Plant Physiol. (1972) 49, 275–276

Short Communication

No Stomatal Response to Ethylene[1]

Received for publication May 25, 1971

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Stomata are sensitive to changes in the intercellular carbon dioxide concentration (2); we do not know the underlying mechanism. CO_2 competitively inhibits many morphogenetic effects of ethylene (1, 3). We therefore studied whether ethylene in turn acts as a competitive inhibitor of stomatal responses to CO_2 and supplemented our studies by applying two other gases, allene (C_3H_4) and nitrous oxide (N_2O), which due to the electronic configuration of their molecules could also compete for the CO_2 receptor in the guard cell.

MATERIALS AND METHODS

Plants. *Zea mays* L., Pioneer var. 395, was grown in a growth chamber in Hoagland solution. The fifth leaves, counted in the sequence of their emergence, were used when plants were 20 to 25 days old. *Pisum sativum* L., var. Progress 9, was grown also in a growth chamber, but in soil.

Gas Mixtures. CO_2-free air was scrubbed with a mixture of $KMnO_4$ and KOH to remove any traces of unsaturated volatile organic compounds. Known amounts of CO_2 were added by gas mixing pumps. Ethylene, allene, or nitrous oxide were bubbled into the air stream through a capillary tube dipping into a 5-ml beaker containing 2 ml of paraffin oil at the bottom of a gas mixing flask. Bubble sizes were determined before and bubble frequencies during the experiments. CO_2-free air as well as the gas mixtures were humidified at room temperature and directed into any one or more of six leaf chambers by means of a series of solenoid valves.

Measurement of Stomatal Aperture and Photosynthesis. Leaf sections were clamped horizontally between pairs of Plexiglas chambers and submerged into a bath kept at 25 C (4). The exposed leaf area was 2.5 cm^2 per pair of chambers; air flow through each of the lower chambers was 19 l hr^{-1}, and the pressure difference between lower and upper chambers was maintained at 10^4 dyne cm^{-2}. The resulting air flow through the leaf sample was a measure of stomatal aperture; it was recorded by means of photoelectric transducers (4). In some experiments the lower chambers were not flushed continuously but supplied with the desired gas mixtures at 10^4 dyne cm^{-2} pressure against the upper chamber. The gas passing the leaf samples was first conducted through the flow meters and then into an infrared gas analyzer for the measurement of CO_2 depletion in the gas stream. The product of CO_2 depletion and gas flow through the leaf gave the rate of net CO_2 uptake.

Ethylene Contamination. The ethylene concentration in the closed experimental system, consisting of a pair of Plexiglas chambers, silicon rubber gaskets, and Tygon tubing (formulation B-44-3) was measured by gas chromatography; it reached about 0.06 μl/l within 30 min after stopping all air movement, with or without leaf samples. At an air flow of 0.2 ml sec^{-1} through the experimental system ethylene could not be detected ($[C_2H_4] < 0.01$ μl/liter). Experiments were therefore only evaluated if the air flow through a leaf sample exceeded 0.2 ml sec^{-1}.

No ethylene could be detected by gas chromatography in the bottled CO_2, allene, and nitrous oxide. From the detection limits of the instrument the following maximal ethylene contents of the administered gas mixtures were computed: CO_2: $< 4 \times 10^{-6}$ μl/liter, C_3H_4: $< 4 \times 10^{-3}$ μl/liter, N_2O: $< 10^{-2}$ μl/liter.

Illumination. A xenon arc lamp provided white light at a quantum flux density of 97 nE cm^{-2} sec^{-1}; in the experiment involving the measurement of CO_2 uptake the quantum flux density was 150 nE cm^{-2} sec^{-1} of photosynthetically useable light.

RESULTS

Ethylene Applied in the Light. Leaf sections of *Zea mays* were exposed to 288 μl/liter CO_2 and white light. When the stomates had reached a steady state aperture ethylene was added to the air stream to give a sequence of $0, 1, 10^2, 10, 10^3, 10^2, 10^4, 10^3, 10^5$, 0 μl ethylene per liter air in steps at 15- to 20-min intervals. Neither air flow through the stomates nor net photosynthesis departed from the controls. The experiment was repeated with CO_2-free air, again without effect of ethylene on stomatal aperture.

Similar experiments were done while stomates were opening. Velocities of stomatal opening did not change when the ethylene concentration in the air (containing 300 μl/liter CO_2) was varied between 0 and as much as 10^4 μl/liter. Air flow through the leaves did not even change when 10^3 μl/liter ethylene flushed the leaf tissue for as long as two hours.

Ethylene Applied in Darkness. Stomata of *Zea mays* were opened by illumination and kept open during a following period of darkness by continuously flushing CO_2-free air through the leaf samples at 0.6 to 0.8 ml cm^{-2} sec^{-1}. When the CO_2-free air was replaced by air containing 10 or 20 μl/liter CO_2, the stomata reduced their aperture but still allowed an air flow of about 0.2 ml cm^{-2} sec^{-1}. Ethylene was then added to give concentrations between 1 and 10^4 μl/liter. Ethylene did not cause changes in the air flow through the stomates, regardless of the CO_2 concentration (0, 10 or 20 μl/liter).

Allene and Nitrous Oxide. Experiments and results were similar to the ones conducted with ethylene. Stomates of *Zea mays* did not respond to applications of allene or nitrous oxide at concentrations between 1 and 10^4 μl/liter.

[1] Research supported by the United States Atomic Energy Commission under Contract AT(11-1)-1338.

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Effects of Ethylene on Stomates of *Pisum sativum*. Experiments similar to those conducted with *Zea mays*, but less extensive, were performed on pea leaves, with equally negative results.

DISCUSSION AND CONCLUSION

The stomates of *Zea mays* and of *Pisum sativum* did not respond to ethylene concentrations between 1 and 10^5 μl/liter. The mechanism of CO_2 action on stomata is, therefore, different from that which competitively inhibits morphogenetic responses to ethylene. The absence of any response to nitrous oxide confirms earlier findings of Slatyer and Jarvis (5), and stomatal insensitivity to all three gases applied, ethylene, allene, and nitrous oxide, suggests that the CO_2 receptor in the guard cells is specific for CO_2. Our results, however, do not rule out the possibility that hydrocarbonate ion is the molecular species that causes stomatal closure in response to CO_2.

Acknowledgments—We thank Dr. D. Simmons, Department of Horticulture, Michigan State University for doing the gas chromatography for us, and Miss Freia Schulz-Baldes and Mr. Douglas Bierer for skillful technical assistance.

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