pendent optical depth of 0.7 normal to the rings (*7–9*). For the size of the disk and rings we adopt the values given by Cook *et al.* (*9*).

Loewenstein *et al.* (*3*) observed Saturn with a passband similar to that of our 400-μm measurement in November 1975 and January 1976, when the ring inclination angle was 20° to 21°. Comparing our new results with the results of those observations, we find T_R (400 μm) = 72 ± 12 K. A similar comparison with the observations of Hudson *et al.* (*10*) in February 1974 (ring inclination angle, ≈ 27°) gives T_R (400 μm) = 92 ± 20 K. In both cases our error estimates are dominated by the uncertainty in the applicability of the martian model used for calibration. An attempt to resolve the rings at 400 μm (inclination angle, ~ 20°) with the 200-inch Hale telescope (*11*) yielded a lower value. Those observations imply an upper limit to the ratio of ring brightness to disk brightness of 0.35. Using our disk brightness temperature, we find that this would require T_R (400 μm) ≤ 52 K. The discrepancy between the results obtained by the two methods may be due to a difference between the A and B rings. Comparisons of total flux at different inclination angles give an average brightness for the rings; the scans made with the Hale telescope should be much more sensitive to the (outer) A ring than to the (inner) B ring.

The 400-μm ring brightness temperature lies between the brightness temperature (~ 90 K) observed for $\lambda \leq 35$ μm (*12*) and the temperatures (≤ 18 K) found for $\lambda \geq 3.3$ mm (*13*). This behavior, combined with the high optical depth of the rings at centimeter wavelengths (*8*, *14*), places severe constraints on the size and composition of the ring particles. The most likely candidates are metallic particles or ice particles with sizes of a few centimeters (*15*), although metallic particles seem less likely on the grounds of cosmic abundance and high density (*8*, *13*). Improved submillimeter observations should help to distinguish between the two compositions and to determine the size distribution.

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16. S.E.W. and R. H.H. are also members of the Department of Physics and R.H.H. and J.K. are also members of the Department of Astronomy and Astrophysics at the University of Chicago; all three authors are visiting astronomers at the Infrared Telescope Facility, which is operated by the University of Hawaii under contract from the National Aeronautics and Space Administration. We thank the staff of the Infrared Telescope Facility for their assistance during the observations. Supported by the National Aeronautics and Space Administration under grant NAG-W-4 and by the National Geographic Society. J.K. and S.E.W. acknowledge support from the Fannie and John Hertz Foundation.

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Responses of Hawaiian Plants to Volcanic Sulfur Dioxide: Stomatal Behavior and Foliar Injury

Abstract. *Hawaiian plants exposed to volcanic sulfur dioxide showed interspecific differences in leaf injury that are related to sulfur dioxide–induced changes in stomatal condutance. Species with leaves that did not close stomata developed either chlorosis or necrosis, whereas leaves of* Metrosideros collina *closed stomata and showed no visual symptoms of sulfur dioxide stress.*

In November 1979, Pauahi Crater, a Hawaiian volcano, erupted (*1*) and provided an opportunity to observe the effects of volcanic fumes on an adjacent forest stand. During the eruption, we observed that *Metrosideros collina* var. *macrophylla* Rock and *Dodonaea eriocarpa* Sm., which are evergreen woody species found throughout the Hawaiian Islands (*2*), appeared to differ greatly in their susceptibility to SO_2, the primary noxious gas in a volcanic plume (*3*). It has long been known that SO_2, an air pollutant in industrialized regions, injures foliage. Unfortunately, investigation of the processes that govern leaf uptake of noxious gases and result in foliar injury has begun only recently. We show here that species differ in stomatal responses to SO_2 and that changes in stomatal conductance during periods of SO_2 exposure are apparently associated with regulating the extent to which foliar injury develops.

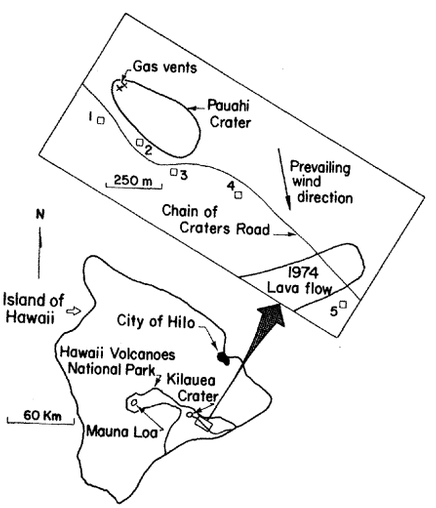

Fig. 1. Map showing the locations of the plots (□) in the Pauahi Crater study site and the site position on the Island of Hawaii. The plot locations reflect a gradient of SO_2 stress that resulted during a crater eruption on 16 November 1979. The crater is parasitic on Kilauea Caldera. Although ground fissures at the north end of the crater occasionally released magma and SO_2, most SO_2 in the study area originated from vents near the floor of the crater. The wind direction was constant in the study area during the eruption.

Long-term exposures to SO_2 at concentrations less than 0.5 parts per million (ppm) can alter the composition of plant communities (*4*), decrease the productivity of agricultural systems (*5*), and cause visible foliar injury (*6*). The nature of these large-scale effects and impairments is related to the effects of SO_2 on plant metabolism. Recent studies show that SO_2 concentrations between 0.1 and 1.0 ppm can cause rapid changes in stomatal conductance for a wide range of plant species, including evergreen shrubs, deciduous shrubs, and herbaceous species (*7*, *8*). Changes in stomatal conductance caused by SO_2 alter the flux rate of

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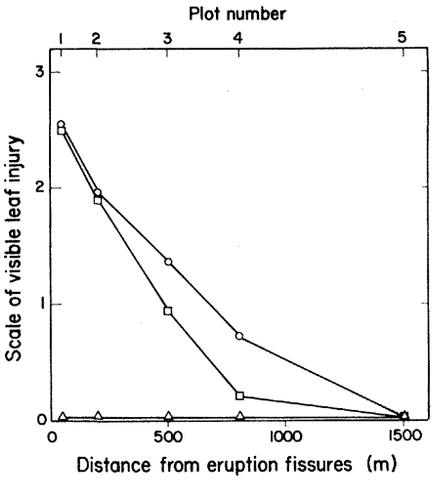

Fig. 2. Visual injury to foliage of *Dodonaea eriocarpa* (○) and young (□) and mature (△) *Metrosideros collina* leaves after exposure to a gradient of volcanic SO_2 stress (*11*). Plotted points are mean values ($N > 49$) for all leaves intercepted on transects in each plot.

gases, including SO_2, between the leaf and air (*8, 9*). Thus, SO_2 stimulation of stomatal opening increases the SO_2 absorption rates, whereas closure decreases the absorption rates. The hypothesis that stomatal responses to SO_2 regulate the uptake of the pollutant and thereby define the potential for foliar injury has been suggested but not tested under natural conditions (*8, 10*).

The Pauahi Crater eruption started on 16 November 1979. Lava flowed for about 40 hours, and SO_2 was released for about 60 hours. On 28 November no SO_2 was detectable in the study area. In order to assess the impact of the volcanic SO_2 on vegetation, we located five ecologically similar study plots downwind from the crater on 18 November 1979 (*11*) (Fig. 1). During the peak of volcanic activity the SO_2 concentrations, at 1 m above the ground, ranged from more than 100 ppm (*12*) at plot 1 near the crater rim to less than 1 ppm at plot 5 located 1.5 km downwind from the erupting vents.

Visible foliar injury for all species in the study area reflected the SO_2 concentration gradient, since injury decreased in plots located progressively farther downwind. The pattern of visible injury for *Dodonaea eriocarpa* (Fig. 2) is typical of response patterns observed for about 20 other species that were abundant in the study area. Thus, most plants were either severely injured or dead in plot 1, whereas the plants in plot 5 appeared to be neither necrotic nor chlorotic.

The susceptibility of *Metrosideros collina* leaves to foliar injury varied with leaf age (Fig. 2). Most young (less than 1 year old) *Metrosideros* leaves near the volcano were severely injured. In sharp contrast to all other foliage in the study area, mature *Metrosideros* leaves exhibited no symptoms of visible foliar injury. Even in plot 1, which was only 50 m from the crater, mature *Metrosideros* leaves had no chlorosis or necrosis.

We measured stomatal conductance for both *Metrosideros* and *Dodonaea* on 20 November (*13*) (Fig. 3). None of the *Dodonaea* leaves in plot 1 survived, but the conductances of this species throughout the remainder of the study area were similar to the values measured in plot 5; that is, they were fully open along the SO_2 concentration gradient. The few young *Metrosideros* leaves that appeared to have survived in plot 1 had about 50 percent of the conductance (stomata were partially closed) that we measured for leaves in plot 5. Mature *Metrosideros* leaves had the greatest range of conductances along the SO_2 concentration gradient and, except for plot 5, had the lowest conductance of all leaves tested. Thus, SO_2 did not alter the conductance of *Dodonaea*, a species that was severely injured in plots exposed to high SO_2 concentrations. Apparently SO_2 caused stomatal closure in mature *Metrosideros* leaves, and these were the most SO_2-resistant leaves in the study area.

Since low conductance values for mature *Metrosideros* leaves could be due to either SO_2-caused injury to stomata or leaf death, we again measured conductance for both species on 28 November (Fig. 3) (no SO_2 could be detected in the study area at this time). These measurements showed that formerly depressed *Metrosideros* conductance values measured for plants receiving the highest SO_2 concentrations had increased to values measured for plants that received little or no SO_2. Had leaf death accounted for decreased *Metrosideros* conductances observed during the eruption, no increase in conductance would have been recorded more than 1 week later. However, changes in conductance values may have resulted from guard cell injury, which was repaired as SO_2 concentrations subsided. A survey of *Metrosideros* on 28 February 1980 showed that mature leaves developed no visible necrosis (*14*).

Mechanisms that plants use to resist the effects of intermittent exposure to SO_2 include stomatal closure to exclude the pollutant, as in the case of *Metrosideros*, or rapid resprouting of leaves subsequent to damage, as in the case of *Dodonaea*. Detoxification or avoidance of SO_2 absorbed into the leaf mesophyll

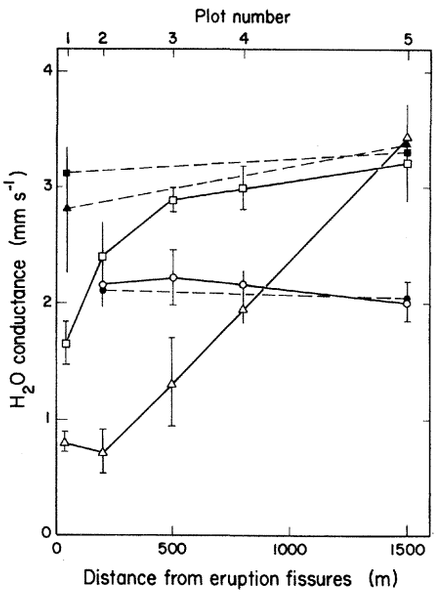

Fig. 3. Mean conductance values (± standard error, $N > 5$) for *Dodonaea eriocarpa* (○) and young (□) and mature (△) *Metrosideros collina* leaves during the late phase of volcanic activity (open symbols) and after a recovery period of about 1 week (closed symbols). Measurements were made in full sunlight when all conditions should be nonlimiting, except for the presence of SO_2.

might be another response of certain plant species inhabiting areas subjected to long-term SO_2 exposure. Knowledge of the diverse mechanisms by which wild plants avoid SO_2 damage should be of value to those who are evolving management strategies in polluted areas for either native or crop systems.

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References and Notes

1. The SO_2 production from the eruption was measured by T. Casadevall, Hawaii Volcano Observatory, Hawaii National Park, who used a Barringer Cospec IV analyzer to detect a minimal production rate of 3000 kg per day. The eruption released about 750,000 m^3 of magma, which is less than 1 percent of the volume produced by major eruptions. About 0.15 percent of the magma weight is gas, of which SO_2 is 6.4 percent by volume [G. A. McDonald, *U.S. Geol. Surv. Bull. 1021-B* (1952)]. Volcanism is thought to contribute 1.5×10^6 tons per year to the global sulfur cycle [W. W. Kellogg, R. D. Cadle, E. R. Allen, A. L. Lazrus, E. A. Martell, *Science* **175**, 587 (1972)].
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11. The topography of the 30-m^2 plots was level, not sloping more than 5 percent. Soils in the study area were from similar volcanic origin. The canopy coverage was analyzed on 18 and 19 November on three transects of 30 m in each plot. Results showed that the dominant tree in the study area is *Metrosideros collina* and that *Andropogon virginicus*, *Styphelia tamaiemaie*, and *Dodonaea eriocarpa* account for 90 percent of the understory canopy coverage in each plot. We assumed that SO_2 caused visible injury to the vegetation near Pauahi Crater, because no other noxious gases are emitted from Hawaiian volcanoes and there were no signs of lava, dustfall, or heat stress in any of the plots. Each time the canopy coverage of a species was recorded along the transects a value ranging from 0 to 3 was also recorded to score visual injury. Scoring was determined as follows: 0, plant with no apparent injury; 1, plant more alive than dead (less than half the leaf area appeared necrotic or the whole plant was slightly chlorotic); 2, plant more dead than alive (more than half the leaf area appeared necrotic or the whole plant was severely chlorotic); and 3, plant was dead.
12. We determined the SO_2 concentrations with five sample measurements, using an MSA universal sampling pump and Gastec analyzer tubes (Mine Safety Appliances Company, Pittsburgh, Pa.) on loan from S. Siegel, Department of Botany, University of Hawaii, Honolulu.
13. Stomatal conductance measurements were made with a Lambda diffusive resistance porometer (Lambda Instruments, Lincoln, Neb.).
14. Survey made by C. W. Smith, Botany Department, University of Hawaii, Honolulu.
15. We thank many scientists at the University of Hawaii (UH) in Honolulu, at Hawaii Volcanoes National Park (HVNP) and at Hawaii Volcano Observatory (HVO) for their help. We are particularly indebted to Drs. C. W. Smith and S. Siegel (UH), K. Baker (HVNP), and T. Casadevall (HVO). We also thank B. Lilley for drafting figures and R. Goldstein for his comments on an early version of this report. Research was supported by an Electrical Power Research Institute grant (RP-1313) to H.A.M.

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Visualization of Specific Angiotensin II Binding Sites in the Brain by Fluorescent Microscopy

Abstract. *The organum vasculosum of the lamina terminalis has been implicated as the site of receptors mediating central responses of angiotensin II. Up to now, this had been based on indirect evidence, but direct visualization of angiotensin II at its site of action has now been achieved by the use of a biologically active fluorescent angiotensin II agonist. The ventricular surface of the organum vasculosum lamina terminalis showed intense fluorescence, which was virtually eliminated by an excess of unlabeled angiotensin II.*

When angiotensin II is injected into the brain, drinking and other responses related to water balance are elicited (*1*). Evidence from both in vivo and in vitro studies implicates the organum vasculosum of the lamina terminalis (OVLT) as the site of receptors mediating these central responses to angiotensin II (*2*). When the ependymal surface of this region is physically blocked by a cream plug, the responses to intraventricularly administered angiotensin II are abolished (*3*). When angiotensin II is injected into the ventricle close to the OVLT, low concentrations are required to produce effects equal to higher concentrations injected into other parts of the brain ventricles (*4*). Lesions in the OVLT area produce a syndrome of transient adipsia and loss of responsiveness to angiotensin II injections (*5*). In addition, hypertension fails to develop in rats after lesions in this area (*5*). Binding assays carried out in vitro revealed specific binding sites for angiotensin II in the OVLT, with increased angiotensin II binding capacity in spontaneously hypertensive rats (*6*). Microiontophoretic studies in anesthetized rats showed specific activation of cells in the OVLT when angiotensin II was applied directly (*7*). These studies, however, provide only indirect evidence that the OVLT is the receptor site for angiotensin II. We now present direct evidence, obtained by a technique that combines the use of a biologically active structural analog of angiotensin II and a competitive binding control in vivo.

Fluorescein thiocarbamyl (FTC) was conjugated to angiotensin II (Meloy Laboratories) in an initial molar ratio of 5:1 in the reaction mixture, with a final maximum ratio of 1:1, by a modification of the method of Nairn (*8*); the *p*H of the reaction mixture was 8.0. The conjugate was separated by gel filtration on a Sephadex G-15 column that had been equilibrated and eluted with 0.001*M* acetic acid. The conjugate was isolated as a homogenous peak corresponding to authentic angiotensin II. Free FTC was eluted 50 ml later. The delayed elution of FTC was due to its property to adsorb on Sephadex G-15 and resulted in a clear-cut separation of the conjugate from free fluorescein.

Ultraviolet spectrum analysis was used to characterize the conjugate and to determine the ratio of FTC to protein. Fluorescent polarization analysis (performed by Meloy Laboratories) demonstrated that no free angiotensin II was present in the conjugate. In a competition assay, the binding of the conjugate to antibodies to angiotensin II was identical to binding of authentic angiotensin II.

Lateral ventricle cannulation was performed on eight adult male Sprague-Dawley rats with use of 23-gauge steel tubing stabilized by Cranioplastic. Rats had at least 3 days to recover from surgery, and patency of the cannulas was tested at least 1 hour before experimentation. Cannulas were judged to be patent if drinking behavior was elicited by a 100-ng dose of angiotensin II delivered in 1 μl of 0.9 percent saline. A 0.96 m*M* stock solution of the conjugate was prepared in 0.9 percent saline so that 1 μl contained a molar concentration tenfold higher than that of the angiotensin II solution containing 100 ng/μl.

Each of five adult male Sprague-Dawley rats was injected with 1 μl of a 1 percent solution of bovine serum albumin (BSA) (to decrease nonspecific binding) and, after a 5-minute interval, with an injection of 1.4 μg of conjugate in 1 μl of saline. Drinking behavior was elicited within 2 minutes after the injection of conjugate. The animals were decapitated during the time of drinking. The brains were removed within 3 minutes and immersed unfixed in liquid nitrogen. Cryostat sections were taken at 16 μm in the horizontal plane, and every fourth section was mounted with a buffered glycerol solution for fluorescence microscopy. Cutting began ventrally at the optic chiasm and included all of the OVLT and the ventral portion of the subfornical organ. To saturate the angiotensin II receptors with excess unlabeled angiotensin II, three rats were injected with 1 μl of 1 percent BSA and 5 minutes later with 8 μl of an angiotensin II solution (6 μg per microliter of 0.9 percent saline). Thirty seconds after injection of angiotensin II, 1 μl of the conjugate solution was injected. After drinking behavior was observed in each animal, the animals were killed and the brains were removed and processed as before.

The brains of all rats receiving the conjugate exhibited intense fluorescence that was limited to the ventricular surface of the OVLT (Fig. 1). The adjacent third ventricle showed low-level fluorescence along the ependymal wall (Fig.

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