Ozone (O\textsubscript{3}) deleteriously affects organisms ranging from humans to crop plants, yet little is understood regarding the underlying mechanisms. In plants, O\textsubscript{3} decreases CO\textsubscript{2} assimilation, but whether this could result from direct O\textsubscript{3} action on guard cells remained unknown. Potassium flux causes osmotically driven changes in guard cell volume that regulate apertures of associated microscopic pores through which CO\textsubscript{2} is supplied to the photosynthetic mesophyll tissue. We show in Vicia faba that O\textsubscript{3} inhibits (I) guard cell K\textsuperscript{+} channels that mediate K\textsuperscript{+} uptake that drives stomatal opening; (II) stomatal opening in isolated epidermes; and (III) stomatal opening in leaves, such that CO\textsubscript{2} assimilation is reduced without direct effects of O\textsubscript{3} on photosynthetic capacity. Direct O\textsubscript{3} effects on guard cells may have ecological and agronomic implications for plant productivity and for response to other environmental stressors including drought.

Ozone (O\textsubscript{3}) in the upper atmosphere is highly beneficial as a UV filter, but O\textsubscript{3} of anthropogenic origin at ground level is a serious pollutant. Elevated ground-level O\textsubscript{3} is associated with impaired human health (1, 2), reduced primary production in forest ecosystems (3), and losses in agricultural yield estimated at $3 billion annually for the U.S. alone (4). However, the cellular targets of O\textsubscript{3} action remain essentially unknown. In plants, O\textsubscript{3} reduces rates of photosynthetic carbon fixation. Such decreases could reflect (I) direct effects of O\textsubscript{3}, and/or the reactive oxygen intermediates generated on O\textsubscript{3} exposure (\ce{\cdot}OH, H\textsubscript{2}O\textsubscript{2} etc.), on the interior, photosynthetic mesophyll cells; or (ii) a decrease in CO\textsubscript{2} availability to the mesophyll cells, as a result of impaired function of guard cells in the plant epidermis. Guard cells control rates of CO\textsubscript{2} entry to the mesophyll by regulating the apertures of the stomatal pores in the leaf epidermis through which gas exchange occurs. Reduced stomatal conductance is commonly observed after O\textsubscript{3} exposure (5, 6), and it is possible that these reductions reflect a primary response of the guard cells to O\textsubscript{3} (7). Alternatively, reduced stomatal conductance could be a secondary response of the guard cells after either O\textsubscript{3} injury of adjacent epidermal cells (8) or alterations in the leaf environment caused by O\textsubscript{3} damage to the mesophyll tissue. In particular, high internal CO\textsubscript{2} concentrations, as result from inhibition of carbon assimilation, are a potent signal for stomatal closure (9) and have been hypothesized to be the proximate cause of the stomatal responses to O\textsubscript{3} (10).

Because the changes in guard cell volume that regulate stomatal apertures are driven in large part by ion uptake and loss, we hypothesized that, if O\textsubscript{3} does directly affect guard cells, it may do so by altering the activity of ion channels in the guard cell plasma membrane. There is indirect evidence that O\textsubscript{3} affects the plasma membrane because altered membrane permeability and ion leakage are frequently detected in leaves and in the unicellular alga Chlorella sorokiniana after treatment with this gas (11). Little research has been performed on the underlying mechanisms, although two studies have demonstrated that membrane vesicles isolated from leaves of O\textsubscript{3}-treated pinto bean (Phaseolus vulgaris L. var Pinto) plants display altered Ca\textsuperscript{2+} transport (12) and a decrease in K\textsuperscript{+}-stimulated ATPase activity (13). However, all of these studies lack specificity with regard to cell type and involvement of molecularly defined transport molecules. In mammals, an effect of O\textsubscript{3} on ion transport in airway epithelial cells also has been suggested by indirect measurements (14, 15) but never tested by direct electrophysiological measurements of ion channel activity.

In response to stimuli that elicit stomatal opening, K\textsuperscript{+} influx increases guard cell osmotic content, resulting in water uptake, a bowing out of the two guard cells that define each stomatal pore, and an increase in stomatal aperture. K\textsuperscript{+} influx occurs through inwardly rectifying K\textsuperscript{+} channels and is driven by membrane hyperpolarization caused by H\textsuperscript{+} ATPase activation and anion channel inactivation. Conversely, stimuli that elicit stomatal closure inhibit H\textsuperscript{+} ATPase activity and activate anion channels, resulting in membrane depolarization that both activates outwardly rectifying K\textsuperscript{+} channels and provides the driving force for K\textsuperscript{+} efflux (16, 17).

In the present study, we used the technique of whole cell patch clamping to directly assess the effects of O\textsubscript{3} on plasma membrane K\textsuperscript{+} channels of guard cells of fava bean (Vicia faba), the most commonly used species for cellular studies of guard cell function. The influence of O\textsubscript{3} on stomatal regulation was also studied in isolated epidermal peels and whole plants.

Materials and Methods

Growth Conditions. Broad bean (V. faba) plants were grown from seeds in 3 parts Metro-mix 360 to 1 part Perlite and were watered 3–4 times per week with 1/2 strength Hoagland solution. Plants used for epidermal peel and patch-clamp experiments were grown in environmental growth chambers under a 10-hr light/14-hr dark cycle with a light intensity of 200 μmol·m\textsuperscript{-2}·s\textsuperscript{-1} and a temperature of 25/23°C. Plants used in the gas exchange experiments were grown in a greenhouse under natural illumination with 200 μmol·m\textsuperscript{-2}·s\textsuperscript{-1} supplemental illumination between 8 a.m. and 8 p.m. The youngest fully expanded leaves from 3- to 4-week-old plants were used in all experiments.

Ozone Exposure. Protoplasts were isolated from three fully expanded leaves as described (18, 19). The protoplasts were exposed to O\textsubscript{3} in a 300 mM K\textsuperscript{+}-phosphate buffer (pH 5.7) containing 2 mM MgCl\textsubscript{2}, 1 mM CaCl\textsubscript{2}, and 40 mM K\textsubscript{2}SO\textsubscript{4} to a final osmolality of 440 mOsm kg\textsuperscript{-1}. Ozone was introduced into the buffer, which was determined not to scavenge O\textsubscript{3} by bubbling 930 ml liter\textsuperscript{-1} O\textsubscript{3} (in O\textsubscript{2}) through the buffer for 30 min.

The buffer was treated with O\textsubscript{3} before cell exposure. Ozone concentration in the buffer was determined spectrophotometrically by using the indigo method (20). In the first experiment (Fig. 1 A and B), the protoplasts were pretreated with the O\textsubscript{3}-containing buffer (33–354 μl liter\textsuperscript{-1}) for <5 min and were immediately transferred to bath solution I (see below) for patch clamping. In the second experiment (Fig. 1 C and D), O\textsubscript{3} was

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containing buffer was added during the patch clamp experiment to protoplasts bathed in the phosphate buffer described above. The initial concentration after adding O₃ to the dish ranged from 10 to 30 µl·liter⁻¹ O₃. The concentration dropped by 75% within 10 s. Because O₃ is so unstable and remained in solution for brief periods of time, it was not possible to obtain a repeatable concentration of the gas in each of these experiments. Despite this variation, similar inhibition of the inwardly rectifying K⁺ currents (I_K,in) was observed throughout the range of O₃ concentrations. It has been observed by others (see ref. 11 for review and references) that relatively high concentrations of O₃ are required to elicit a response when cells or biochemicals are exposed to the gas in vitro. This may relate to the solubility of the gas and the scavenging potential of the cells.

The epidermal peels were exposed to air with or without 1.0 µl·liter⁻¹ O₃ for 3 hr in a custom-made Petri dish with a gas inlet and outlet. The flow through the Petri dish during exposure was 400 ml·liter⁻¹. Whole plants were treated with 0, 0.10, or 0.18
μl·liter⁻¹ O₃ in continuous stirred tank reactors (21) located in a charcoal-filtered greenhouse.

**Patch Clamp Recording.** Two different sets of solutions were used for the patch-clamp recordings presented in Fig. 1 A and B (bath and pipette solution I) and C and D (bath and pipette solution II). Bath solution I contained 100 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 5 mM Mes, 5 mM Hepes at pH 5.6 (adjusted with Tris), and sorbitol to a final osmolality of 460 mOsM·kg⁻¹·water⁻¹. Pipette solution I contained 80 mM KCl, 20 mM KCl, 5 mM MgCl₂, 10 mM Mes, 2 mM EGTA, and 2 mM Mg-ATP (Sigma) (added daily) at pH 7.8 (KOH) and adjusted to 480 mOsM·kg⁻¹·water⁻¹ with sorbitol. Bath solution II contained 10 mM K-glutamate, 1 mM CaCl₂, 2 mM MgCl₂, 10 mM Mes, 0.25–0.50 mM N-methyl glutamine (pH 5.5), and mannitol to a final osmolality of 480 mOsM·kg⁻¹·water⁻¹. This solution was used initially in experiments presented in Fig. 1 C and D because it promoted formation of a high resistance (GΩ) seal. Before O3 exposure of the cells, this solution was exchanged by bath perfusion and was replaced with the phosphate buffer previously described. Pipette solution II contained 100 mM K-glutamate, 2 mM MgCl₂, 0.2 mM 1,2-bis(2-aminophenoxy)ethane-N,N,N,N',N',N'-tetraacetate, 4 mM Mg-ATP, 20 mM Hepes, and mannitol to a final osmolality of 520 mOsM·kg⁻¹·water⁻¹.

Whole-cell currents were measured in response to 1.8-s voltage pulses decremented in −20-mV steps from +63 mV to −177 mV, using an Axopatch-1B patch-clamp amplifier and a TL-1 DMA Interface (both from Axon Instruments, Foster City, CA). Between measurements, the holding potential was kept for 10.25 s at −54 mV and −67 mV, respectively, in the experiments presented in Fig. 1 A and B and Fig. 1 C and D. A schematic of the voltage stimulus is given in Fig. 1 A. The sampling rate was 1 ms. Currents were filtered at a −3dB frequency of 500 Hz by the lowpass Bessel filter of the patch-clamp amplifier. Data analyses were accomplished by using PCLAMP 6.02 software (Axon Instruments). Whole-cell currents were corrected for time-independent or leak currents and were normalized—i.e., expressed in pA/pF—to eliminate differences caused by cell-to-cell variations in surface area (22). Potentials reported have been corrected for liquid junction potentials (23).

**Epidermal Peel Assay.** In stomatal opening experiments, abaxial epidermal peels were floated on 35 mM KCl during 3 hr of exposure to air with or without 1.0 μl·liter⁻¹ O₃. The plants used for isolation of epidermal peels were kept in the dark overnight before each experiment to ensure that stomata were closed. The isolated epidermal peels were simultaneously exposed to light (200 μmol·m⁻²·s⁻¹) in the presence or absence of O₃. In stomatal closure experiments, abaxial epidermal peels were floated on 35 mM KCl/0.5 mM CaCl₂ for 3 hr during exposure to air in the presence or absence of 1.0 μl·liter⁻¹ O₃ under continuous light (200 μmol·m⁻²·s⁻¹). Before the isolation of the epidermal peels for the closure experiments, the abaxial side of detached leaves was exposed to light (200 μmol·m⁻²·s⁻¹) for 2 hr to ensure open stomata. Widths of stomatal apertures were determined with an ocular micrometer and a light microscope (Leitz Dialux 20).

**Gas Exchange Measurements.** Stomatal conductance, assimilation, and A/CO₂ curves (carbon assimilation as a function of the intercellular concentration of CO₂) were measured with a Li-Cor 6400 gas exchange system (Li-Cor, Lincoln, NE). Conditions in the gas exchange cuvette during measurements were 30% relative humidity, 350 μl·liter⁻¹ CO₂ and 800 μmol·m⁻²·s⁻¹ light intensity provided by a red/blue light source (Li-Cor).

**Statistical Analysis.** The patch clamp data were analyzed by analysis of covariance (ANCOVA) using O₃ concentration and time between treatment and measurement of whole cell current as covariates in the first experiment (Fig. 1B). In the second experiment (Fig. 1 C and D), O₃ concentration was used as the covariate in a multiple analysis of covariance (MANCOVA). Multiple analysis of variance (MANOVA) was used to analyze the data from the epidermal peel and gas exchange experiments.

**Results and Discussion**

Two types of whole-cell patch clamp experiments were performed. In the first set of experiments (Fig. 1 A and B), protoplasts were pretreated with O₃ and subsequently subjected to whole-cell patch-clamp analysis. In the second set of experiments, protoplasts were exposed to O₃ after attainment of the whole-cell patch-clamp configuration, such that current responses before and after O₃ exposure could be evaluated on the same cell (Fig. 1 C and D). In both types of experiments, O₃ significantly (P < 0.05) reduced the magnitude of inward K⁺ currents but had no significant effect on the magnitude of the outward K⁺ currents. The reduction in inward K⁺ current over time was significantly greater in O₃-treated than in control cells. The current-voltage relationship of the inward K⁺ currents was negatively shifted by O₃ exposure whereas the characteristic voltage dependence of the channels was maintained (Fig. 1 D). No appreciable effects were observed either on the kinetics of current activation (Fig. 1 A; data not shown) or on ion selectivity, as ascertained by tail current analyses (data not shown).

These results are noteworthy for three reasons. First, they demonstrate specific inhibition of an ion channel by O₃, as has not been previously demonstrated for any biological system. Second, they demonstrate that guard cells are affected by O₃, directly. Third, because the inward K⁺ channels were inhibited, these results lead to the hypothesis that O₃ acts not, or not exclusively, by promoting stomatal closure, as has been widely assumed (6), but rather that O₃ is a potent inhibitor of stomatal opening. These two different loci of action have significantly different agricultural and ecological consequences (see General Discussion).

To test the hypothesis derived from the patch clamp experiments that O₃ significantly inhibits stomatal opening, we measured stomatal apertures in isolated epidermal peels of *V. faba* stripped from the mesophyll tissue and exposed to O₃. Stomatal opening in response to light was significantly inhibited during a 3-hr O₃ exposure (Table 1). Although stomatal opening was inhibited by only 20%, these results have been confirmed in two other species, *Arabidopsis thaliana* and *Nicotiana tabacum*. Ozone exposure (0.30 μl·liter⁻¹ for 3.5 hr) resulted in 20 and 31% inhibition of light-induced stomatal opening in epidermal peels of *A. thaliana* and *N. tabacum*, respectively, with both

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<tr>
<td>0</td>
<td>0.5 ± 0.2*</td>
<td>8.1 ± 0.5*</td>
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<tr>
<td>1</td>
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<td>3</td>
<td>8.4 ± 0.5†</td>
<td>10.6 ± 0.6</td>
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*The 0-hr time point represents a set of peels measured before the initiation of treatments.†Significant difference (P < 0.01) between O₃ and air treatment.
Fig. 2. Stomatal conductance (A and D) and assimilation (B and E) measured on the first fully expanded leaves of V. faba plants during exposure to charcoal-filtered air containing 0 (●), 0.10 (▲), or 0.18 (●) µl·liter⁻¹ O₃ for 4 hr in continuous stirred tank reactors (24). (A–C) The plants were kept in the dark overnight and until the start of exposure to allow observation of the influence of O₃ on light-induced stomatal opening (natural illumination supplemented with 150 µmol·m⁻²·s⁻¹). Both O₃ treatments resulted in significant (P < 0.01) reductions in stomatal conductance and assimilation. (D–F) The plants were kept in the light (natural illumination supplemented with 150 µmol·m⁻²·s⁻¹) for 4 hr before exposure to ensure open stomata at the start of exposure. Only the highest O₃ treatment resulted in significant (P < 0.01) reduction of stomatal conductance (after 3 and 4 hr) and assimilation (after 4 hr). In each replicate experiment, gas exchange was measured on five individual plants for each treatment/time point. (C and F) Assimilation (A) versus intercellular CO₂ concentration (Ci) measured within 60 min after the end of O₃ exposure, on the first fully expanded leaf of two individual plants for each treatment. The average A/Ci curve for the plants exposed to 0.10 µl·liter⁻¹ O₃ was not significantly different from that of plants exposed to charcoal-filtered air whereas plants exposed to 0.18 µl·liter⁻¹ O₃ had significantly lower A/Ci curves (P < 0.05). For all panels, the average of three experiments is shown.
reductions significant at the \( P \leq 0.05 \) level (S. R. Weightman, E.J.P., and S.M.A., unpublished work). The importance of these results is two-fold. First, the data support the hypothesis derived from the electrophysiological measurements that \( O_3 \) inhibits stomatal opening. Second, consistent with the patch clamp results, they demonstrate that intact guard cells are indeed directly affected by \( O_3 \). Although this possibility had been previously inferred (25), no direct evidence had ever been presented to support it. The same concentration of \( O_3 \) did not stimulate significant differences in stomatal closure at any of the individual time points measured. Guard cells retained competence to drive stomatal closure under these circumstances, as evidenced by stomatal closure when control peels were transferred to darkness under otherwise identical conditions [final aperture of \( 1.8 \pm 0.3 \) \( \mu \)m after 3 hr of dark incubation (data not shown)]. Greater stomatal closure of \( O_3 \)-treated epidermal peels was statistically significant when data from all individual time points were pooled (not shown), suggesting that there could be a small \( O_3 \) effect. Our inability to detect effects of \( O_3 \) on outward \( K^+ \) currents could have resulted because the input resistance and variability in electrophysiological recordings precluded detection. Alternatively, \( O_3 \) induction of stomatal closure might have resulted from effects on transporters other than \( K^+ \) channels. For example, opening of anion channels has been proposed to play a central role in stomatal closure by both directly mediating anion loss and by depolarizing the membrane, which then drives loss of \( K^+ \) (26, 27). Taken together, the results of the patch clamp and epidermal peel experiments led to two hypotheses concerning stomatal function in the intact plant. First, we hypothesized that, if leaves were treated with \( O_3 \) under conditions in which stomata initially were closed, subsequent stomatal opening would be impaired. Second, we hypothesized that \( O_3 \)-inhibition of stomatal opening and thus of \( CO_2 \) influx could lead to reductions in photosynthetic rate even in the absence of any direct effects of \( O_3 \) on mesophyll photosynthesis. To test these hypotheses, whole \( V. \) faba plants were exposed to \( 0, 0.10, \) or \( 0.18 \) \( \mu \)l liter\(^{-1} \) \( O_3 \) for 4 hr. Exposures were initiated under darkness, a condition in which stomata are closed and stomatal conductance is low. Subsequent illumination stimulated stomatal opening, resulting in increases in stomatal conductance, as measured through nondestructive gas exchange analysis. Both 0.10 and 0.18 \( \mu \)l liter\(^{-1} \) \( O_3 \) significantly inhibited the increase in stomatal conductance and decreased rates of mesophyll carbon assimilation relative to control plants (Fig. 2A and B). At the end of the exposure, photosynthetic capability of the mesophyll tissue was assessed by \( A_{C_i} \) curves (Fig. 2C). Because \( C_i \) is experimentally manipulated in these protocols, the role of stomatal response in controlling photosynthetic rates is “eliminated.” The \( A_{C_i} \) curves revealed that, at the lower \( O_3 \) concentration, there was no direct effect of \( O_3 \) on carbon assimilation; at a given \( C_i \), assimilation rates were identical in \( O_3 \)-treated and control plants. Therefore, the reduced rates of carbon assimilation seen under those conditions could only be attributed to a reduced availability of the substrate \( CO_2 \) to the mesophyll, as a result of the \( O_3 \)-inhibition of stomatal opening. At the higher \( O_3 \) concentration, a direct effect of \( O_3 \) on mesophyll photosynthesis could be observed as well, detected as a depression in the \( A_{C_i} \) curve; these results agree with previous reports (28–31). The same \( O_3 \) treatments then were applied to plants with initially open stomata (Fig. 2D–F). Only when the photosynthetic apparatus was directly affected by the \( O_3 \) treatment (at 0.18 \( \mu \)l liter\(^{-1} \) \( O_3 \)) as indicated by the \( A_{C_i} \) curve (Fig. 2F), did significant stomatal closure and reduction in assimilation occur (Fig. 2D and E). At the lower \( O_3 \) dose (0.10 \( \mu \)l liter\(^{-1} \) \( O_3 \)) stomata did not close significantly in response to \( O_3 \), despite the fact that this dose sufficed to inhibit stomatal opening (Fig. 2A), and photosynthesis was unaffected. These results further support the conclusion that the process of stomatal opening is more sensitive to \( O_3 \) than is the induction of stomatal closure.

**General Discussion**

The experiments reported here show that \( O_3 \) directly affects guard cells themselves, targeting inward \( K^+ \) channels, and that \( O_3 \) inhibits stomatal opening. These results challenge the prevailing dogma that \( O_3 \), either directly or indirectly, promotes stomatal closure. Moreover, it has been thought that, if stomata are closed at the time of \( O_3 \) exposure, there will be minimal adverse effects because the gas will be unable to penetrate to the interior photosynthetic mesophyll (32, 33). However, our results do not support this theory, showing instead that \( O_3 \) exposure initiated while stomata are closed can depress subsequent carbon assimilation via \( O_3 \) action solely on the guard cells. Our whole plant experiments were conducted with concentrations of \( O_3 \) that can be found in polluted atmospheres (34); this is particularly true for the treatment with 0.10 \( \mu \)l liter\(^{-1} \) \( O_3 \). These studies, therefore, have important agronomic and ecological ramifications, as illustrated by the following three examples. First, stomatal closure during a period of drought may be less readily reversed in \( O_3 \)-exposed plants. This is particularly relevant because the highest \( O_3 \) concentrations are sometimes associated with times of drought; e.g., as was reported in Great Britain during significant droughts in 1976 and 1982 (35). In fact, when ash trees (Fraxinus excelsior L.) received long term \( O_3 \) exposures during periodic drought exposures, there was some evidence that stomatal conductance might recover more slowly during re-watering in \( O_3 \)-treated than control plants (36). Second, in major agricultural regions with high light environments and significant \( O_3 \) exposure—e.g., the South Coast Air Basin of California, which has the most extreme \( O_3 \) levels in the U.S. (37)—midday stomatal closure (“mid-day depression”) often occurs because of the low ambient humidity that results from the high light, high temperature conditions of midday. Because the generation of \( O_3 \) in photochemical smog depends on high solar irradiation (34), \( O_3 \) inhibition of stomatal opening could significantly retard stomatal reopening in the afternoon after this mid-day depression and consequently reduce crop yield. Third, the presence of nighttime \( O_3 \) has been documented in rural locations due to long distant transport from urban sources (34) and at high elevation sites, including sites of ecological importance such as Shenandoah National Park in Virginia (38, 39). Although it has been reported that there can be an adverse impact on biomass accumulation when \( O_3 \) exposure of plants occurs exclusively in the dark (39, 40), this impact was previously explained by the modest stomatal conductance that could be detected under darkness and the concomitant \( O_3 \) uptake that would be expected. Our results suggest that inability of closed stomata to open completely after a dark exposure could be of equal or greater importance in reducing plant productivity.

One possible mechanism for the observed \( O_3 \) effect on guard cell ion currents is direct oxidation of the channel proteins. It is plausible that the expressed amino acids of the inward \( K^+ \) channels are more susceptible to oxidation compared with those of the outward \( K^+ \) channels. The inwardly rectifying \( K^+ \) channel of guard cells, which is shown as the target of \( O_3 \) action in this study, has been cloned from the model plant species *A. thaliana* (41, 42). One next step will be to assess whether alterations, either in ion channel amino acids particularly susceptible to oxidation or in levels of cellular reductants (43, 44, 45), may improve rates of guard cell recovery after the termination of an \( O_3 \) event. It will be of interest to determine whether other guard cell ion transporters are also targets of \( O_3 \) action, although the inhibition by \( O_3 \) of stomatal opening, but not induction of stomatal closure under our conditions, implicates \( O_3 \) targeting of an “opening-
specific” component, for which the inwardly rectifying K⁺ channels is the best example. Alternatively, elevation of cytosolic Ca²⁺ levels after O₃ exposure is another plausible mechanism to explain the electrophysiological responses documented here. Cytosolic Ca²⁺ has been implicated as a signaling molecule in guard cell responses to other oxidative stresses (46) and has been reported to inhibit the inward K⁺ channel (47). Interestingly, an increase in cytosolic free Ca²⁺ levels also has been reported after exposure of human tracheal epithelial cells to O₃ (48). Our identification of a specific ion channel as a target for O₃ action may prompt comparable studies in mammalian systems, leading to improved understanding of and treatment for the disease etiologies exacerbated by O₃. Finally, as implied by our whole plant experiments, genetic alterations in channel properties or cellular signaling events that would lead to accelerated rates of recovery of stomatal function after O₃ exposure could improve plant productivity in geographic regions with significant O₃ exposure.

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1. U.S. Environmental Protection Agency (1996) in Air Quality Criteria for Ozone and Related Photochemical Oxidants (Office of Research and Development National Center for Environmental Assessment, Research Triangle Park, NC), EPA report no. EPA-600/P-93/004. F.