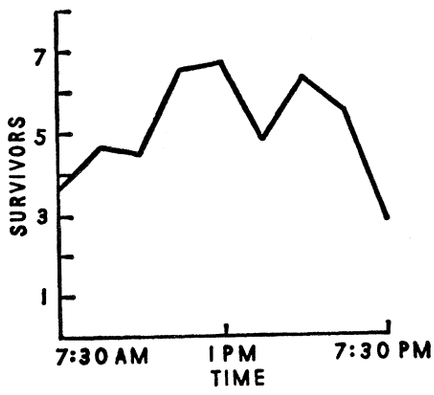

Fig. 1. Survival from pentobarbital sodium as a function of time of day. The curve represents the mean of six replications on widely separated days. Each point represents the mean survival rate of six groups of 18 animals given a dose of 130 mg of pentobarbital sodium per kilogram of body weight, intraperitoneally.

Data were subjected to the arcsin transformation and analyzed by the method of orthogonal polynomials (*3*). Theoretical variances were used to make chi-square tests of significance. Figure 1 shows the mean survival rate derived from the six experiments and Table 1 contains the analysis of variance of the data.

The analysis of variance shows in its upper portion evidence for or against the existence of various trends (linear, quadratic, and others); the lower portion relates to whether a given trend is similar from day to day. In detail, the table indicates first that a linear trend has not been demonstrated, but that this may be due to the heterogeneity between days. The quadratic term has been shown to exist and is similar from day to day. There is very little evidence for the cubic term, and even less for the quartic and quintic; all of these are similar on various days. The sextic term does not exist on the average but shows a high degree of inconsistency; therefore it may on occasion be real. The septic term exists on the average and is reasonably consistent. The octic term does not exist on the average, and there is little evidence of its being inconsistent between days.

Under the conditions of this experiment the LD_{50} dose (*4*) killed substantially more than 50 percent of the animals. This trend, which was so extreme on one day as to allow only one survivor in several groups, may have contributed greatly to inconsistency in the form of the curve between days.

Our data indicate the existence of short-term fluctuations in the toxicity of pentobarbital sodium in a mammalian species. The 4-hour periods at which such observations are usually made, and the even longer period used by Emlen and Kem (*5*) in studying this same drug in the mouse, are too long to reveal the full complexity of the toxicity curve.

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Ozone: Nonlinear Relation of Dose and Injury in Plants

Abstract. *Ozone produces a sigmoidal dose-injury response in sensitive tobacco and pinto bean. A definite threshold concentration and presentation time are required before injury is initiated.*

Ozone was first recognized as a phytotoxic air pollutant of serious concern to agriculture when it was identified as the agent responsible for stipple in grape (*1*) and weather fleck in tobacco (*2*). Visual symptoms and pathological histology of ozone in a number of agronomic plants have been reported (*3*).

Recently, MacDowall *et al.* (*4*) correlated tobacco fleck in the field with amount of oxidant (which they term ozone) by multiplying the dose (time × concentration) by an empirical exposure factor, which they related to evapotranspiration. This exposure factor brought their dose-injury response into a linear relation, as suggested by Middleton (*5*). Using this relation, they found that, for a given total dose, conditions favoring high evapotranspiration would produce greater injury to tobacco than would conditions of high humidity and low wind (days which favor stagnation and large amounts of oxidant). The use of this empirical exposure factor, which does make their data fit Middleton's linear relation of dose to injury, ignores the extensive work that suggests that plants are more sensitive to a given phytotoxic air pollutant when moisture stress is low. Their conditions may favor the production of different types of phytotoxicants and may not necessarily be conditions that sensitize plants to one specific phytotoxicant such as ozone. Work in several laboratories suggests that values for ambient oxidant cannot be interchanged with ozone values.

Menser *et al.* (*6*), in view of Middleton's work (*5*), have also suggested, for four varieties of tobacco, that the relation between ozone concentration and injury is linear after a sensitivity threshold has been reached. They do not have sufficient data, however, to correctly interpret their injury curve; from their graphs, three of the varieties show a definite log-shaped curve for the few concentrations used. Their doses do not go high enough to show the shape of the upper end of the curve.

Middleton *et al.* (*7*) reported data on 4-hour exposures of pinto bean to from 10 to 50 pphm (parts per hundred million) of ozone per hour for a total dose of 40 to 200 pphm. They plotted injury against dose, fitted a linear regression line to their data, and found a significant positive correlation coefficient. Neither the linear regression nor the positive correlation coefficient necessarily demonstrated that a true linear dose-injury relation existed. The work reported by Middleton (*5*) restated the early work (*7*), but gave no additional experimental data on the dose-injury relation. Thus, the concept that plant injury is linearly related to ozone dose has been perpetuated even though it is based on preliminary data and an incomplete experimental design.

A more detailed study of the dose-injury relation of ozone in tobacco (*Nicotiana tabacum* L., var. Bel-W_3) and bean (*Phaseolus vulgaris* L., var. Pinto) was initiated in this laboratory to help explain some of the variability in environmental effects on plant sensitivity to ozone. Plants were grown in growth chambers (with a 14-hour, 27°C day and a 10-hour, 21°C night) and exposed to ozone in greenhouse exposure chambers for periods starting after 5 hours of light. Temperature, light intensity, and humidity were fairly constant during all exposure times. Four concentrations (10, 20, 35, and 55

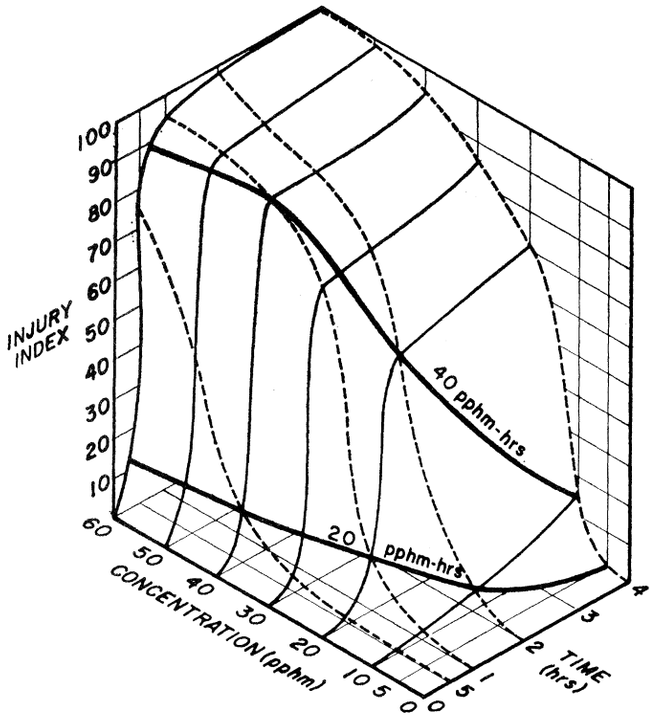

Fig. 1 (left). Interrelations of time and concentration on the sensitivity of pinto bean plants to ozone.

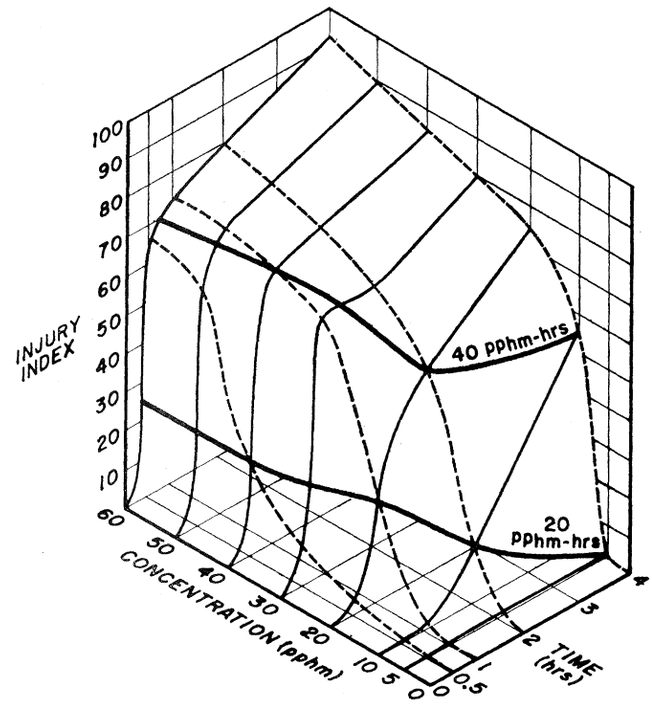

Fig. 2 (right). Interrelations of time and concentration on the sensitivity of tobacco (Bel-W_3) plants to ozone.

pphm as measured by the Mast oxidant recorder) and four time periods (½, 1, 2, and 4 hours) were used, but no exposures were made at 4 hours for the 35- or 55-pphm concentrations, or at 2 hours for the 55-pphm concentration. These 13 doses (time × concentration) were replicated four times on a random basis for a total of 52 exposures. Each exposure consisted of four pots (replications) of tobacco (one plant per pot) and four pots (replications) of pinto bean (two plants per pot). Thus for each dose we had 16 replications for both tobacco and pinto bean. Single exposures were made at 5 pphm for 2 and 4 hours to complete the lower end of the 2- and 4-hour curves.

In preliminary analysis of the results, plotting injury against dose gave two distinct curves for tobacco and three for pinto bean. Doses in the shorter exposures gave significantly greater injury than equal or higher doses given for longer periods. Thus, ozone injury cannot be discussed in relation to a simple dose concept, but must be discussed in relation to both time and concentration.

The results for pinto bean (Fig. 1) and tobacco (Fig. 2) have been presented and show that injury is a function of both time and concentration. The response surface thus generated shows that: (i) the threshold concentration for injury depends upon the length of exposure, for exposures up to at least 4 hours; (ii) a time lag exists after exposure before injury occurs for concentrations possibly as high as 60 pphm; (iii) the response change is rapid during short time intervals when the concentrations are 20 pphm and higher; and (iv) the response change with small concentration changes is rapid after exposure of more than 1 hour. The dose curves at 20 and 40 pphm-hours for both pinto bean and tobacco are shown as wide lines cutting across the response surface. These dose lines show graphically that a nonlinear dose-injury relation does exist, that the dose response is related to time and that it is more sensitive to time for pinto bean than for tobacco.

The steepness of the concentration-injury curve, especially for the shorter time intervals, is much greater than might have been expected. Thus, when we study effects of environmental variables on sensitivity of plants to ozone and we use a critical concentration range, any slight variation in concentration or in conditions within the microenvironment of a leaf could result in a major variation in leaf injury. This may explain some of our injury variations, which have been difficult to understand.

Subsequent experimental work has substantiated the basic results reported in this paper, and preliminary observations suggest that the shape and position of the concentration-injury curve for a given time depends upon a number of environmental variables, including season of the year, or general growth conditions, or both.

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