Fusicoccin and Air Pollutant Injury to Plants

EVIDENCE FOR ENHANCEMENT OF SO₂ BUT NOT O₃ INJURY

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ABSTRACT

Garden peas (Pisum sativum L. cv Alsweet) and a tomato mutant (Lycopersicon esculentum Mill. var flaca) were sprayed with fusicoccin, a fungal toxin affecting membrane transport properties, before exposure to SO₂ or O₃. Tomatoes treated with 10 micromolar fusicoccin and exposed to SO₂ (0.6 microliter per liter for 2 hours) exhibited twice as much foliar necrosis as untreated plants exposed to SO₂. Peas treated with fusicoccin and exposed to SO₂ (0.7 to 1.0 microliter per liter for 2 hours) exhibited 2 to 6 times more injury than untreated plants exposed to SO₂. Peas treated with fusicoccin and exposed to O₃ had less injury than untreated plants exposed to O₃ (0.1 to 0.3 microliter per liter for 2 hours). Several lines of evidence suggested that the fusicoccin enhancement of SO₂ injury is not the result of increased gas exchange, i.e. the tomato mutant has permanently open stomata under all conditions, and in peas fusicoccin had no effect on SO₂ or H₂O flux in plants exposed to 0.12 microliter per liter SO₂. However, a 21% greater leaf conductance in fusicoccin treated versus untreated plants indicated the possibility of some differences in gas exchange for peas exposed to 1.0 microliter per liter SO₂.

The phytotoxicity of SO₂ and O₃ is determined by pollutant uptake, detoxification, and metabolism at cellular perturbation sites (17, 18). The cellular metabolism of the pollutants is especially important since gas-phase conductance alone cannot fully explain plant response to either SO₂ or O₃ (5, 15, 17, 18). Sulfite and bisulfite are the likely SO₂ metabolites transported into cells since the gas is rapidly hydrated following dissolution into water on cellular surfaces (12). The mechanism and importance of sulfate transport into cells in relation to injury has not been directly investigated; however, Bressan et al. (1) suggested that intraspecific variability in SO₂ sensitivity in cucumber resulted from differences in the uptake and transport of SO₂ and sulfite across the plasma membrane. Ozone is less water soluble than SO₂ but may be transported into cells as O₃ (10) or as free oxyradicals which can be produced when O₃ dissolves in water at high pH (5).

The fungal toxin FC² is widely used in studying membrane transport processes. Marrè (9) suggested that FC activated a plasma membrane system which uses ATP for H⁺ extrusion with an accompanying hyperpolarization of the transmembrane electric potential difference. In the guard cells, H⁺ extrusion is coupled with K⁺ uptake stimulating increased cell turgor and stomatal opening (9).

Cation uptake stimulated by FC was associated with the cellular uptake of anions including chloride and sulfate in root or stem segments (9). Sulfate transport (8) and possibly the transport of other anions such as sulfite may occur as a result of passive diffusion associated with cation influx, or may be associated with active (ATP dependent) uptake processes. In a previous study, FC was used to ensure similar stomatal conductance in the light and dark (11); however, the direct effect of FC on SO₂⁻ and O₃⁻ induced injury was not evaluated.

This study used FC to investigate the possible role of cellular transport processes in SO₂ and O₃ phytotoxicity. To separate the effects of FC on cellular transport processes from effects on stomatal conductance, a tomato mutant (Lycopersicon esculentum Mill. var Flaca) was used which has permanently open stomata (7), and peas (Pisum sativum L. cv Alsweet) were exposed in the light when leaf conductance was assumed to be at maximum for both FC treated and untreated plants.

MATERIALS AND METHODS

Peas (Pisum sativum L. cv Alsweet) and the tomato mutant (Lycopersicon esculentum Mill. var Flaca) were grown from seed in an artificial medium (Promix BX³) and watered with North Carolina State University nutrient solution (11). Peas were cultured in controlled environment chambers at light/dark air temperatures and RH of (20.7 ± 0.5°C)/(19.8 ± 1.0°C) and (76 ± 14%)/(90 ± 14%), respectively, and photosynthetic photon flux density of 301 ± 18 μmol m⁻² s⁻¹ for 16 h/d. Tomatoes were cultured in charcoal-filtered greenhouses for 14 d before transfer to chambers with the same environmental conditions as the peas. Plants were sprayed until runoff with FC (10 μM) using an air brush (model 250, Badger Air Brush Company, Franklin Park, IL) and N₂ as a propellant 24 h before pollutant exposure. The FC was dissolved in 0.1 ml ethanol and brought to volume with distilled H₂O and one or two drops of Triton X-100 per 50 ml. The FC concentration was selected as the minimum concentration causing a large increase in the dark conductance to H₂O vapor (11). Control plants were sprayed with a similar solution without FC.

Plants were exposed to SO₂ and O₃ when peas had six fully expanded leaves at 19 to 21 d after seeding, and tomatoes had six to eight leaves greater than 2 cm in length at 26 to 30 d after seeding. Exposures were conducted in small chambers housed in controlled environment chambers (Sherer model GL37-14) (5). Experiments were conducted in the light with environmental conditions similar to those used for unexposed plants.

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2 Abbreviation: FC, fusicoccin.

3 Mention of trade names or commercial products does not constitute endorsement or recommendation for use.
conditions the same as the preexposure plant growth conditions except the air temperature was adjusted to maintain a leaf temperature of approximately 22°C (measured with a fine wire thermocouple on the abaxial leaf surface). Sulfur dioxide (1% in N₂) was metered into the exposure chambers and monitored with fluorescent analyzers (ThermoElectron series 43 and Monitor Labs model 8850). Ozone was generated by passing air over an UV light source and monitored with UV absorption analyzers (Dasibi model 1003AH). Peas were exposed to three levels of SO₂ (0.7, 0.9, 1.0 μl ⁻¹) and O₃ (0.1, 0.2, 0.3 μl ⁻¹), while tomatoes were exposed to only SO₂ (0.6 μl ⁻¹). Plants were moved into the chambers 1.5 h after initiation of the light period and were in the chambers for 2 to 2 h before and after the 2-h exposure. Corresponding control plants received identical treatment except that they were exposed to clean air.

Fluxes of H₂O, O₃, and SO₂ were measured using a whole-plant gas-exchange chamber (11, 14). The H₂O flux was derived from the change in the dewpoint measured with a hygrometer (ElectroMech CTE 84 DR) calibrated at 0°C over ice. Sulfur dioxide, from a permeation tube housed in a constant-temperature oven, was metered into the incoming air stream and monitored with flame photometric analyzers (Meloy models 160 and 185) equipped with H₂S scrubbers. Ozone generated by irradiating the incoming air stream with UV light was monitored with UV absorption analyzers (Dasibi model 1003AH). Both monitors were calibrated prior to use with standards traceable to the National Bureau of Standards. Pollutant concentrations were measured at the chamber inlet and outlet. The change in gas concentration, as it passed through the chamber, was corrected for the sorption of SO₂ and O₃ to the chamber surfaces determined under the same conditions of light (320 μmol m⁻² s⁻¹), air temperature (21°C), dewpoint (15.5°C), and CO₂ concentration (372 μl ⁻¹) as when a plant was present (11, 14). Flux measurements were made in the light. The fluxes of H₂O, SO₂, and O₃ were derived using an analog model (15, 18). The fluxes were approximately constant during the hour prior to measurement.

Leaf injury (necrosis) on the adaxial surface was visually evaluated in 5% increments 3 d after exposure and averaged for all leaves unfolded on the plant at the time of exposure. The injury estimates on plants exposed to the pollutant and FC was adjusted by subtracting the small amount of injury (2 to 3%) on plants exposed to FC alone. Injury from FC to pea leaves generally consisted of a brown necrosis, chlorosis, and epinasty (11). For the highest SO₂ concentration, water vapor conductance was measured on the fourth true leaf of peas using an automatic transient diffusion porometer (Delta T AP-3). The porometer was calibrated with a plate providing standard levels of H₂O vapor conductance from water-saturated filter paper. Adaxial and abaxial conductances were measured on opposite leaflets and total leaf conductance determined by adding conductance values for the leaflets. Leaf conductance measurements were taken during the last 10 min of each 2-h exposure through glove ports on the exposure chambers.

For these studies, two exposure levels (pollutant and controls) were used; half of the plants at each exposure level were sprayed with FC and half with the same solution minus FC. The entire experiment was repeated yielding a randomized block split-plot design with 12 (tomato) or 8 (pea) observations per treatment (13). Analyses of variance and 1-tailed t tests were used to determine differences among means.

RESULTS AND DISCUSSION

Tomatoes treated with FC and exposed to SO₂ had much greater foliar injury than plants not treated with FC. Plants treated with FC had an average of 21% leaf area necrosis compared to only 3% for untreated plants (SE of 4 with 12 observations, statistically significant at p < 0.05). Tomato plants treated with FC but not SO₂ showed some leaf epinasty. Peas treated with FC and exposed to SO₂ in the light displayed 2 to 8 times more leaf injury than plants not treated with FC (Fig. 1A). The increase in injury for FC treated versus untreated peas was statistically significant (p < 0.05) at 0.9 and 1.0 μl ⁻¹, but not at 0.7 μl ⁻¹ SO₂. In contrast to the results from the SO₂ exposures,
peas treated with FC and exposed to O₃ tended to exhibit less injury than plants only exposed to O₃ (Fig. 1B). However, these differences in injury were not statistically significant at p < 0.05 level for any O₃ concentration.

The increased SO₂ toxicity in tomatoes and peas treated with FC could have resulted from increased SO₂ uptake into the plants. In previous studies, foliar injury in general was shown to increase with increasing SO₂ uptake (3, 16). However, several lines of evidence suggested increased pollutant uptake may not be responsible for the increased injury with FC. In the tomato mutant, the stomata remain open under conditions that induce closure in normal tomato plants (7), which would ensure similar SO₂ uptake in FC treated and untreated plants. In peas exposed to a low concentration of SO₂ (0.12 μl 1⁻¹), SO₂ fluxes were similar for both FC treated and untreated plants (Table I). Water vapor flux data also indicated that stomata, which are important determinants of pollutant flux (17, 18) were open to a similar extent in both FC treated and untreated plants. Furthermore, FC enhancement of injury in peas occurred in the light when stomatal opening should be at maximum for both FC treated and untreated plants. At the highest concentration of SO₂ (1.0 μl 1⁻¹) there may have been greater SO₂ flux to leaves of FC treated versus untreated plants as shown by the 23% greater stomatal conductance (Table II). Leaf injury on FC-treated plants was >2× that for untreated plants.

In contrast to SO₂, the trend to reduced O₃ injury in peas treated with FC may be the consequence of decreased O₃ uptake as shown by the decreased O₃ flux at 0.11 μl 1⁻¹ O₃ (Table I), even though this decrease was not statistically significant.

Fusicoccin is known to alter membrane transport processes (8, 9) and it is likely that its influence on air pollution-induced injury is related to this fact. The primary effect of O₃ appears to be an alteration in the plasma membrane itself, affecting permeability and the loss of ions with subsequent effects resulting as consequences of the membrane impairment (5, 18). This mechanism suggests that FC effects on transport processes would not influence plant response to O₃ as the injury does not depend on membrane transport of O₃.

In contrast, SO₂ or its metabolites exert their primary impact within the cell (5, 17). Thus, the FC enhancement of SO₂ injury is most likely the consequence of FC stimulating the transmembrane movement of SO₂ or one of its metabolites across the plasma membrane. This hypothesis is consistent with the suggestion of Bressan et al. (1) that transmembrane movement was the key factor controlling SO₂ injury. Sulfite transport across the plasma membrane has not been studied. However Hampp and Ziegler (4) found a single translocator protein involved in sulfite and sulfate transport across the inner chloroplast membrane. A similar translocator may be present in the plasma membrane regulating both sulfate and sulfite transport. Cram (2) demonstrated that sulfite transport across the plasma membrane was an active process, suggesting that the FC stimulation of sulfite movement across membranes (8) may be associated with its effects on membrane transport. By a similar mechanism, FC could stimulate sulfite transport across the plasma membrane thereby increasing the concentration of toxic metabolites at sensitive metabolic sites within the cell.

In conclusion, these studies demonstrated that FC enhances SO₂-induced foliar injury and that the increased injury may be separate from effects of FC on stomata and gas exchange. Additional research needs to be conducted to precisely evaluate stomatal responses and SO₂ uptake at high concentrations of SO₂ in FC treated versus untreated plants. Research also is needed to conclusively establish the metabolic basis for the increased injury; however, increased sulfite or bisulfite transport into cells is suggested as the mechanism by which FC increases SO₂ toxicity. In contrast, FC had no effect on O₃ injury to plants independent of gas exchange. This selective enhancement of SO₂ may provide a useful tool for investigating the physiology of SO₂ uptake and injury mechanisms of plant response to SO₂ versus O₃ or other pollutants.

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