Physiology of Ecotypic Plant Response to Sulfur Dioxide in *Geranium carolinianum* L.

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**Summary.** Populations of *Geranium carolinianum*, a winter annual plant common in disturbed habitats, vary in their foliar response to sulfur dioxide, and pollution resistance is characteristic of populations sampled from areas in which SO$_2$ has been a prominent stress. The physiological basis of this ecotypic response was investigated using a whole-plant gaseous exchange system in which leaf resistance to H$_2$O efflux and SO$_2$ influx were concurrently monitored. Individual plants of distinct SO$_2$ susceptibility were exposed to pollutant concentrations of either 0.4, 0.6, or 0.8 µL L$^{-1}$ in both the dark and light. Total SO$_2$ flux (µg cm$^{-2}$ h$^{-1}$) to the plant, which is the sum of leaf absorptive and absorptive loss, varied as an inverse function of leaf resistance (s cm$^{-1}$), and the relationship was modeled using linear regression techniques. Total SO$_2$ flux was partitioned to leaf surface and internal fractions using estimation procedures with the regression analysis. SO$_2$ flux into the leaf interior, the pollutant fraction responsible for causing foliar injury, was strikingly similar for resistant and sensitive plants at each concentration. Resistant plants must absorb 30% more SO$_2$ than their sensitive counterparts in order to exhibit comparable levels of foliar injury. Therefore, in *G. carolinianum* the predominant explanation for genetically controlled and quantitatively inherited differences in plant response to SO$_2$ is not variable pollutant flux but rather disparate physiological-biochemical processes affecting pollutant toxicity, cellular perturbation and repair. This conclusion is relevant to understanding how populations of *G. carolinianum* respond to elevated SO$_2$ and may explain the inherent susceptibility of this species compared with plants with which it co-exists.

**Introduction**

Air pollution is increasingly common as an ecologically important component of the environment. Ozone, sulfur dioxide, nitrogen oxides and acidic precipitation, occurring singly or in combination, are no longer solely localized atmospheric phenomena. One consequence of polluted air being a prominent long-term environmental stress is that plants in affected regions may exhibit specific morphological and physiological attributes that enhance survival and reproduction in the polluted atmospheres. These traits arise through either an induced modification of the phenotype (plasticity response) or a change in the populations's gene pool (ecotypic differentiation). In the former case, the capacity to respond in a comparable manner is common throughout the species, whereas in the latter greater pollution resistance at the infraspecific level would be characteristic only of populations thriving in areas experiencing pollution stress.

The evolution of resistant populations (ecotypes) to air pollution is reported in several plant species. In an analysis of variable responses in *Lupinus bicolor* subspecies to ambient air in the Los Angeles Basin, Dunn (1959) found that subspecies from the basin exhibited less foliar injury than their counterparts from non-polluted locations. He hypothesized that greater smog resistance of the L.A. Basin plants arose through the process of natural selection. Similar examples of ecotypic population responses to air pollution are reported for population growth and survival in *Calluna vulgaris* (Sokolowski 1976), enzymatic reactions in *Rumex obtusifolius* (Horsman and Wellburn 1977), yield responses in *Loliwm perenne* (Bell and Clough 1973; Horsman et al. 1979) and reproductive success in *Lepidium virginicum* (Murdy 1979). Although several reports (Catchesyde 1975; Gressel and Segel 1978) concluded that microevolutionary plant responses to chemical residues of anthropogenic origin are unlikely, it appears that genetic adaptation may be a common response of plant populations in habitats experiencing elevated levels of atmospheric pollutants.

One example of genetic adaptation in response to air pollution is the evolution of SO$_2$ resistance within populations of *Geranium carolinianum* L., inhabiting a region experiencing variable SO$_2$ stress for a maximum of 31 years (Taylor and Murdy 1975). In a comparison of the SO$_2$ response of eight different populations of this species that were exposed to SO$_2$ under controlled conditions, those sampled from SO$_2$ stressed locations were more resistant to acute foliar injury than their counterparts from non-polluted sites. Consistent differences in SO$_2$ response between individuals of this species are genetically controlled and quantitatively inherited (Taylor 1978a). Initial investigations (Taylor 1976) reported no difference among *G. carolinianum* plants in their stomatal resistance to H$_2$O flux in both ambient and SO$_2$-polluted atmospheres. These data, specific for H$_2$O efflux, support the hypothesis that pollutant uptake is equivalent between individuals; however, it assumes that SO$_2$ and H$_2$O experience comparable source-to-sink resistances and concentration gradients. This may not be valid, particularly in light of the residual resistance component associated with CO$_2$ assimilation but not H$_2$O efflux (Gaustra 1959; Nobel 1974; Meidner 1975). An analogous residual resistance may affect the influx of SO$_2$ (Mansfield 1973; Barton et al. 1980). Therefore, experiments to assess the uptake of SO$_2$ in resistant and sensitive plants were conducted to investigate how genotypic differences effect changes in a plant's physiological state so that plant to plant variation exists.
Materials and Methods

Plant uptake of SO$_2$ was investigated using an open gas-exchange system (Tingey et al. 1979; Taylor and Tingey 1979) operating on the mass balance principle. The chamber, which enclosed the above-ground portion of the plant, was housed within a controlled environmental unit. To assess gaseous fluxes, air flow rates and concentrations of SO$_2$ and H$_2$O vapor were measured at the chamber’s inlet and outlet. Within the chamber, rapid air mixing was maintained by wall baffles and impeller blades rotated by an externally-mounted electric motor. Fan speed maintained as slight, uniform flutter among all leaves of the plant. The boundary layer resistance of the leaves to H$_2$O efflux was calculated using an energy budget approach (Sestak et al. 1971) and was consistently low at 0.2 s cm$^{-1}$. All gas lines were stainless steel and heated after leaving the chamber to prevent in-line condensation.

From seed sampled from native populations of G. carolinianum, selection has been successful in establishing lines of varying SO$_2$ resistance. This variation is genetically controlled and quantitatively inherited (Taylor 1978a). Each line continues to exhibit variation in SO$_2$ response although the mean, mode and limits of resistance differ among lines. Seed from sensitive and resistant lines was germinated and seedlings were grown in a Jiffy Mix:Perlite (1:2; V:V) mixture. Plants were cultured initially in a greenhouse with maximum day/night temperatures of 28°C and 20°C, respectively. The photoperiod was extended to 16 h per day with HID sodium vapor lamps. A modified Hoagland's nutrient solution (Downs and Hellmers 1975) was applied daily. At least two weeks prior to experimentation, plants were transferred to a controlled environmental unit with a regime similar to that of the gas-exchange chamber. Within the gas-exchange system, the chamber’s air and outlet dew point temperatures were maintained at 27°C and 23°C, respectively (chamber humidity levels of 70-75%); these temperatures were selected to mimic the late spring climatic conditions typical of the Piedmont region of the southeastern United States where the original populations were sampled. Daylight irradiance at canopy level and air temperature were 490 μE m$^{-2}$ s$^{-1}$ and 25°C, respectively; leaf temperature was measured with a fine wire thermocouple (Lange 1965) and was 28°C ± 1°C. Outlet CO$_2$ concentration was 345 ppm ± 20 ppm.

In using the mass balance approach to analyze the rate of gaseous exchange, the fate of SO$_2$ molecules entering the chamber must be assessed. These include (1) reaction with the plant, (2) adherence to the chamber walls, and (3) exiting the chamber unreacted; the last is the ambient concentration to which the plants were exposed. The magnitude of the chamber surfaces as a sink for SO$_2$ is a function of the construction material and H$_2$O content of the air. To determine the SO$_2$ sink capacity of the chamber surfaces, humid air was injected into the chamber to elevate the outlet dew point to levels similar to those created by the plants. The adsorption of SO$_2$ to chamber surfaces increased linearly with dew point (SO$_2$ loss = 2.42 dew point$_{outlet}$ – 26.32, r = +0.985). The data indicate that when outlet dew point levels exceed 20°C (70% R.H.), 20% of the SO$_2$ entering the chamber is adsorbed to the leaf surface. This calculation assumes that resistance to SO$_2$ diffusion through the boundary layer and stomate is equivalent to that for H$_2$O, taking into account the differences in the diffusivity in air of the two gases (Nobel 1974; Unsworth et al. 1976).

Results

An example of the patterns of leaf resistance to gaseous flux (R$_{L}$ and R$_{LH}$) and pollutant flux to the plant over the dark to light regime at 0.4 μl·l$^{-1}$ SO$_2$ is shown in Fig. 1; the patterns were similar for all SO$_2$ exposure concentrations. In the dark, leaf resistance was high (17 and 32 s cm$^{-1}$ for R$_{LH}$ and R$_{LS}$, respectively); with light, resistance decreased within an hour to an 83% lower steady state value. The flux of SO$_2$ to the plant exhibited distinctly different dark and light steady state values, and the pattern is the reverse of that recorded for leaf resistance. Pollutant flux was lowest in the dark (0.8 μg cm$^{-2}$ h$^{-1}$) and increased rapidly with light. The light-associated flux (2.5 μg cm$^{-2}$ h$^{-1}$) was 3 times that observed in the dark. SO$_2$ flux in the dark represents pollutant adsorption to the leaf surface (J$_D$) while that observed in the light is total flux (J$_T$) or adsorption plus absorption.

Concurrent steady state measures of leaf resistance (R$_{LH}$ and R$_{LS}$) and SO$_2$ flux at 0.4 μl·l$^{-1}$ SO$_2$ were collated for night and day conditions within SO$_2$ resistant and sensitive plants (Fig. 2). SO$_2$ flux (J$_T$) was inversely related to leaf resistance.