Air Pollution Affects Pattern of Photosynthesis in Parmelia sulcata, a Corticolous Lichen

Abstract. *Lichen disks kept in flasks contaminated with sulfur dioxide showed morphologic and photosynthetic abnormalities similar to those of lichens from an industrial center in Sweden, but lichens dried out for 4½ to 6 months in the laboratory showed neither. Thus some kinds of lichens may be absent from city environments because of atmospheric pollutants which destroy chlorophyll.*

Absence of corticolous lichens from city environments has usually been attributed to atmospheric pollution, although Rydzak and others (*1*) have suggested a "city desert climate" as the cause. Atmospheric pollution is known to have adverse effects on living organisms; a spectacular manifestation of these effects is the occurrence of "killer fogs" in industrial cities, and recent findings indicating a causal relationship between air pollution and lung cancer point to the dependence of man on the preservation of an uncontaminated reservoir of air. The poisonous gases often present in city air apparently destroy chlorophyll in vascular plants (*2*), and the dearth of lichen vegetation in cities could conceivably be due to the same cause.

If lichen growth and development are indeed limited by pollution of the atmosphere, the study of these plants takes on added significance in that it may therefore be possible to evaluate the degree of air pollution by measuring the abundance of lichens. Our observations in Stockholm (*3*), central Sweden (*4*), northwestern Minnesota (*5*), and eastern Idaho have suggested that some kinds of lichens are limited largely by the degree of air pollution rather than by "city desert" conditions.

We observed previously that different kinds of lichens responded differently to variations in microclimate and air pollution. In northwestern Minnesota, many corticolous and saxicolous lichens—especially some species of *Parmelia, Physcia,* and *Xanthoria*—were more abundant in dry than in humid microclimates (*5*); consequently, they are not likely excluded from cities because of the aridity of the city environment. In central Sweden, we observed that species of these same genera seem to be very sensitive to air pollution (*4*). On the other hand, geocolous lichens of humid regions—for instance, species of *Baeomyces, Cladonia, Collema, Peltigera,* and *Stereocaulon*—seem to be very dependent on high humidity and relatively insensitive to atmospheric pollution.

To aid in ascertaining whether or not lichens can be used as indicators of atmospheric pollution, and if so, how pollution affects them physiologically, we studied photosynthetic patterns in lichens with the Warburg apparatus. We compared (i) lichens collected from an air-polluted area near the center of Uppsala, Sweden, with lichens from three other sites successively further removed from the city; (ii) lichens exposed to an atmosphere artificially polluted with sulfur dioxide gas and normal lichens; and (iii) lichens dried out in the laboratory for several months with fresh lichens. We chose *Parmelia sulcata* for our primary experimental material because of its abundance, its relative insensitivity to microclimatic variation, and its sensitivity to air pollution.

Using a brass die, we stamped disks of lichens 1.72 cm in diameter (and hence 2.32 cm^2 in area) from the bark of *Acer platanoides* or *Tilia cordata* trees at each of the four sites and subjectively rated them from "1" (unhealthy) to "5" (healthy). The disks collected at Ultuna, a rural area about 5 km from Uppsala, scored either 4 or 5, with an average of 4.3; average scores for disks from the other sites were: Ulleråker, 3.7; Pollacksbacken, 3.0; and Slottsbacken, near the center of Uppsala, 2.0.

The lichen disks were placed face down in Warburg flasks and oxygen absorption or evolution was measured by the direct method of Warburg (*6*). A buffer solution was prepared by bubbling, for about 30 minutes, a mixture of 1 percent CO_2 and 99 percent air into a mixture of 85 percent $NaHCO_3$ and 15 percent Na_2Co_3; 0.5 ml of this buffer was placed in the center well of each Warburg flask. The resulting flask atmosphere in which photosynthesis and respiration took place was much richer in carbon dioxide than is the normal atmosphere in nature—according to our measurements about 20 times richer—but for the purposes of this study, this was not of importance. Respiration was measured in the dark, and apparent photosynthesis was measured at 80, 160, and 240 volts supplied to a bank of 14 incandescent lamps of 25 watts each located 80 mm (including 30 mm water) directly below the surface of the disks. The light intensity at the disk surface was approximately 1000, 4000, and 9000 lux, respectively. The water bath was kept at 18°C. Measurements of change in manometer pressure were made every half hour. At least four measurements were made at each light intensity.

For the laboratory study of the effects of SO_2, we collected 12 *Parmelia sulcata* disks on 23 April and a like number on 6 August 1964 from an *Acer platanoides* tree at Ultuna. These were placed in 580-ml flasks in the Uppsala University thallotron (a bank of light and temperature control chambers used primarily for thallophyte studies) and held at 17°C and 3000 lux. A thin (5 mm) sheet of sponge plastic lay on the bottom of each flask and 10 ml of distilled water was added to each. Test tubes containing a small quantity of Na_2SO_3 were placed in nine of the flasks. At intervals of 3 or 4 days, one of the flasks was taken from the thallotron and the sulfite was activated by the addition of an excess of dilute H_2SO_4. A rubber stopper was placed over the flask, and it was returned to the thallotron. The disks that were kept sealed in flasks in the thallotron without the addition of SO_2 served as controls.

In the test tubes inside the thallotron flasks, there were 16.5, 33, 165, 330, 1650, 3300, or 16500 mg of Na_2SO_3. If the solubility of SO_2 was negligible in the dilute acid or in the water absorbed in the sponge plastic, activation of the sulfite with excess acid would yield flask atmospheres containing 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 percent SO_2, respectively. Since SO_2 is soluble in water, the actual concentrations of gas in the flasks was somewhat less than this; these values are nevertheless used in this report to indicate the relative degree of air pollution in the flasks.

Three groups of lichens were used to study the effects of desiccation on lichen physiology. One group of *Lecanora* cfr. *rubina* was collected from rocks on the desert near Rexburg,

Idaho, in May 1963, transported to Sweden by boat, and kept near the window in the laboratory at Uppsala until November 1963 when it was tested with the Warburg apparatus. A second group of *L. rubina* was collected at the same site in March 1964, transported to Sweden by airmail, and tested with the Warburg apparatus only a few days after collecting. The lichens in both groups were liberally watered about 4 hours before they were placed in the Warburg flasks. Disks of *Parmelia physodes* and *Xanthoria parietina* were collected in Uppsala in March 1964; some of them were tested with the Warburg apparatus the same day they were collected, and the remainder were stored in petri dishes in the thallotron until August, when they were tested. The specimens in the thallotron were watered once a week, but one set of petri dishes was left uncovered so that the disks dried out rapidly between waterings while the other set was covered after the watering so that the disks were kept constantly moist, though not excessively wet. Three days after watering, the uncovered set was transferred to a warmer thallotron chamber (27°C) and kept there for 3 more days.

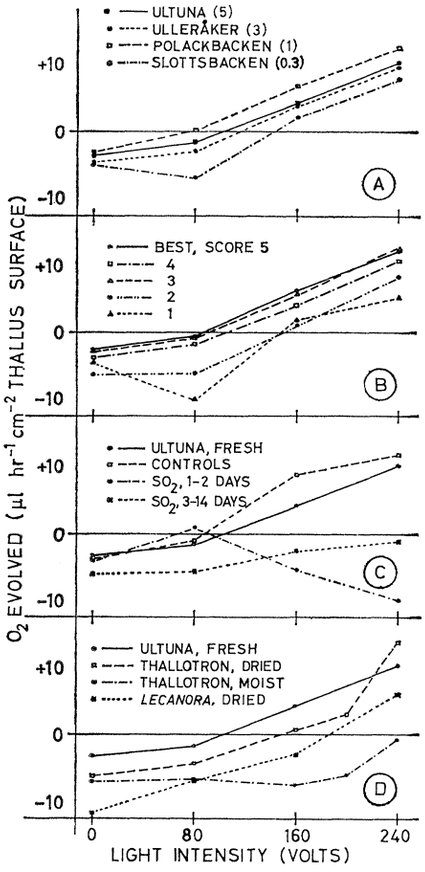

Fig. 1. Apparent photosynthesis in lichen disks measured by the Warburg apparatus. (*A*) Oxygen evolution by disks of *Parmelia sulcata* collected at each of four sites in or near Uppsala, Sweden. The number in parentheses after the name of the collection site is the number of kilometers from the center of the city. Averages of three disks from each site. (*B*) Same data as in *A* but rearranged to give average oxygen evolution for each of the five subjective scores employed. (*C*) Average oxygen evolution by *P. sulcata* disks exposed to artificial atmospheres containing 10 percent SO_2. The control disks were sealed in similar flasks but without the presence of SO_2. (*D*) Average oxygen evolution by *P. physodes* disks and *Lecanora rubina* fragments after drying in the laboratory for 4½ to 6 months. One set of *Parmelia physodes* disks was kept in the thallotron in a humid atmosphere, the other set was kept in a dry atmosphere; both sets were watered weekly.

The lichens collected from the air-polluted sites, especially from Slottsbacken near the center of the city, were different in appearance from those collected from the pure-air sites. The thallus lobes of the former were pale in color, frequently discolored pink, and neither as thick nor as succulent as the lobes of the normal lichens. The photosynthetic patterns produced by the lichens from the polluted areas were also different (Fig. 1, *A* and *B*). As the light intensity was increased from 0 to 80 volts, apparent photosynthesis increased from −4 to −2 ml of O_2 per hour in the lichen disks rated 3, 4, or 5, remained about the same in the disks rated 2, and decreased in the poorest disk, rated 1. With further increase in light intensity, photosynthesis increased in all of the disks at about the same rate.

At each light intensity, photosynthetic rates were essentially constant for the disks subjectively scored 4 or 5, but varied considerably from reading to reading for the disks scored 1 or 2 (Fig. 2). Much of this variation was due to a gradual reduction in photosynthetic rate at the highest light intensity. The standard error seemed to decrease in direct proportion to the square root of the subjective score.

Lichens exposed to artificially contaminated atmospheres also showed morphologic and photosynthetic abnormalities. With heavy contamination (1, 5, or 10 percent SO_2) the lobes became very pale in color, sometimes showed a pink discoloration, appeared dried out, and carried out net respiration at all light intensities (Fig. 1*C*). Exposure to less contamination resulted in intermediate morphological change but no apparent change in photosynthetic rate or pattern. There was, however, a slight (though statistically not significant) increase in standard error proportional to the concentration of the SO_2. Both the morphological and physiological changes occurred quite rapidly, and in this study it made little difference whether exposure to SO_2 was for 15 days or for only 1 day.

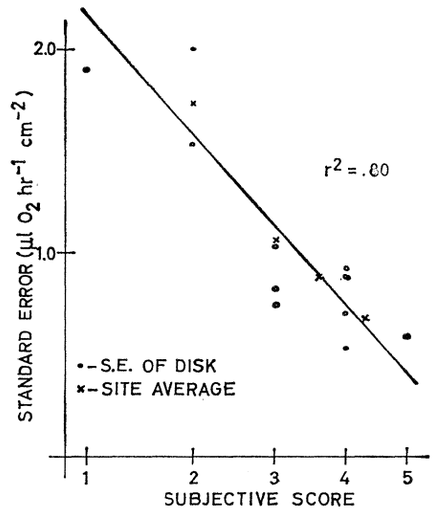

Fig. 2. Standard error of photosynthesis measurements made on disks of *Parmelia sulcata* as associated with the subjective score given each disk.

Lichens exposed to desiccation in the laboratory or in the thallotron for 20 weeks or more were normal in appearance after watering and gave apparently normal patterns of photosynthesis (Fig. 1*D*). Fresh specimens of *Lecanora rubina* produced photosynthetic patterns essentially identical to the curve shown in Fig. 1*D* for specimens of *L. rubina* that had been dried out for over 6 months. Similarly, disks of *Parmelia physodes* (Fig. 1*D*) and *Xanthoria parietina* that had been kept in the thallotron for over 4 months under dry conditions, except for the weekly application of water, produced photosynthetic patterns very similar to those of the freshly collected disks. On the other hand, lichens exposed to relatively uniform moisture and temperature conditions were deeper green in color, softer, and gave abnormal photosynthetic patterns (Fig. 1*D*).

That lichens growing near cities or other industrial centers are morphologi-

cally different from lichens growing in areas where the atmosphere is not polluted is well established (*3, 4, 7*). This study has demonstrated that *Parmelia sulcata*, a corticolous lichen, shows physiological as well as morphological abnormalities when growing near industrial centers, with both rate and pattern of photosynthesis being affected.

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Chromosomes of American Marsupials

Abstract. *Studies of the chromosomes of four American marsupials demonstrated that* Caluromys derbianus *and* Marmosa mexicana *have a diploid number of 14 chromosomes, and that* Philander opossum *and* Didelphis marsupialis *have a diploid number of 22. The karyotypes of* C. derbianus *and* M. mexicana *are similar, whereas those of* P. opossum *and* D. marsupialis *are dissimilar. If the 14-chromosome karyotype represents a reduction from a primitive number of 22, these observations suggest that the change has occurred independently in the American and Australasian forms.*

The order Marsupialia is represented by two major living groups, one in the American continents and the other in Australasia. The American group consists of 69 species (*1*), and the Australasian group consists of about 160 species (*2*). The studies on the chromosomes of the Australasian species are now fairly comprehensive, and they show that the diploid number ranges from 10 to 24, there being a bimodal distribution with peaks at 14 and 22. In contrast, the information on American species is totally unrepresentative; so far only three species have been studied and these all have a diploid number of 22 (*3, 4*). Here we report, first, that a diploid number of 22 is not an invariant property of American marsupials and that a diploid number of 14 occurs in at least two species, namely *Caluromys derbianus* (woolly opossum) and *Marmosa mexicana* (murine opossum), and second, that although several species may have a diploid number of 22 their karyotypes may vary. The latter point is illustrated by a comparison of the karyotypes of *Didelphis marsupialis* (common opossum) and *Philander opossum* (four-eyed opossum).

We have studied the chromosomes of five woolly opossums (three male and two female), four four-eyed opossums (three male and one female), one male murine opossum, and two common opossums (one male and one female). Although only a few individuals of each species were observed, the results are reported in view of the rarity of much of the material. Apart from the common opossums, all specimens were trapped alive in Nicaragua and flown to Philadelphia for observation. The common opossums were trapped in the environs of Philadelphia.

In all species, except the common opossum, chromosome preparations were made from one or more tissues; namely, bone marrow, lung, kidney, skin, and peritoneum. Bone marrow preparations were made directly (*5*). The other tissues were cultured by the method of Basrur, Basrur, and Gilman (*6*), with either Eagle's medium and 15 percent fetal calf serum or medium 1066 with 10 percent lactalbumin hydrolyzate (0.5 percent) and 20 percent inactivated calf serum. In the case of the common opossum, the preparations were made from short-term cultures of peripheral blood leucocytes (7), a modified BGJ culture medium (*8*) being used (0.22 percent sodium bicarbonate); the preparations were incubated at 34°C, since this temperature more closely approximates the mean body temperature of the opossum (*9*).

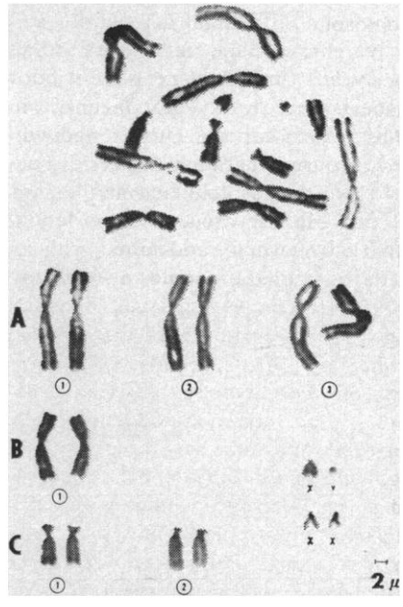

Fig. 1. Karyotype of a cell cultured from the tunica vaginalis from a male woolly opossum (*10*). Sex chromosomes XX from a female wooly opossum are included.

Figure 1 shows a metaphase spread and the karyotype from a male woolly opossum (*10*). Consistent results were obtained within and among animals with respect to chromosome number and karyotype. The diploid number is clearly 14. The karyotype is composed of three pairs of large sub-metacentric autosomes (group A), one pair of medium-metacentric autosomes (group B) and two pairs of medium sub-telocentric autosomes (group C). The X

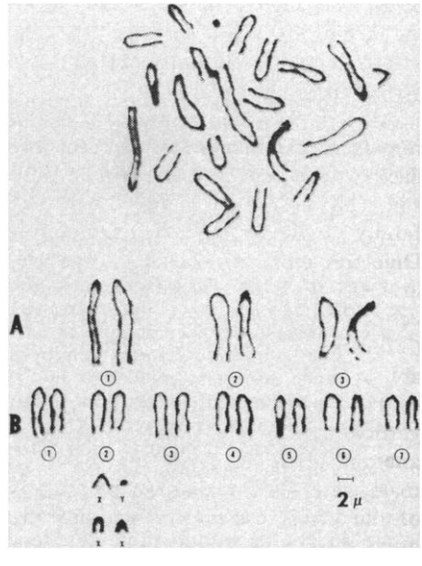

Fig. 2. Karyotype of a cell cultured from the peritoneum of a male four-eyed opossum. The sex chromosomes XX from a female four-eyed opossum are included.