radiation (emanating from the lake bed) which varies from 10 to 50 mr/hr 1 m above the soil (*5*). Adults, eggs, and young of wild birds are now being subjected to controlled sublethal doses at the Atomic Energy Commission's Savannah River Plant to determine the effects, if any, which might be expected at various doses and at key stages in the life cycle (*6*).

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References and Notes

1. S. I. Auerbach and D. A. Crossley, *Proc. 2nd Intern. Conf. Peaceful Uses Atomic Energy*, Paper 401 (1958); E. R. Graham, *Soil Science* **86**, (1958).
2. L. A. Krumholz and A. H. Emmons, *J. Wildlife Management* **17**, 456 (1953).
3. H. H. Willard and E. W. Goodspeed, *Industrial Engineering Chemistry* (Analytical ed.), **8**, 414 (1936).
4. E. Mizzan, *Atomic Energy of Canada Ltd.*, Chalk River Project, PDB 128 (1954).
5. P. K. Lee and S. I. Auerbach, *Oak Ridge Natl. Lab. Rept.* (2755) (1959).
6. This study is a part of radioecological investigations supported by an Atomic Energy Commission grant, contract At (-07-2)-10 with the University of Georgia, and is a joint contribution with the Health Physics Division, Oak Ridge National Laboratory, operated by Union Carbide Corporation for the United States Atomic Energy Commission. I am indebted to Dr. E. P. Odum, University of Georgia, and Dr. S. I. Auerbach and Dr. L. R. Farabee, Oak Ridge National Laboratory, for suggestions and technical assistance.
7. S. I. Auerbach, *Health Physics Ann. Prog. Rept., Oak Ridge Natl. Lab.* (2806) (1959).
8. Since a variety of opinions exist on the maximum permissible concentration for man, a range is given; see R. Bjornerstedt, *Science* **129**, 327 (1959).
9. F. J. Bryant, A. C. Chamberlain, A. Morgan, C. S. Spicer, *J. Nuclear Energy* **6**, 22 (1957).
10. J. L. Kulp, A. R. Schulert, E. J. Hodges, *Science* **129**, 1249 (1959).

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Antiozonants To Protect Plants from Ozone Damage

Abstract. Manganous 1,2-naphthoquinone-2-oxime protected tomato foliage in the field from damage apparently caused by excessive atmospheric ozone. The compound proved to be a very effective antiozonant. The similar cobaltous and manganous chelates of 8-quinolinol were also effective antiozonants. The materials were applied to cloth of the type used to make field tents for shade-grown tobacco. Tomato plants covered with cloth treated with cobaltous 8-quinolinolate were protected against otherwise damaging concentrations of ozone. These materials and methods may afford a useful way to reduce weather fleck of tobacco and other plant injuries caused by excessive atmospheric ozone.

Crop damage from excessive atmospheric ozone is becoming a serious problem throughout the country. Two of the best known examples of ozone damage are stipple of grape in southern California (*1*) and weather fleck of tobacco in Connecticut and Massachusetts (*2*). In New Jersey, ozone damage has been observed on spinach, alfalfa, cereals, red clover, beans, parsley, and grapes (*3*). Recently, Ledbetter *et al.* (*4*) demonstrated that many species of common plants can be readily injured by exposure to ozone.

During 1959, a wide variety of plants in Connecticut, such as tomatoes, potatoes, apples, and many kinds of weeds, showed severe damage after a weather period which resulted in serious fleck on tobacco. The type of injury on the various plants and its coincidence with the appearance of fleck on tobacco indicated that ozone was the damaging agent.

During this period we observed that tomato plants sprayed with a trial fungicide, manganous 1,2-naphthoquinone-2-oxime (*5*), were much less injured than were other tomato plants in the same plot. This suggested that the experimental fungicide is an effective antiozonant. The structural similarity of this compound to 8-quinolinol suggests the possible antiozonant action of the manganous and the cobaltous 8-quinolinolate. The antiozonant action of these compounds was determined experimentally, together with that of diphenyl-*p*-phenylenediamine and other compounds which are used in the rubber industry as antiozonants.

Because these materials have not been thoroughly tested on plants, it became of practical interest to see whether plants could be protected from ozone by covering the plants with standard tobacco shade cloth treated with the compounds.

The testing equipment consisted of an ozone-producing chamber, a gassing chamber, and ozone-measuring meters. Two different ozone-producing chambers were used. Both were glass boxes containing ultraviolet lamps. One box had four "Odor Out" Westinghouse bulbs, producing a total of 0.08 gm of ozone per hour. The other box, for greater ozone production, enclosed a Rayonet Superkill fixture with two high-ozone lamps, producing 2 gm of ozone per hour at full voltage. The ozone-laden air was pumped into either a glass-enclosed gassing chamber for exposing plants or cellophane bags for measuring the antiozonant effect of treated cloth strips. The ozone level in the chamber or bags was measured with a Mast portable atmospheric ozone recorder (model 724-1).

A strip of shade tent cloth dusted with a 25-percent formulation of manganous 1,2-naphthoquinone-2-oxime was sealed into a cellophane bag continuously supplied with ozone. The treated cloth reduced the ozone level from 0.7 to less than 0.1 part per million and held it at the lower level for 24 hours. Other test cloths were prepared as follows: they were dipped into chloroform solutions of 8-quinolinol, air-dried, then dipped into water solutions of the appropriate metal salt to form the chelate complex on the cloth. The cloths were air-dried again. The final weight of compound was 405 mg/m^2 of cloth. Both 8-quinolinolates proved to be good antiozonants, the cobaltous complex being more effective than the manganous complex.

Compounds used as antiozonants in the rubber industry were tested in the same manner as the 8-quinolinolates and proved to be even more effective. Table 1 compares the antiozonant action of the more effective materials with that of zinc ethylenebis(dithiocarbamate). The latter compound is now used in agriculture to protect crops against atmospheric ozone. The data from Table 1 show that the dialkyl-*p*-phenylenediamines and nickel di-N-butyldithiocarbamate are much more effective as antiozonants than is zinc ethylenebis(dithiocarbamate).

Can treated shade cloth protect plants against ozone damage? Young tomato plants covered with untreated shade cloth and gassed with ozone (0.8 part per million) for 4½ hours were severely damaged. Another set of tomato plants covered with tobacco shade cloth treated with cobaltous 8-quinolinolate showed practically no injury after 4½ hours in the ozone gassing chamber.

In tobacco fields, shade cloth treated with these materials may well reduce weather fleck to a considerable extent, although this has not been determined as yet. Because these materials are quite insoluble in water, cloth treated with them should be highly resistant to weathering. The use of catalytic antiozonants, such as nickel di-N-

Table 1. The antiozonant effect of cloth treated with antiozonants used in the rubber industry as compared with that of a cloth treated with zinc ethylenebis(dithiocarbamate), a material now used to protect crops against ozone. The cloths were tested in a chamber constantly gassed with ozone (0.4 to 0.7 part per million).

Lowest reading (in parts of ozone per million) after treated cloth was placed in chamber	Time chamber was held at lowest reading by cloth (hr)
Nickel di-N-butyldithiocarbamate	
0	67
N-Isopropyl-N'-phenyl-p-*phenylenediamine*	
0	18
N,N'-Di-sec-octyl-p-*phenylenediamine*	
0.01	12
Zinc ethylenebis(dithiocarbamate)	
0.06	0.5

butyldithiocarbamate, applied either to cloth covers or directly to plants, eventually may be useful to protect crops other than tobacco from ozone damage (*6*).

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References and Notes

1. B. L. Richards, J. T. Middleton, W. B. Hewitt, *Agron. J.* **50**, 559 (1958).
2. H. E. Heggestad and J. T. Middleton, *Science* **129**, 208 (1959).
3. Anon., *New Jersey Agr.* **42**, 3 (1960).
4. M. C. Ledbetter, P. W. Zimmerman, A. E. Hitchcock, *Contribs. Boyce Thompson Inst.* **20**, 275 (1959).
5. The fungicide (EF 35,429) was obtained from American Cyanamid Co., Stamford, Conn.
6. Samples of the compounds used in this study were obtained from American Cyanamid Co., Stamford, Conn.; Eastman Chemical Products, Inc., Kingsport, Tenn.; U.S. Rubber Co., Naugatuck, Conn.; and R. T. Vanderbilt Co., New York, N.Y.

28 March 1960

Serial Lactic Dehydrogenase Activity in Plasma of Mice with Growing or Regressing Tumors

Abstract. Close correlation has been observed between the serially tested plasma level of a glycolytic enzyme, lactic dehydrogenase, and the growth of several transplanted mouse tumors. The character of the lactic dehydrogenase curve determined by serial blood sampling of the animals during the development of such tumors is found to consist of five separate phases. The first three appear before any visible evidence of tumor growth. Inhibition or regression of tested tumors, induced by therapeutic compounds, was accurately reflected in a corresponding reduction of lactic dehydrogenase activity in the peripheral plasma of the host.

Lactic dehydrogenase activity has been found to be elevated in the serum or plasma of animals with various experimental tumors, and in some patients with malignant neoplasia (*1–3*). It is also increased in the serous effusions that bathe cancer tissue in human beings, and in the media surrounding various malignant cell lines in tissue culture (*2*, *3*).

The serial assay of this enzyme in the blood plasma of mice during the growth of such diverse transplanted tumors as Ehrlich solid carcinoma, sarcoma T-241, mammary adenocarcinoma E-0771, or the Cloudman S91 melanoma shows a close correlation between increasing tumor mass and the progressive elevation of lactic dehydrogenase activity in the peripheral blood. This confirms and elaborates earlier observations of elevation of this enzyme in the serum or plasma of tumor-bearing animals (*1–3*). In addition to an overall correlation of enzyme activity and tumor volume, five phases of the lactic dehydrogenase time curve have been observed, as illustrated in Fig. 1: (i) a latent period between tumor implantation and initial appearance of elevated levels of enzyme in the plasma lasting 24 to 96 hours, depending upon the tumor type and its growth rate; (ii) a rapid increase of the enzyme activity from normal values (approximately 500) to 2000 to 6000, giving a 5- to 10-fold enzyme increase prior to detectable growth of tumor implant (this increase takes place usually during the 2nd or 3rd day after implantation); (iii) a plateau following the initial increase and remaining essentially level for several days; (iv) a second rapid increase in plasma enzyme closely correlated with the logarithmic growth phase of the tumor, but usually initiated prior to measurable growth of the new tumor mass. Such an enzyme increase continues with growth of the tumor up to values of 25,000 to 50,000 units—an increase over normal enzyme values of 50- to 100-fold. The final phase (v) frequently observed is an abrupt fall in enzyme level in the plasma just prior to the death of the tumor-bearing animal. Such a fall has also been seen in terminal cancer patients (*2*). Although Fig. 1 is diagrammatic, it accurately represents the composite data for the control mice in 20 individual experiments. The lactic dehydrogenase activity curve was derived from 707 individual enzyme determinations on 163 untreated Swiss ICR mice implanted subcutaneously with the Ehrlich carcinoma. The bleeding was done serially on the same animals by a modification of the orbital bleeding technique (*4*). The plasma enzyme was assayed by a microtechnique modification of a spectrophotometric procedure described previously (*5*). The tumor growth curve in Fig. 1 is an expression of the average tumor volume, with time, determined by a total of 1172 three-dimensional caliper measurements on the solid Ehrlich carcinomas of these mice, taken from the palpable appearance of the lesion to the death of the animal.

Appropriate correlative alterations of the lactic dehydrogenase activity curve occur when the growth of the tumor is modified by various antitumor compounds. Figure 2 illustrates the development and regression of an Ehrlich carcinoma implanted in an animal undergoing treatment with orthophenylenediamine (*6*) and indicates the corresponding increase and decrease of the enzyme activity in the blood plasma as a function of tumor behavior.

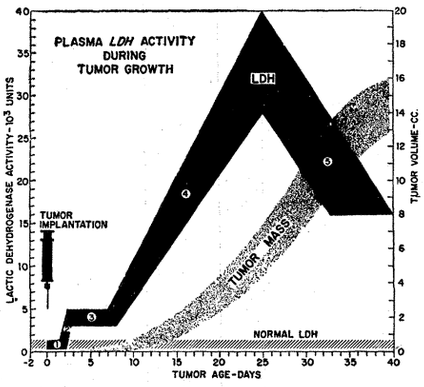

Fig. 1. Correlation of increasing lactic dehydrogenase activity (LDH) in plasma with progressive growth of the solid Ehrlich carcinoma, and illustration of five distinct phases of the enzyme response curve.

The first three phases do not show in this plot since the lactic dehydrogenase determinations were not started until the 4th day following tumor implant, at which time the phase (iii) plateau had already been reached. The data of Fig. 2 were obtained from a single mouse and illustrate the possibility of serial accumulation of enzyme information with these techniques on individual experimental animals, analogous to the close clinical following of a patient.

Although the tumor regressed completely in this experiment and the animal remained tumor free during several months of subsequent observation, the enzyme activity never returned to a completely normal level. This has been confirmed in several experiments in which the enzyme activities in the plasma of mice with regressed tumors slowly approach normal levels after the original precipitous decrease, but never-

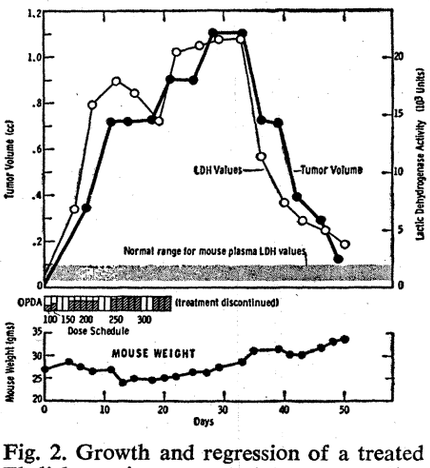

Fig. 2. Growth and regression of a treated Ehrlich carcinoma and the corresponding plasma levels of lactic dehydrogenase activity (LDH).