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EFFECTS OF PESTICIDES ON THE BIOCHEMISTRY AND PHYSIOLOGY OF PLANTS

by

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TITLE

Effect of Pesticides on the Biochemistry

and Physiology of Plants

BY

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This manuscript is dedicated to those who in their presence or in their absense have made it possible, and to those few but very close friends who have made it worthwhile.

D. B.

EXPERIMENT I

Pesticide Interactions in Oats (Avena sativa L. 'Neal')

INDRODUCTION

Part I

The use of pesticides is a common practice in agriculture today. During the last decade, the use of combinations of two or more pesticides on the same crop has become prevalent in an attempt to control a number of pests. When two pesticides are combined, two responses may occur: 1) they may act independently of each other or 2) the action of each pesticide may be dependent upon the presence of the other. The latter is known as an interaction between pesticides, and it is a subject that has been reviewed recently by Putnam and Penner (1974). The reviewers discuss a variety of interactions, among which are included herbicide-herbicide, herbicide-insecticide, and herbicide-fungicide combinations. In addition, interaction between pesticides and plant nutrients are also known to exist. (Adams, 1965; Bingham and Upchurch, 1959; Penner, 1970).

Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) and the organophosphate insecticide phorate (O,O-diethyl-S-(2-(ethylthio) ethyl) phosphorodithiate) interacted to cause loss of cotton (*Gossypium hirsutum* L.) (Hacskaylo et al., 1964) and oats (*Avena sativa* L.) (Nash, 1967). Parks et al. (1972) showed that phorate increased the uptake of prometryne (2,4-bis(isopropylamino)-6-methylthio)-s-triazine) in soybean (*Glycine max* L. Merr.) roots. Disulfoton has been shown to increase the transport of another s-triazine herbicide, atrazine (2-chloro-4-(ethylamino)-6-isopropylamino)-s-triazine) in soybeans (Phatak, 1972). Atrazine and alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide)

interacted synergistically in Japanese millet (*Echinochloa crussgalli* L.Beauv. var. *frumentcea* L.) (Akobundu, 1973). Another *s*-triazine herbicide, cyanazine (2 [[4-chloro-6-(ethylamino)-*s*-triazin-2-yl]amino]-2 methylpropionitrile), and trifluralin (α,α,α-trifluoro-2,6-dinitro -*N*, *N*-dipropyl -*p*-toluidine) interacted antagonistically in oats (Bucholtz, unpublished data). The substituted urea, diuron, and all the *s*-triazines that were discussed are very good inhibitors of photosynthesis (Corbett, 1974).

The purpose of this experiment was to determine if an interaction occurs between combinations of the seemingly unrelated pesticides, alachlor, phorate, and trifluralin with photosynthesis-inhibiting herbicides. After demonstrating the presence of an interaction, an attempt will be made to explain the reason for the interaction. The demonstration and understanding of pesticide interactions could reduce the occurrence of costly losses in the field.

Part II

Several mathematical approaches have been used to indicate the presence of pesticide interactions in plants (Colby, 1968; Gowing, 1959; Hamill and Penner, 1973). Basically, these techniques involve the calculation of expected values of the respones for the combination treatments as a function of the responses produced by each of the pesticides comprising the combination. The presence of an interaction is determined by comparing the value of the calculated expected response with the value of the observed response for the combination treatment. These techniques are dependent on four values (the responses given by both

pesticides applied singly, their combined treatment, and the untreated control) and their variances, and as a result can produce untrustworthy interpretations.

Since the independent variables, pesticide concentrations, of pesticide interactions are quantitative, the response of the plant to pesticides can be expressed as a function of the levels of the independent variables. Carney et al. (1973) have done this for several plant species that were responding to combinations of six herbicides with ozone. Their technique employed regression lines, which are empirically fitted curves, in which the functions represent the best mathematical fit to the observed set of data (Sokal and Rohlf, 1969). The complexity of regression lines vary from the simplest linear function to the most complex response surface. Regression lines can be used to predict a response of the dependent variable to any set of levels of the independent variables within the levels used in the experiment (Cochran and Cox, 1968).

Figure I shows the relationship between the regression lines and the generalized equations describing them. The complexity of these regression lines and equations range from a linear function to a polynominal function which could be used to describe an interaction between two pesticides. The latter is a function similar to the one used by Carney et al. (1973).

The function in Figure I-A describes the dependence of the response, Y, on the level of the independent vaiable, X. This type of function could describe the dependence of a plant's dry matter accumulation on increasing levels of a herbicide, X.

However, as in many biological systems, the response of the plant may not be constant over a wide range of levels of herbicide X. A more likely response of a plant to a herbicide would be that shown in Figure I-B, where the response, dry matter accumulation, is decreased rapidly at low levels of X and continues to decrease, but at a slower rate, at higher levels of X, ie. a curvilinear dependence of Y on X. The search for an interaction requires the introduction of a second variable, Z. If after the introduction of the variable, no interaction occurs, then the function will be similar to that of Figure I-C. The lack of an interaction is indicated by the parallel lines of the graph and by the absence of the cross-products term in the equation (a cross-products term is of the form, $b_n XZ$). If an interaction is present it is indicated by the presence of nonparallel lines and the cross-products term as in Figure I-D. If the response, Y, is dry matter accumulation and if the lines converge as the value of X increases (which occurs when the regression coefficient of the cross-products term is positive) then the interaction is antagonistic. An antagonistic interaction occurs when either of the variables, X or Z, interfers with the other's effect (Sokal and Rohlf, 1969). If the lines diverge as the value of X increases (which occurs when the regression coefficient of the cross-products term is negative) then the interaction is synergistic. A synergistic interaction occurs when either of the variables, X or Z, more than complement the other's effect (Sokal and Rohlf, 1969). Figures I-E and I-F extend these same relationships to curvilinear responses. Figure I-F is the generalized regression line that

would be expected if two pesticides antagonized each other's effect on dry matter accumulation.

EXPERIMENTAL SECTION

A preliminary study was conducted to determine workable concentration ranges for alachlor, atrazine, cyanazine, diuron, methazole (2-(3,4-dichlorophenyl)-4methyl-1,2,4-oxadiazolidine-3, 5-dione), phorate, and trifluralin using oats (Avena sativa L. 'Neal') as a bioassay plant. After concentrations were established, methanol:water (1:3 v/v) solutions of technical grade pesticides were applied at four rates to 520-g samples of a Keith silt loam. This soil contained 2.1% organic matter and 21.4% clay, and its pH was 6.5. The photosynthesis-inhibitor (PSI) herbicides, atrazine, cyanazine, diuron, and methazole, were applied individually with alachlor, phorate, and trifluralin resulting in twelve factorially arranged experiments. After application of the pesticides, sufficient time was allowed for the methanol to evaporate before the soil was mixed to evenly distribute the chemicals. The soil was then placed in 9-cm diameter pots and seeded with approximately 50 oat seeds. Each treatment was replicated four times.

Visual injury symptoms, attributed to the PSI herbicides, were first noticeable ten days after planting. The severity of injury from the PSI herbicides was measured by rating plants and assigning a value ranging from one to five. A value of one was given to a plant with no symptoms, two to a wilting plant (wilting even in moist soil), three to desiccated but still green leaf tissue, four to desiccated leaf tissue but bleached tissue present with green tissue, and five to completely bleached tissue. The plants were rated beginning on the tenth

day and on alternate days thereafter for a total of five times. Regression analyses were performed on the means of these five rating values. In addition, plant height reduction was also rated as a measure of alachlor, phorate, and trifluralin bioactivity. However, height reduction resulting from alachlor, phorate, and trifluralin was unaffected when applied in combination with the PSI herbicides, so these data are not presented.

Nineteen days after planting, the oat shoots were excised at the soil surface. The shoots were dried in a forced-air oven at 70 C for 2 days and then weighed. Regression analyses for both dry weights of shoots and ratings of leaf necrosis were performed for each of the twelve experiments. The regression model used to describe the response surface generated by the chemical combinations was as follows: $Y = b_0 + b_1$ (pesticide) + b_2 (PSI) + b_3 (pesticide)^2 + b_4 (PSI)^2 + b_5 (pesticide) (PSI), where Y equals the dependent variable, either dry weight or rating, b_0, the intercept or the value of the untreated plants, b_1, b_2, b_3, b_4, and b_5 the regression coefficients for the independent variables, the concentrations of pesticide and PSI in µg/g of soil. Since the dry weight and rating values are inversely related, i.e. a smaller dry weight and a larger rating value both indicate a more severe effect of the pesticide or PSI, the b's for the two dependent variables should be of opposite sign. If the b_5 is positive for dry weight or negative for rating, the response of the oats to the combination is antagonistic. Conversely, if b_5 is negative for dry weight or positive for rating the interactions are synergistic.

A pesticide uptake study was conducted using oat seedlings grown in washed sand contained in 250-ml Styrofoam cups. Sand was chosen rather than the soil described previously to facilitate the recovery of the seedlings' root systems. Treatments to the sand before planting included the following: 0.5 µCi of ^{14}C-labeled atrazine, cyanazine, or diuron (specific activity 24.9, 5.6, and 4.1 µCi/mg, respectively, atrazine and cyanazine were labeled in the ring and diuron in the carbonyl carbon) each in combination with nonradioactive alachlor, phorate, and trifluralin at 2, 6, and 4 µg/g of sand, respectively. This study also included pots of washed sand each receiving 0.5 µCi of ^{14}C-labeled phorate (specific activity 13.6 µCi/mg, labeled in the 1 position of the ethyl moiety attached to the sulfur) or trifluralin (specific activity 37.2 µCi/mg, the ^{14}C atom was distributed 85% in the ring and 15% in the $-CF_3$ moiety) in combination with each of the nonradioactive herbicides, atrazine, cyanazine, diuron, and methazole at 0.1, 0.2, 0.8, and 1.0 µg/g of sand, respectively. Additionally, all the ^{14}C-labeled pesticides were applied to the sand without treatments of nonradioactive pesticides. All of the treatments were replicated three times, and six oat seeds were planted in each Styrofoam cup. Nutrients and water were supplied to the sand culture by overhead sprinkling with half-strength Hoagland solution (Hoagland and Arnon, 1950) as needed to maintain good plant growth. No leakage from any of the cups was observed. Eighteen days after planting, the sand was washed carefully from the oat roots. The plants were pressed between layers of paper and dried in a

forced-air oven at 70 C for 2 days. The root lengths were then measured. Autoradiographs of the plants were made by exposing No-Screen X-ray film (Eastman Kodak Company) to the radioactive plants for 2 weeks. The entire plant was then oxidized in a Packard Tri-Carb Sample Oxidizer, model 306, which converts organic matter into CO_2. The CO_2, as it is formed, is trapped in an organic amine solution (Carbosorb), which is then automatically combined with a liquid scintillation cocktail (Permafluor). The radioactive carbon in the ^{14}C-labeled material is converted to $^{14}CO_2$ and is also trapped in the Carbosorb. Radioactivity from the oxidized plant material was assayed in a Packard Tri-Carb liquid scintillation spectrophotometer, model 3320. Counting efficiency of each sample was determined by the external standard technique. The percentage of ^{14}C pesticide taken up was calculated by dividing the radioactivity found in the plants by the amount of radioactivity initially applied to the cups, times 100. A correlation coefficient was calculated expressing the relationship between ^{14}C-labeled pesticide uptake and root length.

A study was conducted involving the foliar application of atrazine to oats growing in soil treated previously with alachlor or trifluralin. A sandy loam soil was treated with alachlor or trifluralin each at 0, 3, or 6 µg/g, potted and seeded as described previously. Ten days after planting, the emerged oat plants received 0, 0.56, 1.12, or 1.68 kg/ha of formulated atrazine 80W delivered in 31 l/ha of Tween 20 with a cabinet