Effects of Simulated Acidic Rain on Retention of Pesticides on Leaf Surfaces

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ABSTRACT

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Commercial formulations of cupric hydroxide, chlorothalonil, and triphenyltin hydroxide (TPTH) fungicides were applied to leaves of potato or snap bean plants. After a 24-hr drying period, the plants were exposed to a single application of simulated acidic rain at a rate of 1 cm/hr. Solutions were acidified to desired levels of pH with a mixture of sulfuric and nitric acids at a 2:1 ratio of nitrate:sulfate and background ions commonly found in rain in the northeastern United States. Increases in acidity of simulated rain accelerated loss of cupric hydroxide and TPTH but not of chlorothalonil from plant leaves. The major influence of acidity occurred in the initial phases of wash-off. Thus, experiments using deionized or tap water as surrogate rain solutions could significantly underestimate the influence of rainfall on wash-off of some pesticides.

Additional key words: acidic precipitation, weathering.

Acidic rain, a condition primarily characterized by elevated concentrations of hydrogen (H^+), sulfate ($SO_4^=$), and nitrate (NO_3^-) ions in rain water, has been identified as an environmental problem, especially in the eastern portions of the United States and Canada (11). With respect to agricultural ecosystems, a major objective of research has been to determine the direct effect of acidic rain on growth and yield of crops so that economic assessments of its impact can be made (8,9). However, acidity in rain may interact with other factors that determine yield of crops. For example, Shriner (14) demonstrated that increased acidity in rain affected development of plant diseases, but the lack of additional studies has made an economic assessment of these effects impractical (17). To further identify interactions between acidity of rain and factors involved in the epidemiology of diseases, we sought to determine whether increases in acidity could influence chemical control of diseases. Specifically, we examined the effect of increased acidity of simulated rain (SR) on the rate of wash-off of selected pesticides from surfaces of leaves.

Rain per se is a major factor in the weathering of pesticides. It can either redistribute or remove compounds from initial sites of deposition (7). Bruhn and Fry (2) determined effects of SR on retention of the fungicide chlorothalonil and concluded that wash-off needed to be quantified and included in their model of the epidemiology of late blight of potato. Experiments to determine effects of rainfall on loss of pesticides from surfaces of leaves, such as those reported by Bruhn and Fry (2), have employed either deionized or tap water as the surrogate solution for ambient rain. Such experiments, however, may not accurately assess the loss of pesticide deposits because potential effects of acidity in ambient rain have not been considered. In this report, we describe the effects of simulated acidic rain on the rate of wash-off of three fungicides from leaves of snap bean and potato.

MATERIALS AND METHODS

Plant culture. Snap beans (*Phaseolus vulgaris* L. 'Provider') were planted in 5-cm square plastic pots containing Cornell Peat-Lite Mix A (1) and thinned to a single plant after emergence. Day and night temperatures in the greenhouse were 24 and 20 C, respectively. Supplemental lighting to provide a 16-hr photoperiod was supplied by high-pressure sodium vapor lamps.

Potato plants (*Solanum tuberosum* L. 'Norchip A') were propagated from rooted cuttings. Plants were grown in 7.5-cm round plastic pots containing Cornell Peat-Lite Mix A in a greenhouse under the same environmental conditions as those for snap beans. All bean and potato plants were irrigated from below to avoid any effects of overhead watering on characteristics of leaf surfaces.

Application of fungicides. Commercially available formulations of triphenyltin hydroxide (Duter 50WP, Thompson-Hayward Chemical Co., P.O. Box 2383, Kansas City, KS 66110), cupric hydroxide (Kocide Chemical Corp., P.O. Box 45539, Houston, TX 77045), and chlorothalonil (Bravo 500, Diamond Shamrock Corp., Agricultural Chemicals Division, 1100 Superior Ave., Cleveland, OH 44114) were tested. Triphenyltin hydroxide (TPTH) was suspended in deionized water at approximately 1,200 μg/ml active ingredient, then applied to the primary leaves of snap bean in two 10-μl spots per leaf (one spot per half leaf). Cupric hydroxide and chlorothalonil were suspended in deionized water at approximately 1,200 and 500 μg/ml, respectively, then sprayed to runoff onto potato plants with a DeVilbiss paint spray gun (T6A Series 502, DeVilbiss Co., Toledo, OH). All applied solutions were allowed to dry for 24 hr before exposure to SR. The concentration of fungicides deposited on foliage coincided with recommended rates of application (12).

Application of SR. SR solutions were applied via stationary hydraulic nozzles (RA-2, Delevan Corp., 811 4th St., West Des Moines, IA 50265) located 3 m above the test plants. Plants were placed on turntables (2 m diam) and rotated at 3 rpm in the outer portion of the spray cone to ensure even application of treatments. When operated at a water pressure of 1.2 kg/cm, the rate of SR was approximately 1 cm/hr and the mass median diameter of droplets was 0.33 μm. Polypropylene cups were located on each turntable to determine the amount of SR deposited in each event.

Experimental design. The effect of acidity of SR on the wash-off of fungicides from plant foliage was determined by taking leaf samples for residue analysis at time intervals during exposure of plants to one SR event. For TPTH, a 4×3 factorial design was used; plants were exposed to four levels of acidity at pH 5.6, 4.6, 3.8, and 3.0 and samples for residue analysis were taken after 30, 60, and 120 min of exposure (0.5, 1.0, and 2.0 cm of rain, respectively). For chlorothalonil and cupric hydroxide, a 3×3 factorial design was used; plants were exposed to three levels of acidity at pH 5.6, 4.2, and 2.8 and samples for residue analysis were taken after 15, 30, and 60 min of exposure (0.25, 0.5, and 1.0 cm of rain, respectively).

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Each trial, which consisted of a test of one fungicide and exposure to only one rain event, was repeated three times.

Determination of fungicide in residues. At least three plants were harvested at each sampling time and at least two separate residue analyses were made per plant. Residues of TPTH were determined by yeast bioassay (16). Leaf disks (11 mm diam) were taken from the original spot of application, frozen at −12 C for at least 24 hr, then placed individually into 10 ml of potato-dextrose broth (Difco Laboratories, Detroit, MI 48232) that had been inoculated with a suspension of yeast bud cells (1×10^5 cells/ml of *Saccharomyces cerevisiae* Meyen ex Hansen). The bioassay tubes were incubated on a rotary shaker (85 rpm) at ambient temperature (20–22 C) for 20 hr, then the optical density of the culture at 450 nm was determined. The optical density was converted to the amount of TPTH present on the leaf via a standard dose-response curve (produced with known concentrations of TPTH) and the final results expressed as μg TPTH/cm^2 of leaf surface.

Residues of cupric hydroxide were determined by atomic absorption spectroscopy (6). Ten leaf disks (11 mm diam) were taken from each plant with a stainless-steel punch and placed in 10 ml of 0.1 N nitric acid and extracted for 1 hr. The extract was filtered (Whatman No. 42) and the concentration of cupric ion determined directly with a Perkin-Elmer 205A atomic absorption spectrophotometer (Norwalk, CT 06856) at 324.8 μm. When necessary, the extract was diluted with 0.1 N nitric acid. Absorbancy readings were converted via a standard curve to μg Cu^{++}/cm^2 of leaf.

Residues of chlorothalonil were determined by gas chromatography using an electron capture detector (3). Ten leaf disks were taken from each plant, placed in 20 ml of acetone, and extracted for 0.5 hr. Then, 2 ml of this extract was diluted to 10 ml with acetone, and aliquots of 1 μl were injected into the gas chromatograph, a Varian Series 3700 (Palo Alto, CA 94303) equipped with a Ni^{63} electron capture detector. The column was 0.4 × 1.0 cm, 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport. Operating conditions were injector temperature at 250 C, column temperature at 220 C, detector temperature at 300 C, and the flow of carrier gas (argon-methane) at 50 ml/min. Technical chlorothalonil, recrystalized from acetone, was used as the standard. Peak areas were calculated by a Hewlett-Packard 3390A integrator (Cupertino, CA 95014). The concentration of chlorothalonil in the extract was converted to μg chlorothalonil/cm^2 of leaf.

Statistical methods. A factorial analysis of variance was conducted for each fungicide to determine main and interactive effects of duration and pH of SR on the amount of fungicide remaining on leaves (13). The shape of response surfaces was determined by partitioning the treatment sums of squares for each main effect into orthogonal contrasts for regression (15). A significant linear contrast indicated a linear relationship between the response and the treatment. Significant higher order contrasts (quadratic and cubic) indicated curvature in response. Samples for residue analyses that were made prior to the start of SR treatments (0 min, Tables 1 and 2) were used to determine the amount of fungicide initially deposited on leaves. These values within trials had no variance in relation to the treatment factors and thus could not be included in the statistical analyses. The initial values, however, were useful in calculating the percentage of fungicide remaining on the leaf at each sampling time to aid in illustrating the relative effects of treatments on the kinetics of the rate of wash-off of each compound.

RESULTS

Chlorothalonil. The only significant main effect on wash-off of fungicide was duration of SR (Tables 1 and 3). Therefore, values for all levels of pH were pooled to determine effects of duration on the kinetics of wash-off. A large amount of chlorothalonil was removed from potato leaves between the start of the treatment and the first sampling time; at 15 min of exposure, only 57% of the initial deposit remained. As indicated by the significant linear contrast for duration, the rate of removal was constant between 15 and 60 min of exposure. However, the residue was only reduced to 45% of the initial deposit by the end of the exposure.

Cupric hydroxide. Both main effects of duration and pH of SR significantly affected wash-off (Tables 1 and 3). With respect to the effect of duration, deposits of cupric hydroxide were more tenacious than those of chlorothalonil to SR at pH 5.6; at 15 min of exposure, 92% of the initial deposit of cupric hydroxide remained on potato leaves. Curvature in response was indicated between 15 and 60 min so that the rate of wash-off decreased with time.

The response to pH of SR was curvilinear between 15 and 60 min of exposure, indicating that the rate of wash-off increased as pH of SR increased. The lack of interaction between duration and pH of SR indicated that the curves were similar at each level of pH. Thus, the major effects of acidity in rain must have occurred between the start of the event and the first sampling time (15 min).

TPTH. Both main effects of duration and pH of SR significantly affected wash-off (Tables 2 and 3). With respect to the effect of duration, deposits of TPTH were less tenacious than those of cupric hydroxide to SR at pH 5.6; at 30 min of exposure, 45% of the initial deposit of TPTH remained on snap bean leaves, whereas at 30 min of exposure, 77% of the initial deposit of cupric hydroxide remained on potato leaves. As indicated by the significant linear contrast for duration, the rate of removal was constant between 30 and 120 min of exposure. The residue was only reduced to 36% of the

TABLE 1. Amount of fungicide in initial deposits and in residues after exposure of potato plants to different durations of one simulated rain event and at different levels of acidity

Acidity level Simulated rain duration (min)[a]	Chlorothalonil[b] Trial 1	Trial 2	Trial 3	Av.	Cupric hydroxide[b] Trial 1	Trial 2	Trial 3	Av.
pH 5.6								
0	4.5	3.6	4.6	4.2	12.5	13.5	16.4	14.1
15	2.4	2.0	2.5	2.3	12.6	11.9	14.5	13.0
30	1.9	2.0	2.3	2.1	9.1	11.3	12.0	10.8
60	2.0	1.4	2.2	1.9	9.6	10.1	11.1	10.3
pH 4.2								
0	4.5	3.6	4.6	4.2	12.5	13.5	16.4	14.1
15	2.4	2.0	2.8	2.4	11.4	11.4	13.4	12.1
30	2.2	1.6	2.9	2.2	9.1	11.1	10.1	10.1
60	2.2	1.5	2.3	2.0	6.6	9.4	9.6	8.5
pH 2.8								
0	4.5	3.6	4.6	4.2	12.5	13.5	16.4	14.1
15	2.4	2.1	2.9	2.5	7.6	8.0	5.8	7.1
30	1.9	2.1	2.3	2.1	2.3	7.3	4.8	4.8
60	1.5	1.6	2.2	1.8	1.9	4.1	4.0	3.3

[a] Rate of deposition from nozzle was 1 cm/hr.

[b] Values are micrograms of fungicide per square centimeter of leaf surface. Each value is the average of at least six samples.

the initial deposit by the end of the exposure, however. Hence, as observed with chlorothalonil, the effect of rain on wash-off was greatest between the start of the SR event and the first sampling time.

The response to pH of SR was linear between 30 and 120 min of exposure (Table 3). The lack of a significant interaction between duration and pH of SR indicated that the curves were similar at each level of acidity. Thus, as observed with cupric hydroxide, the effect of acidity also must have occurred between the start of the SR event and the first sampling time.

DISCUSSION

The wash-off of fungicides from plant leaves was accelerated by increases in acidity in rain for two of the three compounds tested. The sensitivity of a compound to wash-off by deionized water (pH 5.6) did not necessarily preclude sensitivity to acidity of SR. For example, chlorothalonil and TPTH were much less tenacious than cupric hydroxide to rainfall at pH 5.6, but chlorothalonil was the only compound not removed more readily by additional acidic rain treatments. Because the fungicides were commercial formulations, their composition was unknown. Thus, speculation on exact mechanisms of action of wash-off due to acidic components in rain was not possible. However, the shapes of the response curves for wash-off with respect to pH of SR differed among fungicides, indicating that presumption of a single mechanism was not valid (Table 3).

Portions of the rain event where critical reactions occurred were identified by analysis of the effects of treatments on the kinetics of wash-off. The lack of interaction between duration and pH of SR indicated that the curves for wash-off at each level of acidity were parallel after the first sampling time (15 min of rain for chlorothalonil and cupric hydroxide and 30 min of rain for TPTH). Thus, the principal impact of acidity on processes of wash-off must have occurred in the interval between the start of SR and the first sampling time. Lauver and McCune (10) have provided a quantitative description for the time-course removal of sodium fluorescein particulates from foliage of soybean plants. By analyses of fractions of material washed off plants they were able to identify four phases of removal. In the first phase, water accumulated on the foliage with only a slight amount of material removed. In the second phase, the rate of removal increased to a maximum as the storage capacity of the foliage was reached and superficial water with suspended or dissolved material was displaced from the foliage. This occurred 2.5 min after the start of the rain event. The third phase was characterized by an exponential decline in the rate of removal. In the fourth phase, no additional material was removed. The rate of rainfall used to identify these phases was similar to that in our investigation. Further investigations should emphasize reactions occurring in the early phases, particularly with respect to suspension or dissolution of deposits during initial accumulation of solutions by foliage. Other factors that influence mechanisms of wash-off should also be investigated: relative humidity, intensity of rainfall, and residence time of the deposit (4).

It should be noted that the effect of acidity of SR could be detected after only one application of an SR event. Thus, the significance of these results can be estimated by relating these effects to values of pH measured in ambient rain events. Data for ambient rain events were obtained from the MAP3S monitoring network for the site in Ithaca, NY, for June, July, and August during 1979–1982 (5). Among 81 events recorded, the values for pH appeared normally distributed and ranged from 4.8 to 3.4, with a mean and median of 4.0; one-half of the observations occurred between pH 4.2 and 3.85, with one-fourth below 3.85. Clearly, for compounds sensitive to increases in wash-off due to increased acidity in rain, use of deionized water or tap water as surrogate solutions would be inadequate and would lead to underestimating the rates of wash-off due to ambient rain.

These results indicate several consequences for management practices used to control diseases in areas where rainfall is acidic. First, the effectiveness of a compound may be decreased if its rate of wash-off is accelerated by acidity in rain. Consequently, costs of production may be affected either by a reduction in the efficacy of applied sprays or by an increase in the amount of material needed to maintain an adequate level of control.

Second, epidemiological models may need to be reevaluated. Increased cost of pest control has spurred the development of

TABLE 2. Amount of fungicide in initial deposits and in residues after exposure of snap bean plants to different durations of one simulated rain event and at different levels of acidity

Acidity level Simulated rain duration (min)[a]	Triphenyltin hydroxide[b] Trial 1	Trial 2	Trial 3	Av.
pH 5.6				
0	15.0	15.0	15.0	15.0
30	6.4	6.1	7.6	6.7
60	6.1	6.1	2.9	5.0
120	8.0	5.8	2.3	5.4
pH 4.6				
0	15.0	15.0	15.0	15.0
30	7.1	4.1	4.7	5.3
60	5.5	3.6	2.9	4.0
120	4.1	2.0	2.4	2.8
pH 3.8				
0	15.0	15.0	15.0	15.0
30	4.8	2.8	4.6	4.1
60	3.3	2.7	2.6	2.9
120	1.0	2.7	2.0	1.9
pH 3.0				
0	15.0	15.0	15.0	15.0
30	1.2	2.1	0.0	1.1
60	0.0	2.0	0.0	1.7
120	0.0	0.0	0.0	0.0

[a] Rate of deposition from nozzle was 1 cm/hr.
[b] Values are micrograms of fungicide per square centimeter of leaf surface. Each value is the average of at least six samples.

TABLE 3. Analyses of variance for the effects of duration and level of acidity of simulated rain on the amount of fungicide in residues on plant leaves

Source of variation	df	Mean square[a] Chlorothalonil	Mean square[a] Cupric hydroxide	df	Mean square[a] Triphenyltin hydroxide
Trials	2	1.0516	7.8885**	2	4.9751
Duration of rain	2			2	
Linear	1	1.1391**	45.9086**	1	16.5753**
Quadratic	1	0.0321	6.2790*	1	2.5014
Acidity of rain	2			3	
Linear	1	0.0013	176.4694**	1	120.7394**
Quadratic	1	0.0921	24.0817**	1	2.4663
Cubic	...	...	...	1	1.0524
Duration × acidity of rain	4	0.0278	0.3714	6	0.5613
Residual	16	0.0456	1.3444	35	1.5541
R-square		0.83	0.93		0.83
Coefficient of variation		10.0	13.1		37.6

[a] Mean squares that give ratios greater than the tabulated values for the *F* distribution at $P = 0.05$ and $P = 0.01$ are denoted by * and **, respectively.

cost-effective spray programs based on models that integrate the growth of plant and pest with the loss of pesticide caused by rainfall (3). Consequently, models generated from wash-off experiments will need to consider the effects of increased acidity and perhaps other components in rain on the retention of pesticides on surfaces of leaves. Additionally, data on the interaction between acidic rain and development of diseases (14) will be required to improve the accuracy of disease progress estimates. Hence, the potential impact of acidic rain on the epidemiology of diseases and subsequently on the cost of production could be significant and should not be overlooked in future economic assessments.

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