amounts of methanol in diethyl ether), and their identity was verified by thin-layer chromatography on silica gel G, by spectrophotometry (*5*), and by biological assay (inotropic response in rat heart, and antagonism of this by aldosterone). The glycosidic compounds obtained from the plant and insect extracts showed no loss of identity or inotropic activity on acid and base hydrolysis.

Cardiac glycosides are present in all northern species of *Asclepias* said by Brower (*1*) to lack these compounds, and in two other northern species of *Asclepias* studied (Table 1); these compounds are thus available to insects feeding on various portions of the plant. The seeds are the richest source of these noxious compounds in three of the species examined. The indigenous monarchs (*6*), which are leaf feeders in the larval stage, seem to have sequestered cardiac glycosides from these northern species of *Asclepias*. It has also been shown that *Danaus plexippus* is not atypical. *Oncopeltus fasciatus* (*7*), *Lygaeus kalmii kalmii, Lygaeus kalmii angustomarginatus, Tetraopes tetraophthalmus*, and *Tetraopes oregonensis*, all of which feed upon northern *Asclepias*, contain cardiac glycosides (*7*). Also, fifteen other species of *Asclepias* (from diverse parts of North America) possess cardiac glycosides (*8*). Thus, Brower is incorrect in assuming that the northern species of *Asclepias* lack cardiac glycosides. The geographical range of *Asclepias* possessing cardiac glycosides is indeed much more extensive than indicated by Brower (*1*).

However, since Brower (*1*) reports that the northern species of *Danaus* feeding on *Asclepias* is not unpalatable, then some alternative explanation must be sought to explain his birds' behavior. Several possibilities seem to exist. It is possible that the northern species of *Asclepias* lack specific glycosides that are present in the southern species of *Asclepias*. In the five northern species examined (at least 15 compounds in seeds, and at least six in leaves and pods) and one tropical species (at least 12 compounds) tested there are a large number of compounds that react positively to *m*-dinitrobenzoic acid plus NaOH. Further, in the 15 additional asclepiads studied some three to ten cardiac glycosides have been detected and Kupchan *et al.* (*9*) report at least nine cardiac glycosides (positive to *m*-dinitrobenzene plus NaOH) in *A. curassavica*. Until the specific identity of all these glycosides is established, qualitative chemical differences in the host plants cannot be ruled out.

It is also possible that the various species of *Asclepias* differ in the concentration of component glycosides. Brower (*1*) mentions three specific glycosides in *A. curassavica* and in *Danaus* fed on this plant, but it is not certain that these are either the most important or the sole glycosides involved in effecting impalatability. Indeed, the present study on limited material has detected at least eight compounds positive to *m*-dinitrobenzoic acid plus NaOH in indigenous northern *Danaus*.

The blue jay *Cyanocitta cristata bromia* is the major avian predator employed in studies of palatability (*1, 2, 10, 11*). One would expect interspecific variation in the responses of (avian) predators to foods containing cardiac glycosides, a factor which could complicate the palatability spectrum. Further, it is also possible that there is a threshold concentration of glycosides that the predator must ingest to induce emesis: such a threshold may again involve specific glycosides or total concentration of cardenolides in the insects. We do not know whether *Danaus* feeding on *Asclepias* simply accumulates glycosides according to the type and concentration in the plant, or if it has specific selective or concentration mechanisms.

Not until the chemical composition of the various asclepiad hosts is accurately determined and the insect physiology thoroughly known, will it be possible to deduce the true basis of the palatability spectrum observed by Brower. The present results suggest that it will be more complex than originally postulated.

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7. *Oncopeltus fasciatus* concentrates cardiac glycosides in its ventral metathoracic stink glands, and in previously undescribed dorsolateral meso- and metathoracic and dorsolateral abdominal glands. I thank Dr. D. Feir and Mr. R. Cannings for specimens of insects from Missouri and Ontario.
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Ozone Uptake by Bean Leaves

Abstract. *The removal of ozone from the air by bean leaves is regulated by the same factors that control the exchange of water vapor between leaves and the atmosphere.*

Ozone is one of the principal air pollutants causing damage to plants in the United States (*1*). The uptake of ozone by leaves is essentially an exchange of a gas between leaves and the atmosphere. As such, this uptake must be regulated by the same factors that control the exchange of other gases, such as water vapor. The loss of water vapor from leaves by transpiration is commonly analyzed in terms of resistance, seconds per centimeter (*2*). Thus, we measured ozone uptake by leaves, converted these data to resistance to ozone uptake, and compared this resistance to water-vapor loss.

The rate of removal of ozone was measured as the time t (in seconds) for the concentration C to decline from 200 to 150 parts per billion (ppb) within a closed, illuminated, 75-liter chamber equipped with a fan. Temperature within the chamber was 15°C ± 1.5°. The concentration of ozone in the chamber was increased by pumping in air irradiated by ultraviolet lamps in a separate box. When the desired concentration was reached, the ozone-rich air

stream was stopped, and the subsequent rate of depletion was determined. Ozone concentrations were measured with a Mast ozone meter.

The concentration of ozone declined at $(k_l + k_c)$ units per second when plants and pots of soil were present. The rate of ozone depletion may be expressed as

$$dC/dt = -(k_l + k_c)\,C \qquad (1)$$

$$(dC/dt)/C + k_c = -k_l \qquad (2)$$

The k_l represents the rate at which ozone is removed by the leaves, and the k_c the rate at which ozone is removed by the chamber, pots of soil, and defoliated stems. If t_l is required for the change in C when the intact plant is present and t_c when the leaves have been removed, then

$$k_l = \ln(200/150)/t_l - \ln(200/150)/t_c \qquad (3)$$

In a typical observation, t_l and t_c were 26 and 39 seconds, respectively.

The observed removal of ozone by a particular leaf in a chamber is proportional to leaf area, A, and inversely proportional to chamber volume, v. The proportionality factor can be written as a resistance, r.

$$k_l = A/(vr) \qquad (4)$$

or

$$r = A/(vk_l) \qquad (5)$$

The resistance, r, in seconds per centimeter, is now characteristic of a single square centimeter of leaf lamina. Since it is in the same units as used to express resistance to loss of water vapor by leaves, we can now compare the resistances that regulate ozone uptake with those that regulate transpiration.

Transpiration is regulated by a boundary layer, r_a, around the leaf, and a stomatal resistance, r_s, in the leaf (*2*). We evaluated r_a by measuring the rate of ozone removal by dummy "leaves" made of squares (5 by 5 cm) of cheesecloth soaked in a saturated solution of the ozone reactant diacetyldihydrolutidine (*3*). These cloths have an r_s of zero and simulated ozone removal by leaves without cuticle, epidermis, or stomata. Hence, we used the observed value of 0.6 sec/cm as the r_a for ozone. If ozone uptake is regulated by the boundary layer and stomata, then $(r - r_a)$ equals stomatal resistance for ozone.

Next we evaluated r_s by an independent means. Between observations of ozone removal, we measured resistance

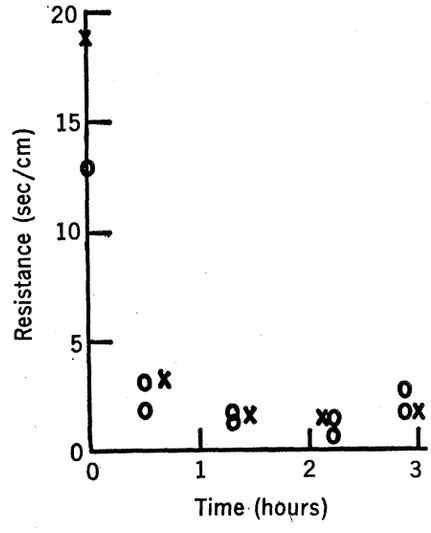

Fig. 1. The course of leaf resistance as shown by a diffusion porometer (crosses) and uptake of ozone (circles) after the lights were turned on at 0 hours.

to transpiration with an agitated-diffusion porometer (*4*) that has been calibrated in absolute units (*5*). We converted the porometer resistance for water into estimates of the resistance, r_s, for ozone by the following equation.

$$r_{s,o_3} = (D_{H_2O}/D_{O_3})\,r_{s,H_2O} \qquad (6)$$

D represents the coefficient of diffusion in still air. Since we do not know D_{O_3}, we substituted D_{CO_2} for D_{O_3} because CO_2 has about the same molecular weight as ozone. If, as we expect, ozone and transpiration are regulated by the same factors, the two estimates of stomatal resistance, $(r - r_a)$ from ozone uptake and r_{s,O_3} from the porometer, will be the same.

A series of resistances, $(r - r_a)$ and r_{s,O_3}, were measured as follows. Eight bean (*Phaseolus vulgaris* L.) plants, 10 to 14 days old, with two primary leaves each and growing in four polystyrene cups of soil were placed in the chamber in the dark. The leaves had a resistance (r_{s,O_3}) of 19 sec/cm, which indicated a slow leakage through cuticle and nearly closed stomata of leaves in the dark. The 610 cm² of leaf removed ozone with an $(r - r_a)$ of 13 sec/cm, which indicated an ozone reduction little faster than by evaporation.

The plants were then illuminated to open the stomata, and porometer readings and ozone depletion rates were taken periodically. The resistance measured by ozone depletion is nearly identical to that measured by the porometer (Fig. 1).

In another experiment, bean plants were illuminated until their stomata were open wide. Depletion rates of ozone and water were then observed, the lights were turned off, and the rates were observed intermittently as the stomata closed. Again the resistances as given by the porometer closely approximated those calculated from ozone depletion.

Whether the observations were taken as the stomata opened or closed, the resistances measured either by porometer or by ozone uptake were remarkably alike, which indicated the predominant role of stomata in controlling the uptake of ozone. In other words, the cuticle or contaminants on the leaf surface destroyed little ozone. Furthermore, the near equality of the resistances indicates that the ozone and water vapor travel the same path, although in opposite directions. Since the surfaces of the cells beneath the stomata are wet with nearly pure water, the path for water vapor is essentially from those surfaces through the stomata. The equality of the two resistances implies that ozone is reduced to a very low concentration at surfaces of the substomatal cells. Since the uptake of ozone and transpiration of water are regulated by the same factors, and because we have realistic simulators of transpiration from natural canopies (*6*), we can calculate the cleansing of the atmosphere.

The close relation between the factors regulating both ozone uptake and transpiration help to explain the fact that the degree of ozone injury to tobacco in the field could be correlated with ambient outdoor concentrations of ozone only when adjustment was made for the ratio of transpiration to atmospheric water demand (*7*). We see this adjustment as an index of stomatal opening and a confirmation of the control of ozone uptake by stomata.

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