Effect of Environmental Factors on Phosphamidon Degradation

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Because field studies indicated an external environmental effect on the rate of degradation of phosphamidon in plants, laboratory studies were undertaken to measure the magnitude of this effect. The results indicate that the length of light day does not affect the rate of degradation of phosphamidon and its metabolites, but that a minimum light period is necessary. For each 15° rise in temperature between 60° and 90° F., the rate of disappearance is increased by approximately 2 days. At all concentrations, metabolites of phosphamidon are degraded faster than the phosphamidon itself.

In residue analyses of phosphamidon (1 - chloro - 1 - diethylcarbamoyl-1-propen-2-yl dimethyl phosphate) on spinach and broccoli, carried out in Maryland in 1960 and 1961 (*2*), there seemed to be an effect of temperature on the length of time phosphamidon took to disappear completely. This would be important in determining time limitation between treatment and harvest of the crop in various localities and in different seasons of the year. Experiments were carried out in the laboratory during the summer of 1961 to determine in a quantitative manner the effects of temperature and length of day on the degradation of phosphamidon in plants.

Materials and Methods

Experiments were carried out in two phases, the temperature phase and the length of day phase. In both phases, the growth chambers of the Horticulture Department of the University of Maryland were utilized. In these chambers, both temperature and length of day can be controlled artificially. For the temperature phase, snap beans and potatoes were planted in the greenhouse on May 26. After 41 days in the greenhouse, the plants were transferred to the growth chambers. Both the beans and the potatoes were in the blossom stage at this point. Three growth chambers were used, one at 60 ± 3° F., another at 75 ± 3° F., and the third at 90 ± 3° F. The length of day was constant at 14 hours of light alternating with 10 hours of darkness. The plants were left in the chambers for 2 days before being treated. Treatment was made by dipping each plant into a solution of 2 quarts of phosphamidon in 50 gallons of water for 7 seconds. Duplicate analytical samples were taken immediately after drying and at 1, 3, 4, 5, and 8 days after treatment of both beans and potatoes.

The plants for the length of day phase of the experiment were planted on June 12. After 36 days in the greenhouse, when the plants were in bloom, they were moved to the growth chambers. Again three growth chambers were used, one with 1 hour of light alternating with 23 hours of darkness, another with 12 hours of light and 12 hours of darkness, and the third with 23 hours of light alternating with 1 hour of darkness. The temperature was constant in the three chambers at 75 ± 3° F. After 2 days in the chambers, the plants were dipped in a solution of 2 quarts of phosphamidon in 50 gallons of water for 7 seconds. Duplicate samples for analysis were taken at each sampling at 1, 3, 5, 7, 9, and 11 days after treatment.

After completing the analysis of plants from these two experiments and finding extremely high residues throughout the sampling period, the temperature experiment was repeated with a lower concentration of phosphamidon to determine if concentration had any effect on the slopes of the disappearance curves. The plants for this experiment were planted in the greenhouse on August 16. All experimental conditions were the same as in the first temperature experiment except that in this experiment plants were treated with 0.5 pint of phosphamidon in 50 gallons of water by dipping for 7 seconds in the solution. Duplicate samples of each kind of plant from each growth chamber were taken 0, 2, 4, 6, 8, 10, and 12 days after treatment.

Samples were composed of one or more whole plants each; for beans they

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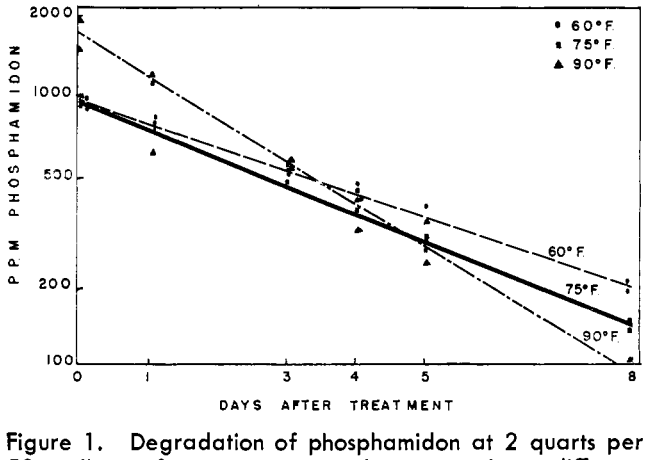

Figure 1. Degradation of phosphamidon at 2 quarts per 50 gallons of water on snap beans at three different temperatures and constant length of day

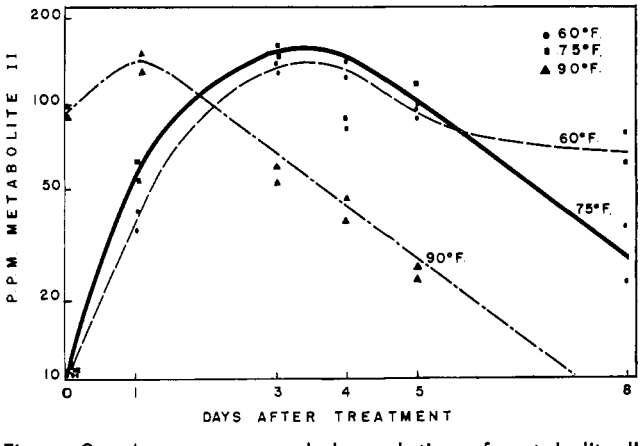

Figure 2. Appearance and degradation of metabolite II when phosphamidon is applied at 2 quarts per 50 gallons of water to snap beans at three different temperatures and constant length of day

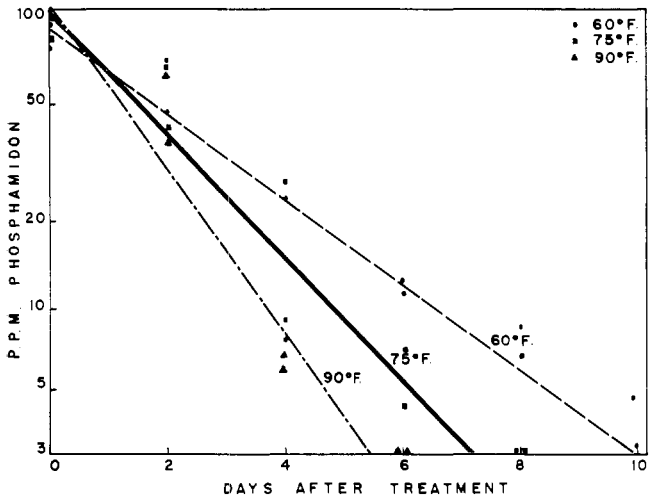

Figure 3. Degradation of phosphamidon at 0.5 pint per 50 gallons of water on snap beans at three different temperatures and constant length of day

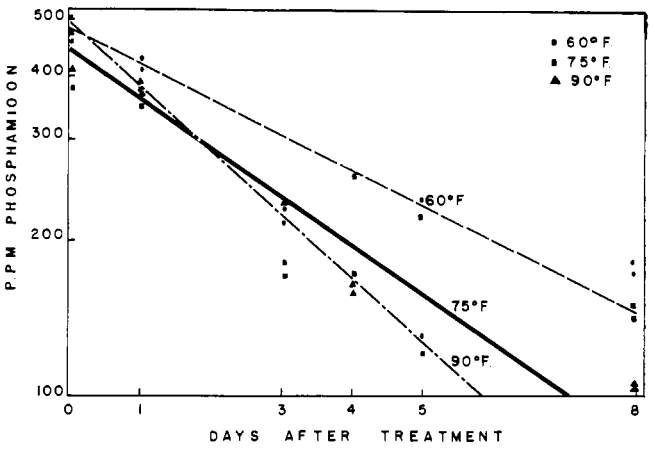

Figure 5. Degradation of phosphamidon at 2 quarts per 50 gallons of water on potatoes at three different temperatures and constant length of day

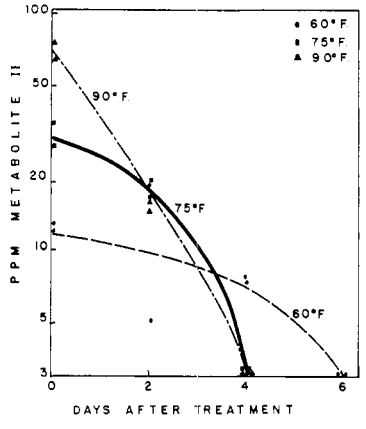

Figure 4. Appearance and degradation of metabolite II when phosphamidon is applied at 0.5 pint per 50 gallons of water to snap beans at three different temperatures and constant length of day

varied from 35 to 135 grams and for potatoes, from 60 to 165 grams. The majority of bean samples weighed between 75 and 85 grams, while most potato samples weighed between 90 and 105 grams.

After sampling, plants were immediately extracted without being stored in the freezer, and the extracts were stored at 40° F. The method of extraction and analysis was that for total phosphorus using the molybdenum blue reaction as adapted by Anliker and Menzer (*1*).

Results and Discussion

The results of the temperature experiments are given in Figures 1 through 7, and the results of the length of day experiment are given in Figures 8 through 11. In all cases except the lower concentration temperature experiment in potatoes, curves are given for both phosphamidon and metabolite II, desethylphosphamidon, in each phase of the experiment.

The effect of temperature on the degradation of phosphamidon in snap beans and potatoes is obvious from the figures. This effect is due, in part, to the behavior of the phosphamidon with respect to its metabolic products. At higher temperatures, metabolite II is produced faster than at lower temperatures in snap beans, and this, in turn, means that phosphamidon is being degraded faster. The opposite is the case in potatoes. Figure 3 shows that 15 degrees difference in temperature causes a difference of approximately 2 days in the time required for complete breakdown in snap beans at the concentration used. This is also true in potatoes (Figure 7). There is a regular gradation of slopes and half-lives as the temperature is changed in both beans and potatoes.

The effect of length of day on the degradation of phosphamidon is not so pronounced, however. In snap beans, there is not a linear relationship between the speed of the degradation of phosphamidon and the length of day involved. This appears to be true of metabolite II also, as would be expected. In potatoes, length of day does not seem to play even as great a part as in snap beans. The initial slopes of the curves for the three lengths of day are approximately the same. However, it appears that after a certain period of time under conditions of very short lengths of day the rate of breakdown of phosphamidon is definitely reduced. In other words, the curve begins to level off (Figure 10). This is probably a direct result of the disruption of the plant's normal metabolic processes resulting from the short period of light. Under field conditions, length of day would have little effect on the degradation of phosphamidon since the extremes of day lengths used in this experiment are not encountered.

The effect of concentration on the rate of degradation of phosphamidon can be seen from the slopes of the lines at the two concentrations used. One must conclude that the concentration does play an important part in the speed at which the plant is able to completely break down the phosphamidon. The actual rate is also affected, as seen from comparing the slopes of the lines obtained at the two concentrations. This indicates that the breakdown of phosphamidon is dependent on some limiting factor within the plant itself and is not simply a function of temperature, light, or other environmental factors. This is further borne out by comparing the behavior of metabolite II at the two concentrations. At the higher concentration, the metabolite builds up slowly to a limiting factor and then degrades, while at the lower concentration the metabolite is produced in its highest concentration almost instantaneously. Also, the degradation of metabolite II is faster than that of phosphamidon; it never appears alone, but always in the presence of phosphamidon.

The initial deposit of phosphamidon on snap beans in the temperature experiment at the 2 quarts per 50 gallons of water concentration varied between 1936 and 880 p.p.m. On potatoes, it varied between 619 and 340 p.p.m. At the lower concentration of 0.5 pint per 50 gallons of water in the temperature experiment, the initial deposit varied between 154 and 59 p.p.m. on snap beans and between 108 and 81 p.p.m. on potatoes. At the higher concentration, the residue had degraded to the 100 to 300 p.p.m. range by 8 days after treatment. At the lower concentration, the phosphamidon was completely degraded by 12 days after

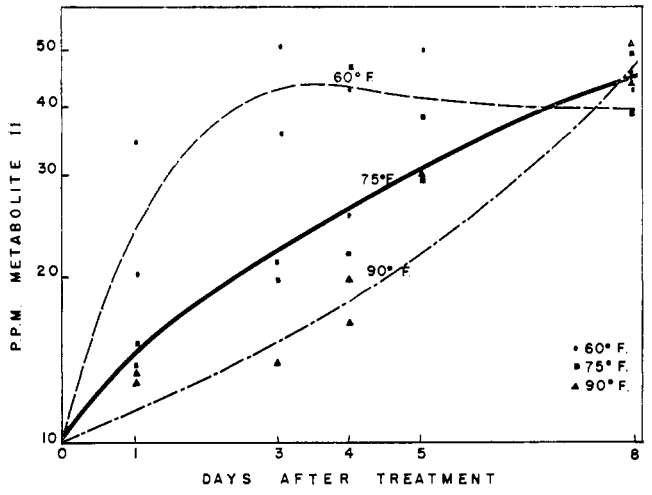

Figure 6. Appearance and degradation of metabolite II when phosphamidon is applied at 2 quarts per 50 gallons of water to potatoes at three different temperatures and constant length of day

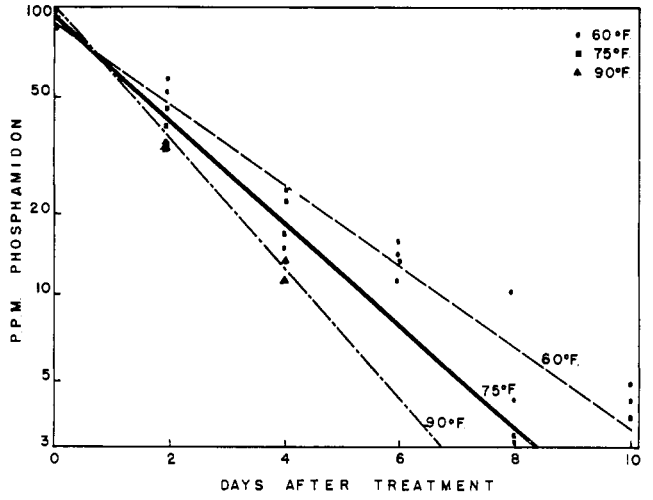

Figure 7. Degradation of phosphamidon at 0.5 pint per 50 gallons of water on potatoes at three different temperatures and constant length of day

(No curve could be drawn for metabolite II from dota obtained)

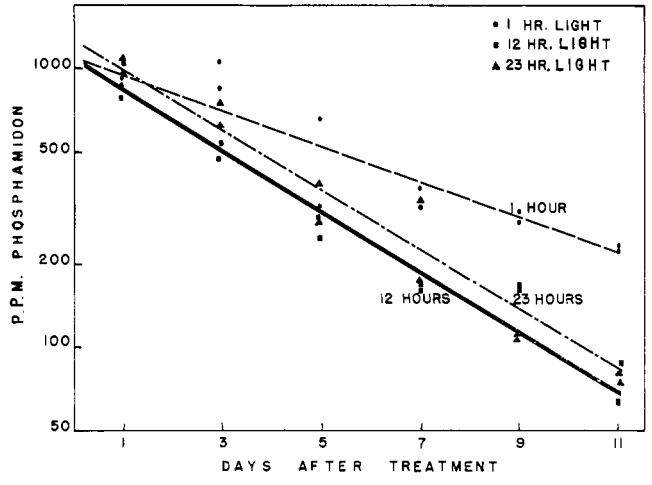

Figure 8. Degradation of phosphamidon at 2 quarts per 50 gallons of water on snap beans at three different lengths of day and constant temperature

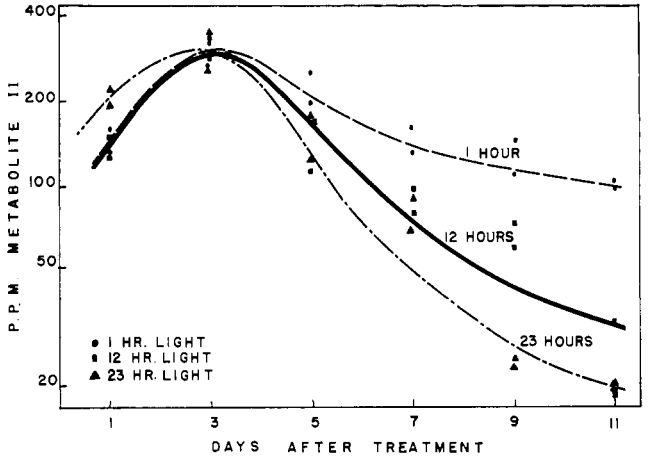

Figure 9. Appearance and degradation of metabolite II in snap beans when phosphamidon is applied at 2 quarts per 50 gallons of water; three lengths of day and constant temperature were used

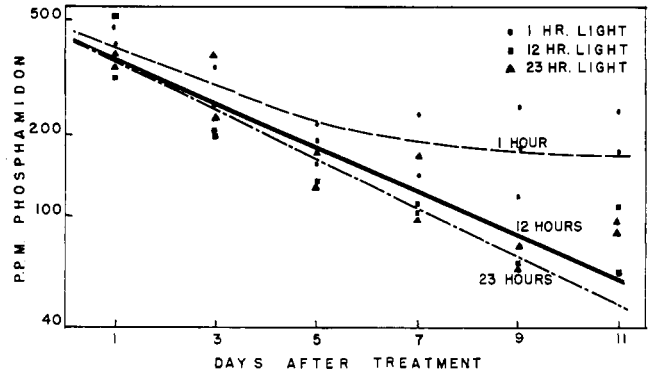

Figure 10. Degradation of phosphamidon at 2 quarts per 50 gallons of water on potatoes at three different lengths of day and constant temperature

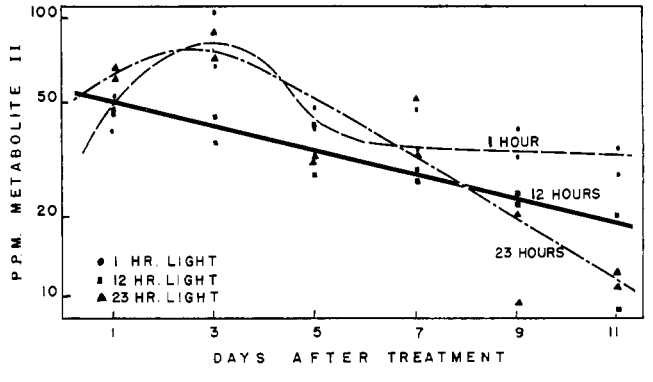

Figure 11. Appearance and degradation of metabolite II in potatoes when phosphamidon is applied at 2 quarts per 50 gallons of water; three lengths of day and constant temperature were used

treatment. In the length of day experiment, the initial deposits varied between 1467 and 876 p.p.m. on snap beans and between 547 and 301 p.p.m. on potatoes. Here the deposit was in the 70 to 300 p.p.m. range at 11 days after treatment.

These data have a very direct application to the use of phosphamidon under field conditions and support the previous observations made on spinach and broccoli. In practice, one will have to consider the conditions of temperature under which phosphamidon is used. More time will have to be allowed between last application and harvest when colder temperatures are expected. However, the effects observed herein are greatly magnified because of the high concentrations of phosphamidon used. At dosages used in the field, one would not expect to find such pronounced differences because the deposits of phosphamidon are considerably less.

Acknowledgment

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Literature Cited

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(2) Menzer, R. E., Ditman, L. P. *J. Econ. Entomol.*, **56**,No. 1 (1963).

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INSECTICIDE ANALYSIS

The Quantitative Determination of Heptachlor in Pesticide Formulations by Gas Chromatography

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By using lindane as an internal standard, heptachlor can be determined quickly and accurately with an argon ionization detector. A method for determining heptachlor by gas chromatography has been developed and applied to several commercial products. The calibration curve can be prepared in 1 hour when four points are plotted. Additional samples can then be run in 15 minutes. The method is easy, fast, and accurate.

SPECIFIC CHEMICAL ANALYSES of pesticides are often difficult, time consuming, and cumbersome. Gas chromatography has made it possible to determine many pesticides accurately and rapidly. Previous work by Coulson, Cavanagh, and Stuart (*1*); Zweig and Archer (*5*); and Hughes and Freed (*2*) has shown the advantages of this method. More recently, the availability of stationary phases capable of withstanding high temperatures and the sensitive argon ionization detector have made the technique more useful.

Repetitive injections of a given volume of a sample by means of a syringe give variable peak heights unless extreme care is exercised. This variation is undesirable in routine quantitative work. Experience has proved that different analysts employ different injection techniques, and that even when the techniques were standardized, reproducibility was poor. Therefore, an internal standard or marker was employed. In the present work, an unsuccessful attempt was made to use a nonhalogenated internal standard; however, when a halogenated compound was employed, the results became very useful.

Apparatus and Reagents

Gas Chromatograph. Barber-Colman Model 10 equipped with a radium sulfate ionization detector and 5-mv. recorder.

Stationary Phase. General Electric SE-30 silicone gum (5% w./w.) on Chromosorb W (80-100 mesh) packed in a borosilicate glass column (6 feet × 1/4 inch, I.D.).

Syringe. A 10-μl. No. 701N Hamilton syringe was used to inject the samples.

Reagents. Benzene (analytical reagent grade) was used as a solvent for heptachlor (analytical reference grade assaying 99.5%) and lindane (technical grade assaying 99.0%).

Procedure

The internal standard technique introduced by Ray (*3*) was used since it is the most accurate of all methods, ±1% being easily achieved. A detailed discussion of the use of an internal standard was made by Wesselman (*4*).

Standard solutions of lindane and heptachlor were prepared and chromatographed under the following conditions: column temperature, 180° C.; detector temperature, 235° C.; flash heater, 315° C.; argon pressure, 20 p.s.i.; cell voltage, 1000; electrometer gain, 1×10^{-7}; sample size, 1 μl. Under these conditions, lindane has a retention time of 7 1/2 minutes and heptachlor 12 1/6 minutes.

To benzene solutions containing 4 mg. of lindane per ml., heptachlor was added to make concentrations of 5.5, 4.5, 3.5, and 3.15 mg. per ml. In these standard solutions, the ratio of lindane to heptachlor was 1.19, 1.73, 2.59, and 2.93, respectively. The standard curve was obtained by plotting the log of the ratio of the lindane peak height to the heptachlor peak height against the concentration of heptachlor. In all cases, the curve is a straight line.

When assaying materials of unknown concentration, they must be dissolved in the proper volume of benzene containing 4 mg. of lindane per ml. so that the concentrations fall within the limits of the calibration curve; however, the curve can be extended to cover a greater range if desired. In practice, the authors have found it expedient to use the same calibration curve while varying the dilution of the sample being assayed.

To assay heptachlor in a commercial fertilizer (Greenfield Triple Action Crab Grass Killer), 8 grams of the fertilizer was extracted with chloroform for 2 hours in a Soxhlet extractor, the extract was evaporated to dryness, and the heptachlor residue was dissolved in 10 ml. of benzene containing 4 mg. of lindane per ml. of benzene.

Results

After the standard curve was prepared, six lots of heptachlor solution were assayed in duplicate using this method. These solutions having a theoretical concentration of 226 mg. of heptachlor per ml. were found to contain 227, 230, 228, 233, 230, and 224 mg. per ml.

The method was also applied to three lots of technical grade heptachlor (72.0% heptachlor). Duplicate assays showed that the samples contained an average of 71.0, 72.0, and 72.9% heptachlor.

When six lots of commercial fertilizer were analyzed, they were found to contain 0.454, 0.468, 0.471, 0.460, 0.456, and 0.435% heptachlor. These results are in good agreement with the theoretical amount of 0.468% heptachlor.