in figure 1 and indicate roughly the same response to temperature as that of photosynthesis when the fluctuations in rate are not observed.

Dark respiration of treated and untreated plants increased with temperature; the Q₁₀ was 2.0. Although dark respiration of leaves of treated plants was 30% higher than that of untreated plants per unit area, the respirations per unit dry weight of the leaves were not significantly different. Dark respi-

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ration was always less than 1 mg CO₂ per hour per 100 cm². So only a minor correction of the measured rate of photosynthesis was necessary.

Two possible explanations of the effect of decenylsuccinic acid upon photosynthesis are obvious. One might speculate that at lower temperatures CO₂ fixation of untreated plants decreases because activity of the enzymes in the chloroplast is decreased. Then to explain the above results one would have to assume that decenylsuccinic acid increased the activity in the cold of the various enzymes involved in photosynthesis. So far, however, only inhibitory effects of decenylsuccinic acid on enzymatic processes have been observed. Respiration of excised root tips (G. Yelenoski, private communication) as well as respiration of pea stems (D. Penny, private communication) is decreased after treatment. Inhibition of photophosphorylation and of reduction of triphosphopyridine nucleotide of spinach chloroplasts in the light by decenylsuccinic acid has been observed by P. A. Siegenthaler and L. Packer (private communication). The slightly lower rate of photosynthesis of treated leaves could be explained by these observations. Another explanation for the unchanged rate of photosynthesis at low leaf temperatures has to be sought.

The second possible hypothesis is that treatment with decenylsuccinic acid increases CO₂ permeability of the leaf cell membranes, especially at low temperatures. Treatment with decenylsuccinic acid does increase water permeability of the root cell membranes of young bean plants and does decrease the temperature dependence of water permeability (6). The increased number of pores available for water transport could permit more rapid transport of CO₂, and research on the red blood cell demonstrates that a very high membrane permeability, for both water (1) and for dissolved gases, O₂ and CO₂ (10) is possible.

To test this possibility, the resistances to transport of CO₂ into the leaf were analyzed by measuring leaf temperature, transpiration, and photosynthesis. The resistances to transport of CO₂ of the external air and of the stomata (rₐ + rₑ) and of the mesophyll (rₑₑ) can be calculated by means of the formula:

\[ rₐ + rₑ = \frac{[H₂O]_{int} - [H₂O]ₐ}{T} \frac{D₁₂o}{D₂co₂} \]

and \[ rₑₑ = \frac{[CO₂]ₐ - [CO₂]ₑₑₐir}{P} \]

where \([H₂O]_{int}\) and \([H₂O]ₐ\) are the water vapor concentrations (cm³ vapor · cm⁻² · air) inside the leaf and in the external air, and \(D₁₂o\) and \(D₂co₂\) are the diffusion coefficients for water vapor and CO₂ in air, 0.24 and 0.14 cm² · sec⁻¹ respectively. \(T\) is transpiration (cm³ H₂O vapor · cm⁻² · sec⁻¹), \([CO₂]ₐ\) and \([CO₂]ₑₑₐir\) refer to the concentrations (cm³ CO₂ · cm⁻² · air) of the external air and of the chloroplasts, and \(P\) to photosynthesis (cm³ CO₂ · cm⁻² · sec⁻¹). The water vapor inside the leaf is assumed to be saturated, and the CO₂ concentration of the chloroplasts is assumed to be zero; these assumptions are discussed at length by van den Honert (3, 4) and Gaatra (2). Of course one might assume a significant CO₂ concentration of the chloroplasts which decreases with temperature. This implies an increase in transport resistance for CO₂ with temperature for the treated leaves. I think this is unlikely since diffusion as well as active transport of CO₂ will increase with temperature within the temperature range studied.

An example of the calculation of the values of \(rₐ + rₑ\) and \(rₑₑ\) will be given for the untreated plant at 15° leaf temperature. The transpiration rate was 0.195 g · h⁻¹ for a leaf area of 39 cm². This corresponds to 0.005 g · cm⁻² · h⁻¹ or 0.00183 cm³ H₂O vapour · cm⁻² · sec⁻¹. The concentration of the water vapour of the air entering the leaf chamber was 3.1 g · m⁻³ (air temperature 14.6°, relative humidity 25 %). Taking into account the amount of water produced by the leaf (0.195 g · h⁻¹) and the rate of the air flow through the chamber (3.25 liter · min⁻¹), the concentration of the water vapour of the air was corrected to 4.1 g · m⁻³. The concentration of the saturated water vapour inside the leaf was 12.8 g · m⁻³, so the difference in density between leaf and air was 8.7 g · m⁻³ or 0.0114 cm³ · cm⁻³. The value of \(rₐ + rₑ\) is then 6.24 sec · cm⁻¹ for water vapour and 10.7 sec · cm⁻¹ for CO₂ diffusion. The rate of photosynthesis was 6.9 mg CO₂ · dm⁻² · h⁻¹ or 10.3 × 10⁻⁶ cm³ CO₂ · cm⁻² · sec⁻¹ in air with 350 ppm CO₂. The value of \(rₑₑ\) is then 34.1 sec · cm⁻¹ and the value of \(rₑₑₑₑ\) 23.4 sec · cm⁻¹.
The effect of the root treatment on the bean stomata was small. In a leaf disc assay, on the other hand, decenylsuccinic acid inhibits opening of stomata (17). Evidently with application to the roots, no appreciable amount of the chemical had reached the guard cells, which are found only in the lower epidermis. Transport of the chemical through the spongy parenchyma to the lower epidermis probably is slow. This explanation can be tested in a tobacco leaf, which has stomata on the upper surface in close contact with the densely packed palisade parenchyma and stomata on the lower surface, separated by a layer of spongy parenchyma. The cut ends of petioles of excised tobacco leaves were placed in 10⁻⁴ M decenylsuccinic acid. One hour after beginning the treatment, the stomata of the upper surface were closed, whereas those of the lower epidermis showed their normal opening response, even 24 hours later. Since transport of decenylsuccinic acid is evidently rapid from vessel to upper guard cells of a dichotomous leaf, but very slow to the lower guard cells, slowness of transport may be the reason the interior of the bean leaf was modified with little effect upon its hypostomatus guard cells.

The value of \((r_a + r_s)\) was fairly constant between 7 and 30°C, contrary to observations on other plants (13, 16). Apparently stomata of bean plants are not much affected by temperature in this range. Above 30°C the value of \(r_a + r_s\) increased rapidly, indicating closing of the stomata. Differences between \((r_a + r_s)\) in treated and untreated plants were small, except that at temperatures below 10°C stomata of the untreated plants tended to close.

A striking effect of the treatment on the resistance to transport from the mesophyll cell wall to the chloroplast \((r_{me})\) was observed (fig 2). The \(r_{me}\) value of the treated plants was not affected by temperature. The curves of the untreated plant and of the plant whose treatment was discontinued 13 days after it began and 5 days before \(r_{me}\) was observed showed a temperature dependent part with the \(Q_{10}\) of about 0.25. Above a certain critical temperature, depending on the treatment, no effect of temperature on \(r_{me}\) could be observed. This minimum value of \(r_{me}\) was about 9 sec cm⁻¹, which value is in agreement with other observations (2, 11, 12). It varied from 6 up to 17 sec cm⁻¹ in my experiments. The critical temperature for minimum \(r_{me}\) is less than 5°C for

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**Fig. 2.** Effect of temperature on photosynthesis, transpiration, the resistance to transport of CO₂ of the stomata and the external air \((r_a + r_s)\) and the resistance to transport of CO₂ of the mesophyll \((r_{me})\). •—•, Untreated plant, leaf area 39 cm². ○—○, Plant with treatment discontinued 5 days before the experiment, leaf area 32 cm². ▲—▲, Treated plant, leaf area 20 cm².
treated plants, 15° for plants with discontinued treatment, and about 25° for untreated plants.

Thus the effect of decenylsuccinic acid on the $r_{me}$ value can apparently be explained as an increase of CO$_2$-permeability of the mesophyll cell membrane. It follows that the minimum value of $r_{me}$ (9 sec$^{-1}$ cm$^{-1}$) refers to the resistance to transport of CO$_2$ inside the cytoplasm, while the mesophyll resistance in excess of 9 sec$^{-1}$ cm$^{-1}$ is located in the plasmamembrane itself. Finally, the results indicate that the reduction in photosynthesis of untreated bean leaves at low temperatures is due to an increased resistance to transport of CO$_2$ rather than a decreased enzymatic activity of the chloroplasts.

Summary

Photosynthesis of bean leaves (Phaseolus vulgaris var. Bountiful) is markedly decreased by temperatures below 20°. When the roots of the plants are watered daily with 10$^{-3}$ M decenylsuccinic acid, no decrease in photosynthesis is observed from 25° to 5°. An analysis of the resistances to transport of CO$_2$ has been made and the effect of the treatment is explained as an induced increase in carbon dioxide permeability of the plasma membranes of the mesophyll cells.

Literature Cited