FUNGICIDE REDISTRIBUTION ON POTATO LEAVES

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THESIS

This is to certify that the

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A. J. Hooker
Major professor

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ABSTRACT

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ON POTATO LEAVES

By

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Redistribution of fungicide from the point of application on a potato leaf was determined by electron probe analysis, direct observation of deposits on leaf cuticle, and by bioassay using the late blight fungus, Phytophthora infestans. Because of suitability for electron probe analysis, metallic fungicides, Kocide 101 containing cupric hydroxide and Du-ter containing triphenyl tin hydroxide, were used throughout.

Using electron microprobe analysis, there was no detectable fungicide migration from 0.001 ml fungicide on the potato leaf surfaces before or after weathering. Direct optical examination also showed clearly defined rims at the margin of the droplets of measured or sprayed copper fungicides, but those of tin fungicides were less well defined. There was no evidence of movement of fungicide from the original droplet.

Sporangial germination of Phytophthora infestans on the potato leaves was inhibited within 5 mm of measured fungicide droplets of Kocide 101 and Du-ter. Toxicant had

diffused none or only slightly from the original droplet as measured by spore germination. However, infection of the fungus was conspicuously inhibited 15-20 mm or more from the fungicide droplets. Infection hyphae were apparently more sensitive to different toxicants than were sporangiospores. The detectable level of copper and tin in these bioassay trials was apparently below the level of sensitivity of the electron probe.

The initial effluents from fungicide sprayed leaves markedly reduced germination of sporangiospores. Effluents collected after 5 or more hours weathering were low or lacking in toxicity. Biofilm and Pinolene added to technical compounds of tin and copper were slightly less effective than the commercial fungicieds.

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INTRODUCTION

Several workers have suggested that poor coverage of plant foliage by fungicide may be somewhat compensated by redistribution of initial deposits. It is generally recognized that most fungicides are redistributed to some extent following the initial application. The extent to which this takes place is not completely understood.

When deposits of Bordeaux mixture on the potato leaves were washed, redistribution was demonstrated by Bjöling and Sellgren (1957). Since 1965, Hislop has reported a series of papers on 'the redistribution of fungicide on plants', in which he demonstrated fungicide redistribution in the laboratory and field. Hislop and Cox (1970) demonstrated redistribution of various fungicides applied to detached broad-bean leaflets by laboratory bioassay with Botrytis fabae. The results showed that the protected areas on the leaves inoculated after washing were often much greater than on unwashed leaves.

Complete coverage of fungicide on the leaf surface is difficult to accomplish in field application, and redistribution of initial deposits is necessary for sufficient control of disease. Concentrated low volume application is economically important in the field, and since coverage is not complete, redistribution becomes important in disease control. Therefore, information concerning the extent to whcih redistribution takes place is highly desirable.

The object of the present work was to measure redistribution of copper and tin on the potato leaf surface, and to verify local redistribution (Björling and Sellgren, 1957; Hislop and Cox, 1970). To accomplish this, direct observation with optical microscope, electron probe analysis, and bioassay with Phytophthora infestans were employed.

LITERATURE REVIEW

Perhaps the first observation of fungicide redistribution was made by Millardet in 1882 and published in 1885. Copper sulfate and lime were dissolved in water and applied with a brush on grape leaves to prevent pilfering. Reduction of mildew of the vine on treated leaves followed. Quoting from the translation by F. J. Schneiderhan, "it is not necessary that the leaves be wholly covered by the mixture. I believe it safe to assert that a single spot of it on each leaf is sufficient". Ricaud and Paulin, 1884 (Large, 1940) found that grapes growing on poles treated with copper sulfate to prevent wood decay showed less downy mildew than neighboring grapes on untreated supports. Hamilton et al. (1943) showed that rains can wash a protectant from a heavily covered area and drop it onto unprotected areas below. Hislop (1965, 1966a, 1967, 1969) has described distant redistribution of fungicide experimentally in the laboratory and in the field.

In contrast to the concept of distant redistribution, Björling and Sellgren (1957) used the term 'local redistribution'. They found that best protection against late blight was obtained by the smallest droplets of Bordeaux mixture, and control was interpreted as the result of a local redistribution of Bordeaux particles to unprotected leaf areas by dew or rain. Morgan and Russell (1965) suggested

redistribution of active fungicide from initial deposits of dodine and captan in a trial of diluted fungicides applied in form of much smaller droplets for control of apple scab. Hislop and Cox (1970) worked on local redistribution of various fungicides by bioassay with broad bean leaflets and by leaf printing techniques. They observed that fungicidal particulate and soluble material will spread in water from the initial deposit and that the protected areas on leaves inoculated after washing were often much greater than on unwashed leaves.

The extent of redistribution was related to the inherent toxicity, physical form, duration of weathering, and tenacity of fungicides (Hislop and Cox, 1970), and wettable powder formulations were to some extent resuspended in washing water, and frequently redeposited in previously unprotected areas.

Goossen (1958) presents data to show that copper concentration is higher on lower leaf surfaces of sprayed leaves after a rain than before. He suggests that fungicide should not be highly tenacious as fungicide transfer from one part of the plant to another is an important aspect of control. Hislop and Cox (1970) confirmed Goossen's suggestion, and reported that most fungicides tested were probably moved over the leaf surface in particulate form. The effect of time of washing on redistribution was examined by varying lengths of time before drying and inoculation. The results showed that protection from redistributed fungicide decreased the longer leaves were washed.

Differences in protection from particle size fractions may be related to tenacity or toxicity effects (Hislop and Cox, 1970). Assay with Alternaria tenuis confirmed that small particles were more toxic from large particles. They also found that redistribution from large fungicide drops applied with a microliter syringe was better from the more concentrated suspensions than from dilute suspensions. Rich (1954) found that during weathering of dried Bordeaux deposits, the larger the initial deposit, the smaller was the percentage lost, but the larger the deposit of zineb, the greater was the proportion of its mass lost by weathering.

None of the wetting agents representing non-ionic, anionic, and cationic types, enhanced redistribution (Hislop and Cox, 1970). At low concentrations (Somers, 1957), representative wetting agents of anionic, cationic, and non-ionic type slightly lowered the amount of copper spray deposit at 'run-off', but in the absence of supplements, spray retention increased with increasing spray concentration and with increasing wettability of the surface.

High concentrations of leaf exudates sometimes found in rain and dew were inhibitory to germination of Botrytis cinerea spores (Kovács and Szeöke, 1956). Germination of Alternaria tenuis was slightly inhibited by the presence of leaf exudates (Hislop, 1966a), and soluble copper in leaf washing was relatively non-toxic. Biedermann and Müller (1952) observed inactivation of copper by leaf excretions.

MATERIALS AND METHODS

Preliminary studies of fungicide deposition were made with smooth leaf surfaces of pepper and sugar beet and with the hirsute leaf surface of potato. No marked differences existed in drop distribution nor tenacity on leaves of the plants tested. For this reason subsequent tests were made with leaves of potato, Solanum tuberosum L.

Bioassay for fungicidal action was made with the fungus, Phytophthora infestans (Mont.) deBary, which is the incitant of the late blight disease of potato.

Spray materials were prepared in distilled water as described (Table 1).

Distribution of copper and tin fungicides was determined by electron probe analysis of potato leaf surfaces. Drops of fungicide, 0.001 ml volume, were placed between the lateral veins on the upper surface of potato leaflets using an Hamilton microliter syringe. The drop location was identified by placing two drops of ink one on each side of the fungicide drop. After drying, leaves were subjected to:

1. No further treatment
2. Weathering for varying periods in a moist chamber using an atomizer which delivered to the leaf surface 0.25 to 1.5 mm of water per hour. During weathering the leaflet was arranged so that the tip pointed downward at approximately 30 degrees.

Later, interveinal pieces were cut from the area between the lateral veins of the leaflet. One end of the leaf piece was cut diagonally to indicate clearly the upper surface where the fungicide had been applied (Fig. 1). In order

Table 1.--Fungicide and surfactant preparations.

Product, manufacturer, and description	Amount/liter
Fungicides	-
Kocide 101, 2 lbs/25 gal, cupric hydroxide (86%) plus surfactant, Kennecott Copper Corp., Houston, Texas	9.576 gm
Cupric hydroxide (Technical), 2 lbs/25 gal, no surfactant, 100% active, Kennecott Copper Corp., Houston, Texas	9.576 gm
Du-ter, 0.6 lbs/25 gal, triphenyl tin hydroxide (47.5%) plus surfactant, Thompson-Hayward Chem. Co., Kansas City, Kansas.	3.051 gm
Triphenyl tin hydroxide (98%), no surfactant, Thompson-Hayward Chem. Co., Kansas City, Kansas	1.525 gm
Surfactants	
Pinolene (Nu-Film-17), 2 pints/100 gal, nonionic, Miller Chemical and Fertilizer Corp., Hanover, Pennsylvania	10.000 gm
Biofilm, 6 oz/100 gal, anionic-nonionic blend, Colloidal Products Division, Kalo Laboratories, In., Petaluma, California	0.466 gm

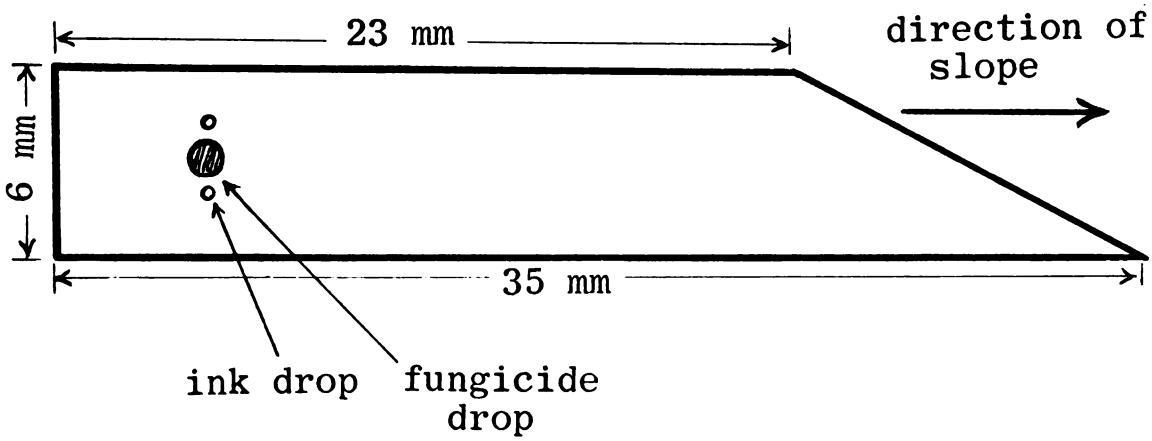


Figure 1.--Piece of interveinal leaf tissue prepared for electron probe analysis.

to protect the surface, each piece was separately placed in a small piece of Kleenex tissue, then into flat paper towels. For obtaining dry, flat sections a glass plate was put on the paper towels for several days.

Analysis of copper was made with an electron probe (Applied Research Laboratories Model EMX-5na) set for K alpha 1.537 Å, 0.05 micro amperes, 25 KV. Tin was determined at L alpha 3.595 Å, 0.1 micro amperes, 25 KV.

Measured droplets as well as droplets on leaf surfaces following spraying were examined optically. A glass microscope slide covered with an adhesive film was adpressed to the leaf surface. The epidermis of the sprayed leaf surface remained fastened to the glass slide as the leaf was stripped away.

Preparation of leaf epidermal surfaces for microscopic examination:

(1) A small amount of Duco cement or Elmer's Clear Cement was dropped on a slide glass and immediately spread with a glass rod or wooden stick to make a thin film on the surface. After approximately 1 minute a small amount of rubber cement was spread on the top of the film of Duco cement using the same method as described earlier. It was important that the rubber cement be smeared before the Duco cement dried out. These two kinds of cements must not be mixed. Neither rubber cement nor Duco cement alone will serve. Duco cement and Elmer's clear cement seemed to be equally good. It was better to make the film of the rubber cement slightly thicker than the film of Duco cement. This composite film may be used up to several months after preparation.

(2) The sample leaf was placed on the slide, prepared with adhesive and the leaf surface was pressed on the film with the thumb. The pressing was done so the leaf surface was completely in contact with the film. Then the leaf was peeled from the slide leaving the epidermal layer attached to the Duco film.

(3) Permanent preparations lasting a few months at least may be made by dropping Duco cement on the epidermis and covering this with a cover glass.

Preparation of sporangiospores of P. infestans:

Fresh young sporangiospores were obtained as follows:

(1) Matured leaves of the potato cultivar, 41956, were placed on damp paper towels on moistened Sphagnum moss in a wooden flat.

(2) One drop of the sporangiospore suspension was inoculated each leaflet. The flat with inoculated leaflets was kept in a polyvinyl bag to maintain high moisture and placed in a growth chamber at 19-20°C.

(3) After 2 days the inoculated leaflets were soaked briefly in distilled water, and shaken gently. Thereafter, they were treated similarly every one or two days.

(4) Four to five days after inoculation, typical whitish masses of sporania appeared on the lower surface of the leaflets. Thus, sporangiospores were less than one or two days old, since the most recent rinsing in distilled water had removed the mature spores.

Device for collecting sporagia of P. infestans:

Spores were collected from infected leaves in a moist chamber using vacuum from an aspirator with the device illustrated (Fig. 2). Initially a few ml of water were placed in the collector to serve as a spore trap. Solutes from diseased leaf tissue were avoided in the collected spore sample as spores were moved directly into the reservoir of water by air current from the place of formation, the sporangiospores, which are well above the substrate. By this means desiccation was avoided as spores were immediately suspended

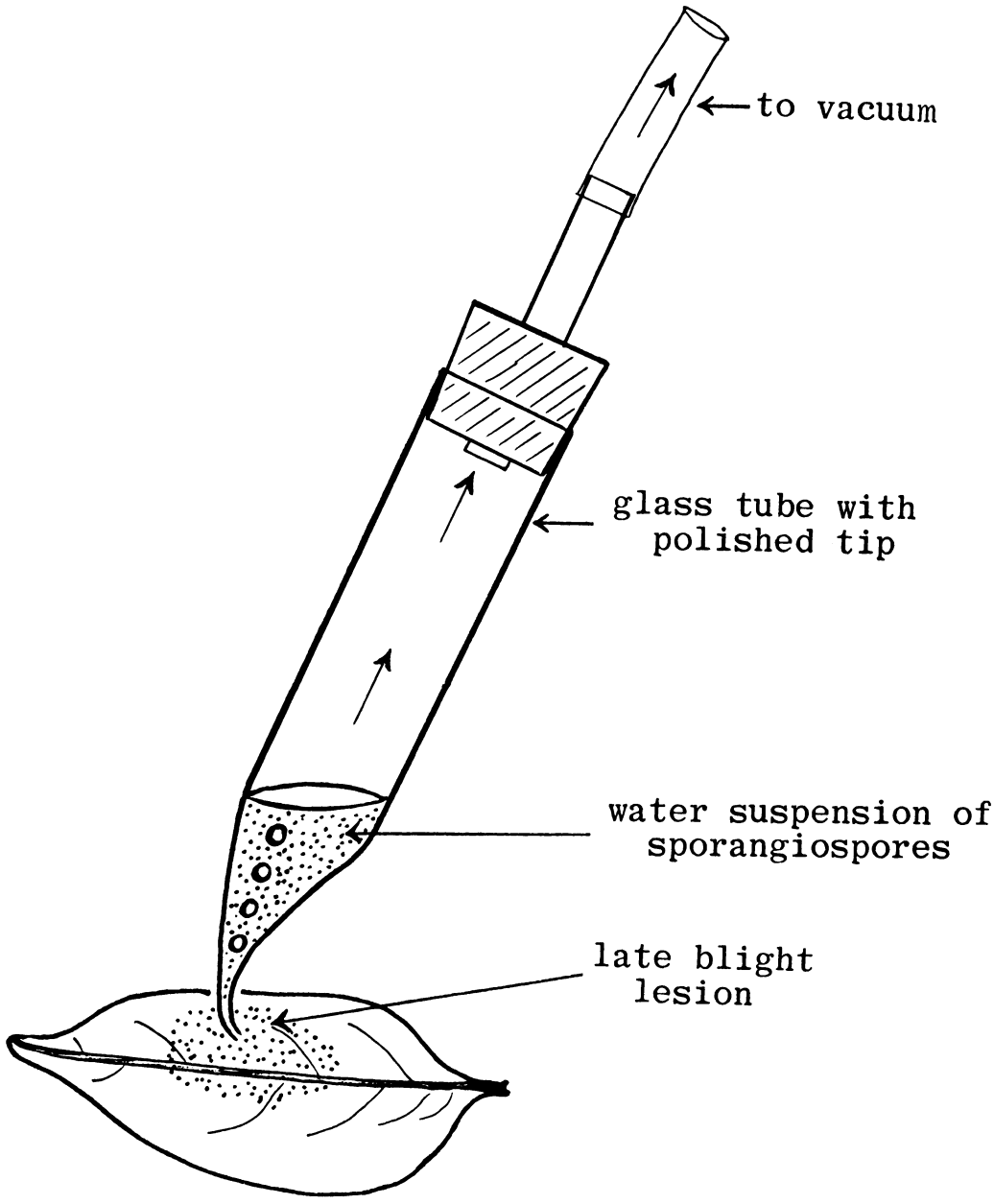


Figure 2.--Device for collecting sporangiospores of Phytophthora infestans free from decayed leaf tissue.

in a liquid after removal from the sporangiospores. Sporangiospores in suspension were transferred to the test systems promptly after collecting.

RESULTS

Electron probe examination of fungicide drops: Distribution of fungicides as measured by the electron probe was essentially similar on either smooth leaved plants, pepper and sugar beet or on an hirsute leaf such as that of potato.

Sprays of Kocide 101 and Du-ter were prepared in concentrations of 2 lbs and 0.6 lbs commercial fungicide per 25 gal respectively. Individual droplets of 0.001 ml volume contained approximately 0.00001 gm of copper fungicide and 0.000003 gm of tin fungicide respectively. These amounts of copper and tin were well within the level of sensitivity of the electron probe. Concentrations of copper and tin are shown in counts per second on the accompanying graphs as 3,000 and 300 for Cu and 100 for Sn. Each unit on the horizontal (X) axis indicates 0.1 mm along the surface of the leaf. Recorder sensitivity for copper was changed during the analysis. Where copper was apparently lacking on the leaf surface and the recorder pen was at or near the base line, sensitivity was set at 300 counts per full scale and where concentrations were high, sensitivity was changed to 3,000 counts per full scale.

Electron probe determinations were usually begun within the drop and quantitative measurement made in a straight line along the leaf sample between the larger lateral veins (Fig. 4). Analysis of certain drops (Figs. 3, 5, 6) were made starting outside the drop and extended through the drop.

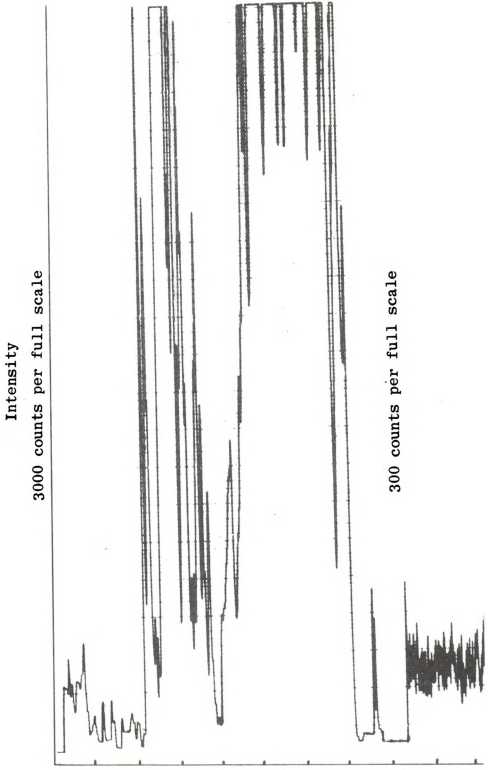


Figure 3.--Electron probe analysis through drop of Kocide 101 on potato leaf surface before weathering. Where copper concentration was high 3000 counts per full scale was used. Where counts approached the base line sensitivity was changed to 300 counts per full scale.

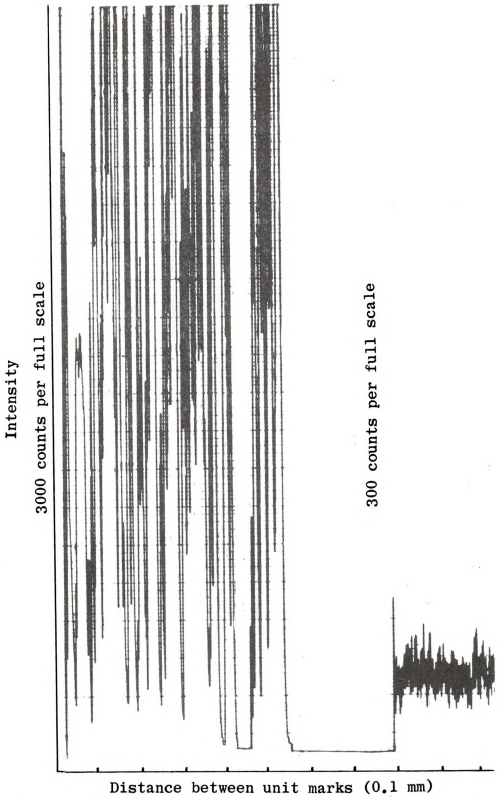


Figure 4.--Electron probe analysis from within drop of Kocide 101 on potato leaf surface after 4 hours weathering.

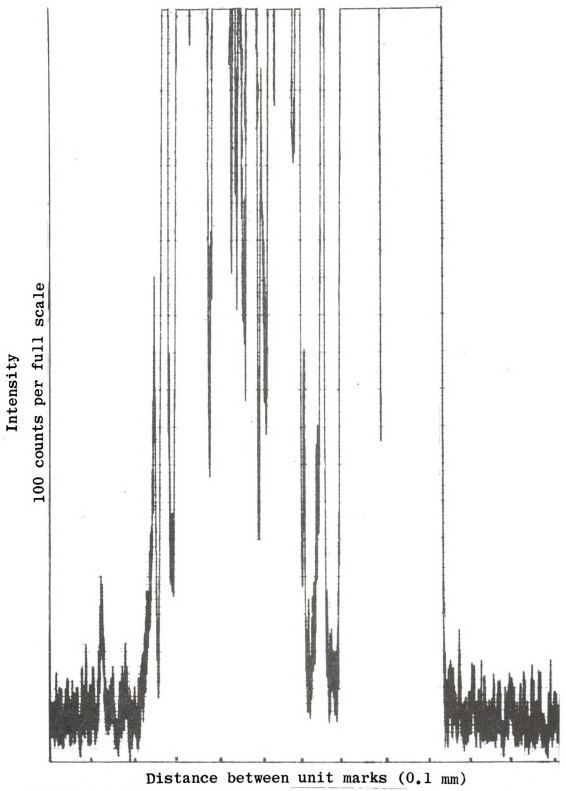


Figure 5.--Electron probe analysis through drop of Du-ter on potato leaf surface before weathering.

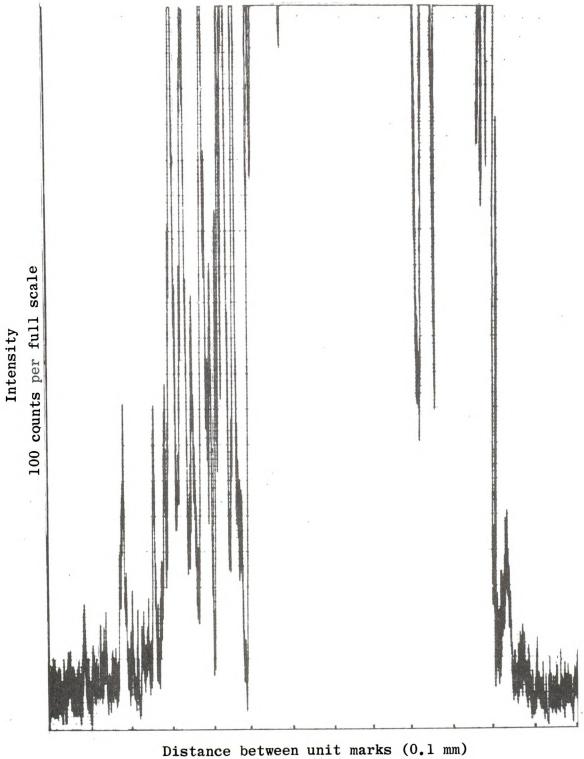


Figure 6.--Electron probe analysis through drop of Du-ter on potato leaf surface after 4 hours weathering.

Copper and tin deposits of commercially available Kocide 101 (Figs. 3, 4) and Du-ter (Figs. 5, 6) respectively, were characterized on leaves by clearly defined margins. These margins were distinct on the electron probe with or without weathering. Concentrations of metal abruptly fell to the base level at the drop edge. The edge of the drop could be visually distinguished in the optical microscope of the electron probe, and the border identified simultaneously on the electron probe graph. Apparently from or around a single drop of commercial fungicide there was no well defined spreading over the leaf in quantities which were detectable by the electron probe.

Both fungicides frequently formed a dense rim around or in portions of the perimeter of the drop. Within such a drop the central area had metal in varying concentrations and the concentration on the rim was higher than that near the center. This rim was not consistently present nor was it entire and continuous. Evidence of such a heavy rim is shown on the right margin of the droplet in Figs. 5, 6 and to a lesser extent in Fig. 3.

Occasionally specks of copper were present near the drop. These are believed to have been bounced from the deposit by the electron beam during electron probe examination. This was evident by microscopic observation of the specimen during migration of the specimen through the electron beam.

Drops of either fungicide with Biofilm or Pinolene again had clearly defined margins but the marginal rim was not

quite so evident. There apparently was some movement from the point of original application to surrounding leaf surface. Possibly the movement could have been described as a crawling type of surfactant action. Copper or tin so moved from the original drop did not adhere well to the surface and was apparently not as effective as measured in bioassay.

Droplets with Biofilm and cupric hydroxide (Fig. 7) had a well defined margin but copper had migrated outward in concentrations sufficient to have been recorded in the probe. Droplets of Pinolene with cupric hydroxide (Fig. 8) were similar. Droplets of triphenyl tin with Biofilm (Fig. 9) or with Pinolene (Fig. 10) had a more gradual slope at the margin than those with the commercial Du-ter fungicide and also there was apparently a low level deposition outside the droplet.

Sensitivity of the electron probe: Parallel bioassay trials suggested that active toxicant existed immediately outside the droplet. This level of copper or tin may have been below the level of sensitivity of the electron probe and, therefore, may not have been detected in the previous tests. To determine the sensitivity of the electron probe, serial dilutions were prepared with Kocide 101 and Du-ter. Droplets (0.001 ml volume) were placed on potato leaves as previously described. Kocide 101 in the droplet as in the previous studies (2.265 gm commercial fungicide/235 ml; 2 lbs/25 gal) could be detected at dilutions of 1/1000 but not a 1/10,000. Du-ter (0.72 gm commercial fungicide/236 ml;

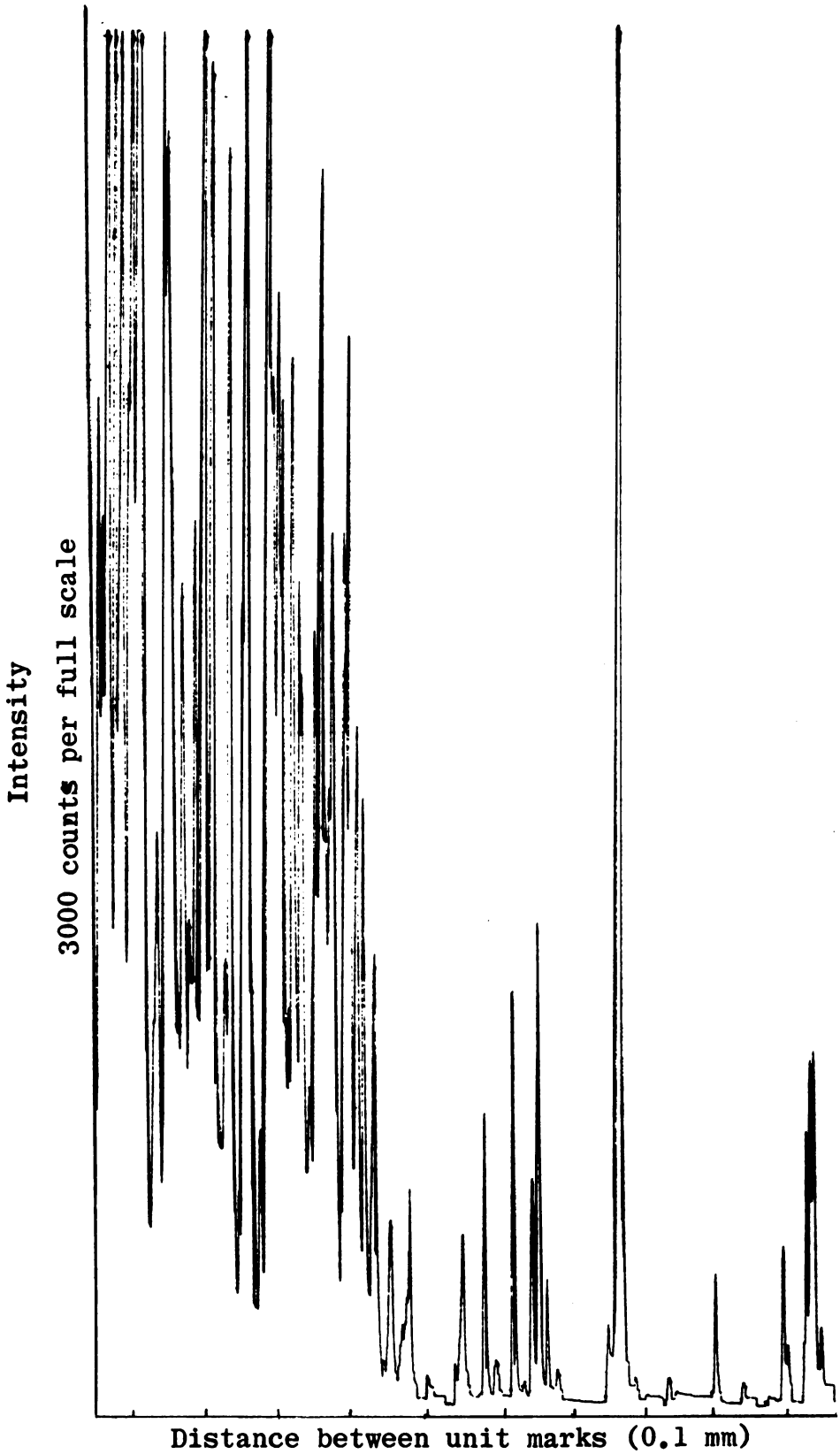


Figure 7.--Electron probe analysis from within drop of cupric hydroxide with Biofilm on potato leaf surface before weathering.

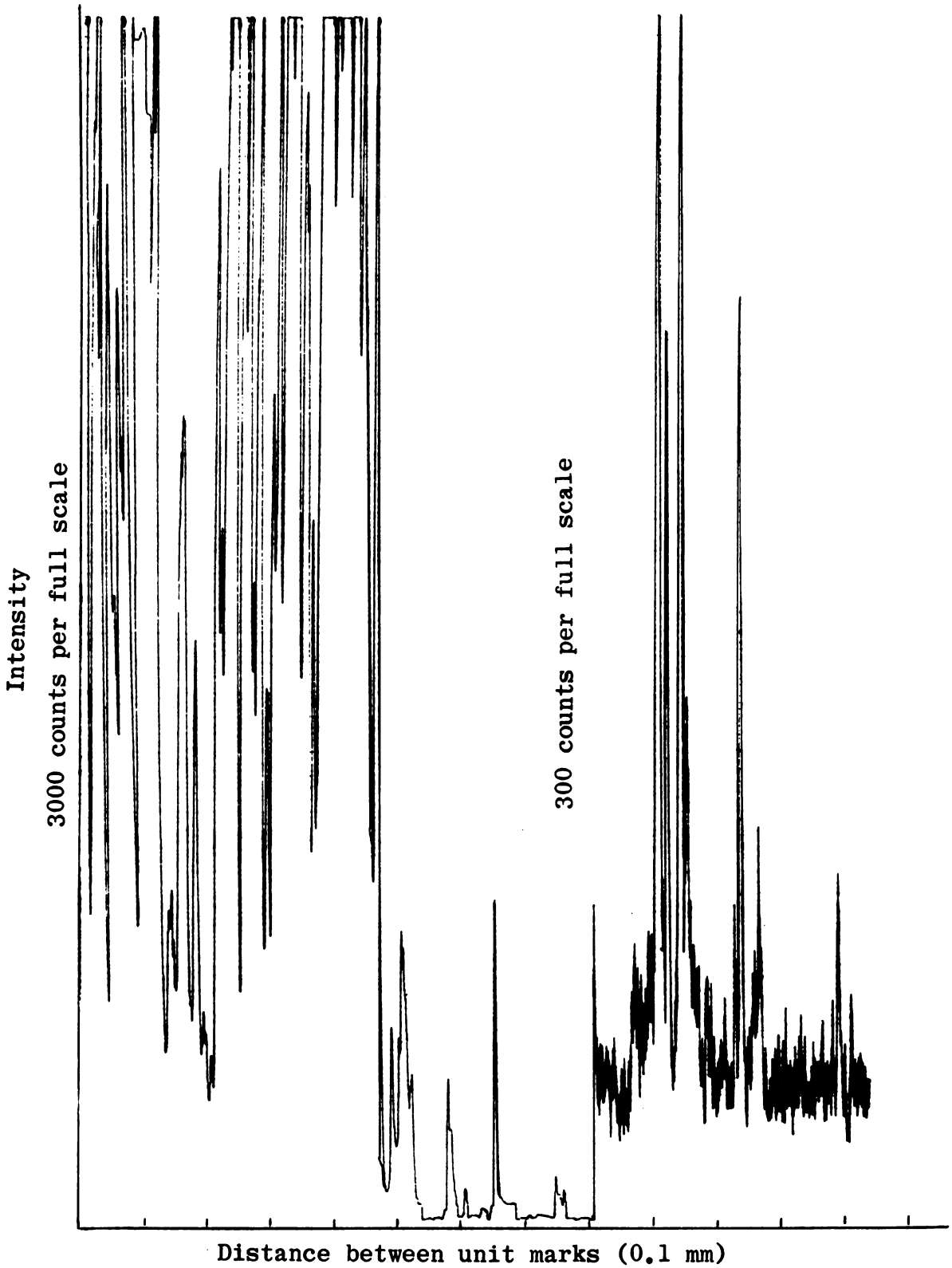


Figure 8.--Electron probe analysis from within drop of cupric hydroxide with Pinolene on potato leaf surface after 4 hours weathering.

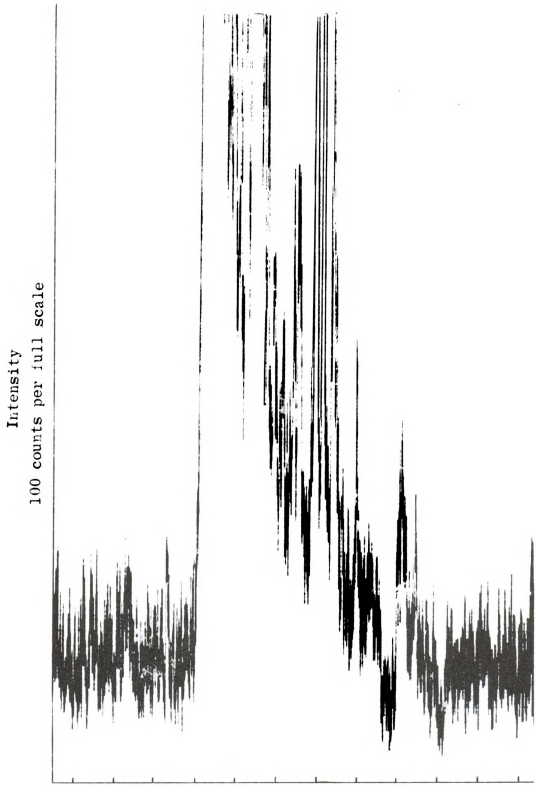


Figure 9.--Electron probe analysis through drop of triphenyl tin hydroxide with Biofilm on potato leaf surface after 4 hours weathering.

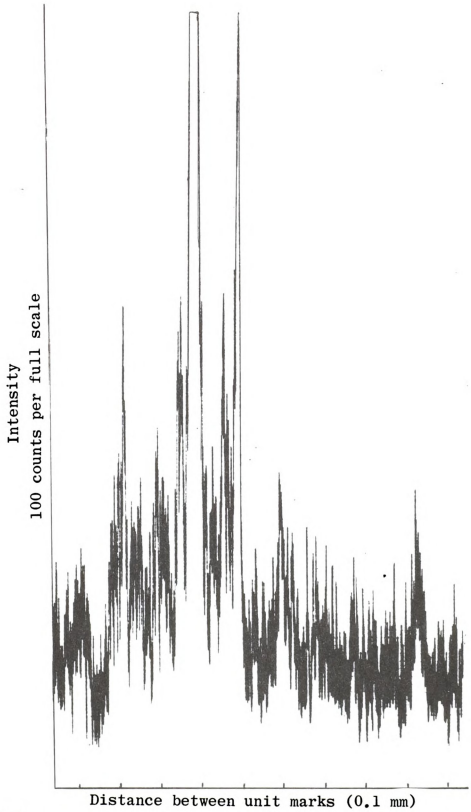


Figure 10.--Electron probe analysis through drop of triphenyl tin hydroxide with Pinolene on potato leaf surface after 4 hours weathering.

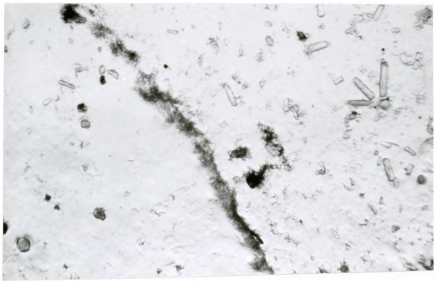
0.6 lbs/25 gal) was evident at 1/10 dilution on one trial and at 1/100 dilution in a second trial. The original droplet before dilution contained 0.0095975 mg and 0.0030508 mg of Kocide 101 and of Du-ter respectively. It was concluded that the concentration of toxicant determined by bioassay was below the level of sensitivity of the electron probe.

Microscope examination of fungicide drops: Direct optical examination of spray deposits was accomplished by stripping away the upper leaf surface with the adhesive film described earlier. A number of droplets from each preparation were observed and attempts made to assess variation between them. Photographs of representative deposits are shown (Figs. 11A, B).

Copper droplets could be examined with relative ease. Triphenyl tin hydroxide was more difficult to observe on the leaf surface due probably to an initial concentration considerably less than that of copper and because it was some what less opaque. Measured droplets of tin compounds (0.001 ml) could be seen but observations of sprayed droplets were not satisfactory

A rim of heavy spray concentration at the margin of the measured drop was present with commercial Kocide 101 and Du-ter (Fig. 11 A). This rim was not completely continuous, but in general, was clearly defined, and the rim was not lost after mild weathering. Sprayed droplets of copper compounds frequently had a heavy rim on at least one side and a less conspicuous margin elsewhere (Fig. 11 B).

A



B

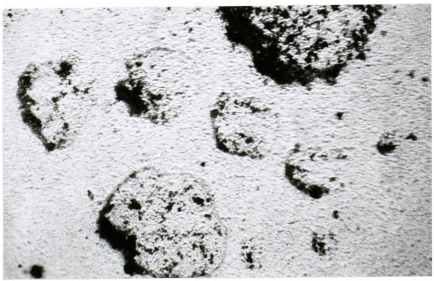


Figure 11.--Fungicide deposit on potato leaf epidermis. A: Du-ter measured droplet, and B: Kocide 101 spray. (Magnification 210 x).

With both spreaders, Biofilm and Pinolene, technical compounds lacked the clearly defined rim and the drops became optically less dense on weathering. These spreaders did not perform better than the spreaders in the commercial fungicides under test. There was a noticeable loss of fungicide following a few hours of gentle mist. Droplet margins were not as well defined and were often identified only with difficulty.

These observations support those of the electron probe but were not considered as precise.

Germination of Phytophthora infestans sporangiospores at distances from the fungicide drop: Drops of fungicide (0.001 ml volumes) were placed on potato leaf surfaces. Kocide 101 was prepared at 2 lbs/25 gal so that a drop (0.001 ml) contained 0.0095975 mg Kocide 101. Du-ter was prepared 0.6 lbs/25 gal to contain in 0.001 ml 0.0030508 mg commercial fungicide.

Fungicide droplets were dried on the leaf surface and exposed to mild weathering in a mist chamber for 5 hrs. The amount of mist was approximately 0.36 mm per hr. Leaf surfaces on which the fungicide drop was placed were oriented at a 30° angle so that the drainage during weathering would run down the surface to be examined. The leaf was then surface dried in air and a strip approximately 1 cm wide and 2.5 cm long was cut so that the fungicide drop was at one end of the strip. The strip was then cut into segments 5 mm wide but left attached as shown (Fig. 12). This was done to preclude

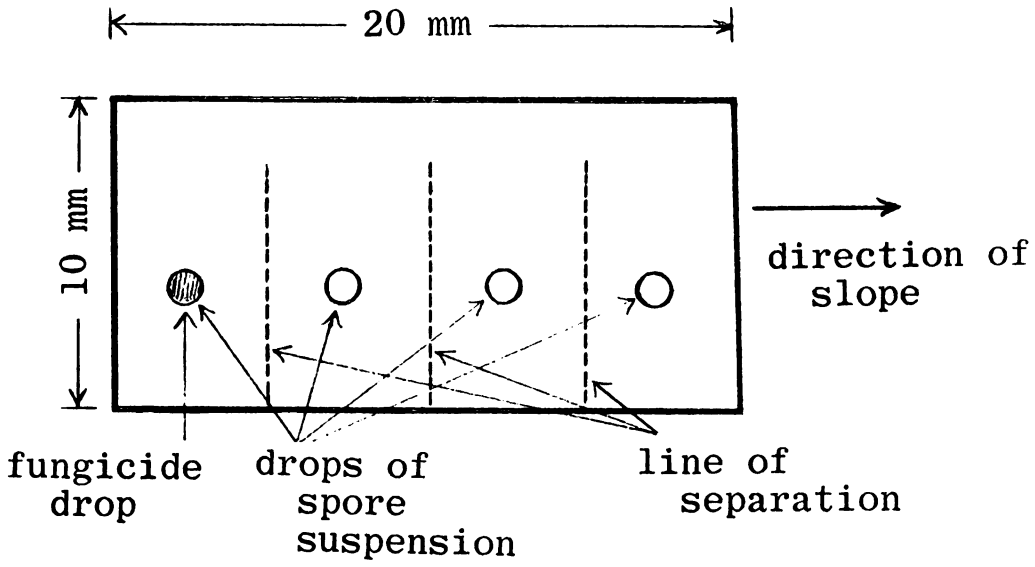


Figure 12.--Plan for fungicide application on potato leaflet surface, separation of segments, and sporangiospore application.

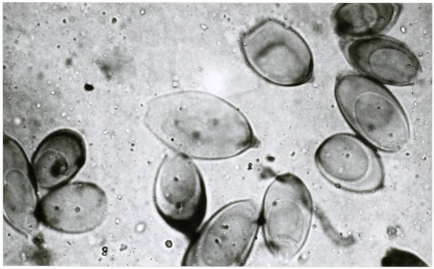
washing of toxicant across the leaf during application of spore suspensions. The spore suspension was prepared using the spore collector shown (Fig. 2), and sporangiospore concentration was set at 500 or more spores per 0.001 ml. A drop of spore suspension, 0.001 ml, was then placed at 0, 5, 10, and 15 mm from the original fungicide drop. The segments were then incubated in a moist chamber for 48 hrs.

The leaf surface with the spores was air dried after which the epidermis with the attached spores was stripped from the leaf using the procedure previously described. The strip was then stained with Ziehl's carbol fuchsin.

Spore germination counts were made to determine the percentages of spore germination near the original fungicide droplet and at 5, 10, and 15 mm from it. At each location 50 sporangiospores were counted and the percent

germination determined. Empty sporangial cases or those with germ tubes were considered to have germinated (Fig. 13A).

A



B

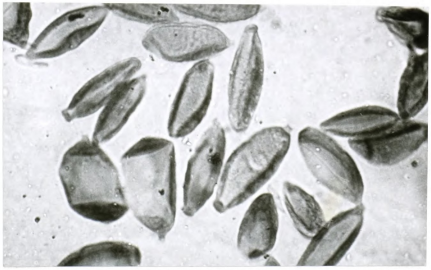


Figure 13A: Germinated Phytophthora infestans sporangiospores showing empty spore cases. These spores were removed from the leaf surface using adhesive.

B: Nongerminated spores. Note the shrivelled protoplast adhering to the inside surface of the spore wall. (Magnification, 840 X).

The protoplast of nongerminated sporangiospores adhered closely to the walls on one side (Fig. 13B) and accurate counting was difficult. Data presented are the combined average results from 3 trials and data from each trial consisted of two fields of 50 sporangiospores. Results with both fungicides as well as the copper and tin compounds with surfactants, Biofilm and Pinolene, are presented for Kocide 101 (Table 2) and Du-ter (Table 3).

Germination was consistently low with both fungicides applied on or very close to the drops (0 mm distance from drop). Similar results were obtained with cupric hydroxide and triphenyl tin hydroxide with either spreader. Kocide 101 was relatively nontoxic at the 0 hour time 5-15 mm from the drop. Toxicity increased with 5 to 24 hours of weathering. Cupric hydroxide was relatively nontoxic 5-15 mm from the point of application with either Biofilm or Pinolene.

Du-ter was effective at the 0 hour 5-15 mm from the drop. It was relatively nontoxic 5-15 mm from the point of application after 5-24 hours weathering. Triphenyl tin hydroxide with Pinolene was toxic at 5-15 mm at 0 hour. However at later times it was relatively nontoxic away from the drop. Triphenyl tin hydroxide with Biofilm somewhat less toxic at 5-15 mm before weathering and was relatively nontoxic after weathering.

Table. 2.--Germination of *P. infestans* sporangiospore at distances from the Kocide 101 drop [1]/ on potato leaf.

Treatment and duration of weathering	Germination at various distances from fungicide drop [2]/			
	0 mm	5 mm	10 mm	15 mm
0 hour				
No fungicide	33%	27%	29%	22%
Kocide 101	1	20	18	21
Biofilm + cupric hydroxide	0	22	23	26
Pinolene + cupric hydroxide	1	30	24	22
5 hours				
No fungicide	34	44	34	42
Kocide 101	2	8	19	20
Biofilm + cupric hydroxide	0	16	15	11
Pinolene + cupric hydroxide	1	21	20	23
24 hours				
No fungicide	34	34	36	33
Kocide 101	1	2	8	8
Biofilm + cupric hydroxide	0	13	12	12
Pinolene + cupric hydroxide	0	22	22	30

[1]/ Fungicide drop of 0.001 ml contained 0.009575 mg of Kocide 101 (2 lbs/ 25 gal).

[2]/ Combined spore germination data from counting 2 fields of 50 sporangiospores each for each of 3 trials.

Table 3.--Germination of *P. infestans* sporangiospore at distances from Du-ter crop[1] on potato leaf.

Treatment and duration of weathering	Germination at various distances from fungicide drop[2]			
	0 mm	5 mm	10 mm	15 mm
0 hour				
No fungicide	14%	17%	12%	14%
Du-ter	0	9	7	3
Biofilm + triphenyl tin hydroxide	0	15	15	9
Pinolene + triphenyl tin hydroxide	3	9	11	8
5 hours				
No fungicide	19	23	19	19
Du-ter	1	15	16	16
Biofilm + triphenyl tin hydroxide	1	21	24	26
Pinolene + triphenyl tin hydroxide	3	18	15	18
24 hours				
No fungicide	25	27	20	20
Du-ter	2	11	13	17
Biofilm + triphenyl tin hydroxide	1	16	24	14
Pinolene + triphenyl tin hydroxide	4	11	13	16

[1] Fungicide drop of 0.001 ml contained 0.0030508 mg Du-ter (47.5% active) (0.6 lbs/25 gal).

[2] Combined spore germination data from counting 2 fields of 50 sporangiospores each for each of 3 trials.

Infection of Phytophthora infestans near the fungicide drop on potato leaf: Distribution of fungicide around the point of application was determined by demonstrating infection sites in a cleared potato leaf.

A potato terminal leaflet was cut along the midrib into two parts. A drop (0.001 ml) of fungicide was placed on the center of the upper surface of one of the half leaflets. The opposite half leaf recieved no fungicide. After surface drying, both half leaves were weathered for 30 minutes by atomizer spraying with approximately 0.36 mm of water per hour. Both half leaflets were surface dried in air, and sprayed with a sporangiospore suspension. The spore suspension was obtained by suspending spores from diseased leaves in glass distilled water. The half leaflets were kept in a dish with moistened filter papers, and incubated for 48 hours in a growth chamber at 19-20°C.

After incubation half leaflets were boiled in water for 5 minutes, then heated in 95% ethyl alcohol until they were clear. Infection sites as indicated by numerous small necrotic spots then become visible. In cross section of the potato leaf, necrotic areas appeared as shown in Fig. 14.

Incidence of infection was determined by placing a grid (Fig. 15) on the surface of the half leaflets and counting infection points within each of the concentric areas with under a bacterial colony counter.

The protected area of the leaf surface was usually more or less circular around the point of application suggesting diffusion from the droplet (Fig. 16). The circular

Figure 14.--Cross section of potato leaf showing the extent of necrosis after 48 hours of incubation in these trials. (Magnification, 210 X).

pattern was not a constant feature presumably because drainage patterns distributed the toxicant irregularly over the surface.

The number and frequency of infection sites is presented (Table 4). Each number is the average infection of at least 10 half leaves. Infectivity was usually precluded

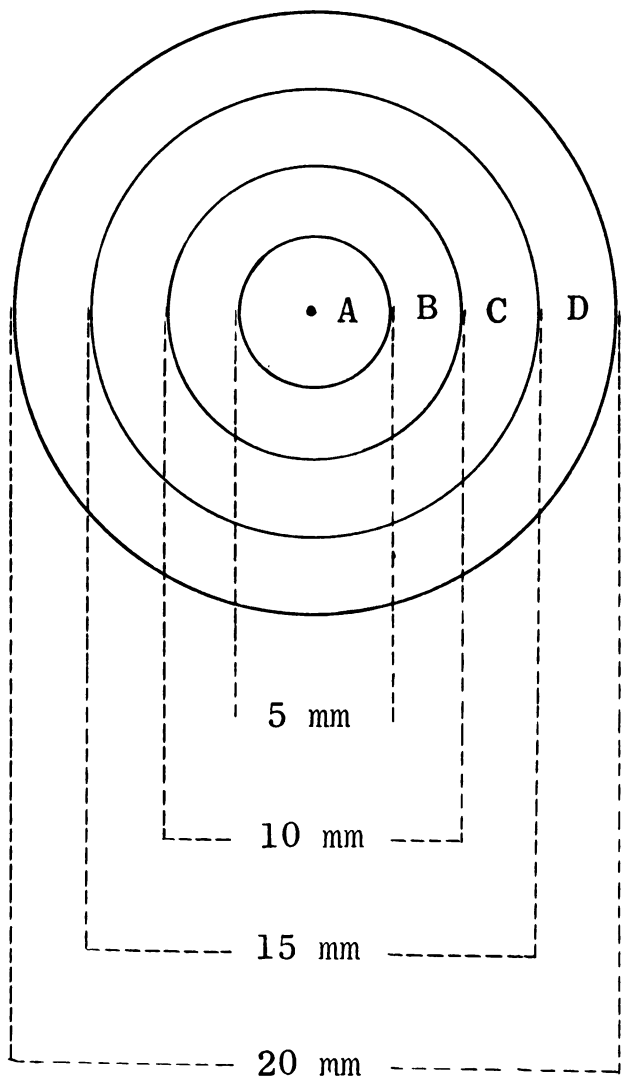


Figure 15.--Grid pattern used in quantitatively determining incidence of infection at distances from the fungicide drop.

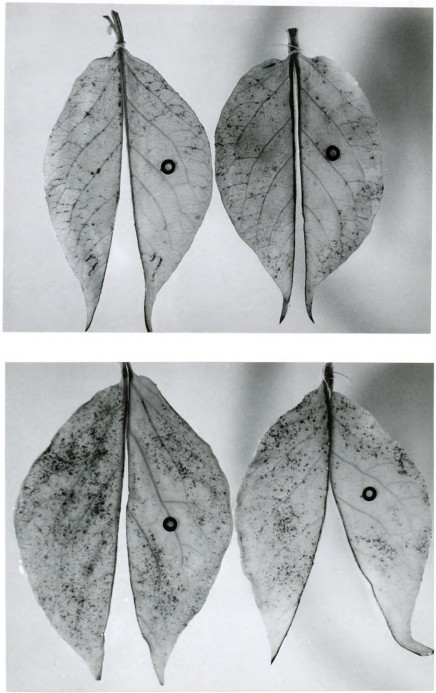


Figure 16.--Phytophthora infestans infection sites on potato leaves after 48 hours incubation and clearing in ethanol. Fungicide (0.001 ml) was placed on the leaf at the black circle, weathered 0.5 hrs, surface dried, and sprayed with spore suspension A, Kocide and B, Du-ter. (Magnification 1 X).

Table 4.--Infectivity of P. infestans at distance from a fungicide drop[1/].

Toxicant[2/] and date of test	Infection points[3/] at distances from droplet application 0-5 mm (A)		6-10 mm (B)		11-15 mm (C)		16-20 mm (D)[5/]		Control 0-20 mm A+B+C+D
	No.	CFA[4/]	No.	CFA	No.	CFA	No.	CFA	
Kocide									
7/24/71	0.5	8	6	33	14	44	34	78	74
7/25/71	0.1	2	2	8	7	22	15	36	68
7/26/71	0.0	0	2	10	8	24	19	43	108
Du-ter									
6/8/71	0.0	0	3	16	9	30	22	51	137
6/9/71	0.5	7	13	67	37	117	50	114	208
6/10/71	0.7	11	6	27	21	55	9	21	101
6/11/71	0.3	4	4	23	17	54	42	96	288

1/ Fungicide (1) applied in 0.001 ml droplet to half leaf of potato, (2) surface dried, (3) weathered, 30 minutes in mist chamber (0.36 mm water/hr), (4) surface dried, (5) sporangiospores of Phytophthora infestans sprayed on leaf, (6) incubated in an illuminated mist chamber approximately 48 hrs, (7) leaf cleared by boiling in 95% ethyl alcohol.

2/ Kocide 101 droplet (2 lbs/25 gal); 0.0095975 mg applied in 0.001 ml volume.
Du-ter droplet (0.6 lbs commercial (47.5% active) /25 gal); 0.0030508 mg (47.5% active) applied in 0.001 ml volume.

3/ Brown spots indicating early infection counted in a circular area at distance shown from the point of application. Area of A is 20 mm^2; B, 59 mm^2; C, 98 mm^2; D, 137 mm^2 respectively. These total 314 mm^2, the area of the control and comprise 6, 19, 32, and 43% respectively of the total area surveyed.

4/ CFA: Correction for area differences between the area of observation and the area of the control.
Factors are for: A, 16.0; B, 5.3; C, 3.2; and D, 2.3.

5/ In certain instances leaflets were not sufficiently large to permit observation in area D. Average values in this column are calculated only from leaves of sufficient size. Values for the control were corrected also for this area difference.

in circle A at the point of fungicide application. A few infection sites were counted in this area for both of the fungicides. Positive evidence was obtained of fungicidal action preventing infection by P. infestans some distance from the point of application. Usually infectivity was progressively reduced from the 16-20 mm, zone D, towards the zones, C, B, and A. Occasionally infection in the control area was not as high as that of the outer zones near the fungicide drop. The exact reason for this is not apparent, however, it is generally difficult to obtain a consistently high level of uniformity of infection with P. infestans.

A single drop of fungicide protected a considerable area of the leaf surface. Quite frequently infection was very low on the entire half leaf treated with a single drop of fungicide. This suggested that for practical purposes protection would have been adequate under field conditions where inoculum load per leaf was not excessive.

Germination of Phytophthora infestans spores in fungicide at different concentrations: Information was desired concerning the toxicity of the fungicides, Kocide 101 and Du-ter, in dilute solutions to sporangiospores. This information should give some indication of the concentration on the leaf surface capable of retarding germination and possibly infection.

Fungicides were serially diluted (Table 5). A drop of fungicide (0.01 ml) was placed in a van Tieghem cell. Sporangiospores less than 48 hours old, obtained from infected

Table 5.--Toxicity of fungicides in water suspension to *Phytophthora infestans* sporangiospores.

	distilled water control	% germination of sporangiospores at concentrations (ppm active) indicated			
Du-ter		475	47.5	4.75	0.475
test 4/10/71	33, 54[3/]	0, 0	0, 0	9, 9	22, 34
test 4/18/71	44, 57	0, 0	2, 3	20, 9	44, 70
test 5/1/71	71, 59	0, 0	1, 4	4, 5	8, 13
Kocide[2/]	control	1000	100	10	1
test 5/2/71	39, 11	0, 0	14, 6	9	50, 54
test 5/3/71	75, 86	0, 0	0, 0	0, 0	79, 71
test 5/8/71	2, 13	0, 0	0, 0	0, 0	11, 0
test 5/9/71	81, 85	0, 0	0, 0	0, 0	67, 72

[1/] Commercial Du-ter (47.5% active).

[2/] Kocide 101 100% active in the preparation.

[3/] Paired numbers indicate percent germination in 100 spore sample from 2 separate van Tieghem cells.

potato leaves were transferred directly by using a bevelled wooden applicator stick. The bevelled edge was dampened with glass distilled water and touched to the mass of Sporangiospores on the surface of the diseased leaves. Since spores over a constant area were thus harvested the number of spores transferred was relatively constant. Over 1000 sporangiospores were present in each van Tieghem cell. By this method of transferring spores, the concentration of toxicant was not modified by adding inoculum in water suspension.

The sporangiospores in a van Tieghem cell were incubated in a refrigerator at 6-7 C for one hour, then removed to a growth chamber at 19-20 C for 24 hours. After incubation, 100 random spores were counted to calculate the percent germination. Empty sporangial cases and sporangiospores which had germinated with germ tube were considered to have been germinated spores (Table 5).

The percent germination of spores obtained by this method was extremely variable and was slightly higher than with the suction tube method described (Fig. 2).

Germination of Phytophthora infestans in effluents from fungicide sprayed leaves: Estimation of toxicity of effluents from fungicide sprayed leaves was done by determining percentage of germination of sporangiospores. Leaves from green house grown plants were sprayed with fungicide to approximate good field coverage and then surface dried. They were placed in a moist chamber and exposed to fine water droplet precipitation of approximately 0.35 mm per hour.

Effluents dripping from the leaflet tips were collected in small vials at intervals during a 96 hour weathering period. 0 hour indicates the time of collecting the first few drops of effluent at the beginning of the weathering period, and the other effluents were collected at the several times indicated in (Tables 6, 7).

Toxicity of leaf effluents was determined using sporangiospores obtained from the surface of potato leaves infected with late blight using the spore collector (Fig. 2). A concentrated suspension of more than 1,000 sporangiospores per 0.001 ml were obtained as counted with a standard blood haemocytometer.

Drops of effluent, 0.009 ml, were transferred to a cover slip. An 0.001 ml drop of spore suspension was added and the droplet was mixed with a small pointed strip of Parafilm M manufactured by the American Can Co. The cover slip was then inverted over a van Tieghem cell. Empty sporangial cases or those with germ tube were counted after incubation in the refrigerator at 7-8 C. Results of tests with Kocide 101 (Table 6) and with Du-ter (Table 7) are shown. Controls involving no fungicide were prepared using effluents from unsprayed leaves collected in a manner similar to that described for the fungicide trials.

The initial effluent of both copper (Table 6) and tin (Table 7) was toxic but after 5 hrs., effluents were low in toxicity and lacking in toxicity after longer periods. Neither surfactant modified the behavior of either toxicant in these trials.

Table 6.--Toxicity to Phytophthora infestans of effluents from potato leaves sprayed with cupric hydroxide + different surfactants at time intervals during weathering.

Duration of weathering (hours)	Germination of sporangia*																			
						Cupric hydroxide with spreader indicated														
	No fungicide					Commercial spreader (Kocide)					Biofilm spreader					Pinolene spreader				
	4/12	4/21	6/16	9/13	Av	4/12	4/21	6/16	9/13	Av	4/12	4/21	6/16	9/13	Av	4/12	4/21	6/16	9/13	Av
0	23	32	65	60	45	9	22	26	0	12	0	9	34		14	12	0	10		7
5	46	81	83	51	65	51	72	51	42	54	50	55	49		51	44	66	42		51
24	72	83	88	54	74	62	70	78	49	65	49	56	21		42	44	62	53		53
48	76	84			80	77	64			71	51	65			58	88	77			83
72	95	103			99	90	84			87	95	88			92	96	91			94
96	95				95	99				99	117				117	116				110
24 + 0**			86	72	79			63	36	50				81	81				86	86
24 + 5**			94	69	82			65	85	75				103	103				93	93
24 + 24**			100	---	100			93	---	93				45	45				76	76

*Each figure represents the number of empty sporangia in a total of 200 sporangia counted in each of 2 van Tiegem cells (100 sporangia per cell). Different dates indicate results of identical tests repeated 4 times. Each test was prepared in duplicate on each day.

**Leaves were weathered for 24 hours, air dried, and returned to the moist chamber. A second effluent was collected at the time indicated following the + sign.

Table 7.--Toxicity to *Phytophthora infestans* of effluents from potato leaves sprayed with triphenyl tin hydroxide + different surfactants at time intervals during weathering.

Duration of weathering (hours)	Germination of sporangia*																					
	No fungicide						Triphynyl tin hydroxide with spreader indicated															
							Commercial spreader Du-ter)						Biofilm spreader					Pinolene spreader				
	5/13	5/16	5/28	5/31	9/13	Av	5/13	5/16	5/28	5/31	9/13	Av	5/13	5/16	5/28	5/31	Av	5/13	5/16	5/28	5/31	Av
0	90	79	56	96	60	76	1	0	0	3	0	0.8	0	0	0	0	0	17	2	0	0	5
5	56	113	44	105	51	74	17	15	22	69	43	33	15	16	0	30	15	49	101	30	99	70
24	58	116	70	125	54	85	23	84	1	41	55	41	35	78	49	80	61	47	79	51	116	73
48	76	129	58	118		95	55	85	27	104		68	52	86	28	119	71	82	98	67	103	88
72	79	120	88	108		99	53	99	87	111		88	43	51	87	114	74	71	117	71	112	93
24 + 0**					72	72					47	47										
24 + 5**					69	69					61	61										

*Each figure represents the number of empty sporangia in a total of 200 sporangia counted in each of 2 van Tieghem cells (100 sporania per cell). Different dates indicate results of identical tests repeated 5 times. Each test was prepared in duplicate on each day.

**Leaves were weathered for 24 hours, air dried, and returned to the moist chamber. A second effluent was collected at the time indicated following the + sign.

Inconclusive evidence in these trials suggested that a toxic substance may have been eluted from untreated potato leaves at the 0 hr. time. The initial effluent (0 hour) caused some reduction in germination. After a few hours, however, the effluent was no longer toxic.

DISCUSSION

The extent to which fungicide redistribution is important in potato disease control has not been accurately measured. It is generally accepted that redistribution takes place somehow. The two fungicides studied, Du-ter (triphenyl tin hydroxide) and Kocide 101 (cupric hydroxide), were selected because of suitability for electron probe analysis.

Direct observation of the fungicide drop by visual observation and electron probe analysis suggest that the nature of the droplet margin and appearance of the droplet was not appreciably changed by surface water action on the leaf. Droplet margins were clearly defined and discrete, and no detectable fungicide had migrated. Optical examination supported the electron microprobe analysis, and showed clearly defined rims in the margins. Kocide 101 and Du-ter seemed to be very tenacious, and the deposit did not resuspend in mild weathering.

By direct observation and bioassay of washed deposit, Hislop and Cox (1970) found that wettable powder formulations were resuspended and frequently redeposited in previously unprotected areas. They also pointed out that Bordeaux and Burgundy mixtures were very tenacious and were not resuspended. Redistribution in my observations must have been below the level of sensitivity of the electron probe because protective

action away from the fungicide drop was demonstrated by subsequent bioassay with P. infestans.

Sporangial germination on the leaves close to the fungicide drop was very low and 5 mm or more from the drop, germination approximated that of the control. This suggests that toxicant has little effect a short distance from the fungicide droplets. This also supports the electron microprobe analysis and optical observation. Again the level of toxicant redistributed from the drop of fungicide was apparently very low and not sufficient to prevent germination of sporangiospores.

Lower concentrations of toxicant redistributed from the fungicide drop were demonstrated using infection as the criterion for protection. Infection tests around the funicide droplets (Table 4), demonstrated that protected areas were considerably larger than the area occupied by the original drop of fungicide. Infection was inhibited 15-20 mm from the fungicide droplet. Thus, high fungicidal protection seems to be due to greater sensitivity of germ tubes and infective hyphae than that of sporangiospores. There are a number of reports comparing mycelial and spore sensitivities to fungicides. Diphenyl, for example, does not inhibit spore germination of several species of fungi, but it is inhibitory to hyphal growth of those organisms (Ramsey et al., 1944; Horsfall, 1956). The process of infection by germ tubes from fungus spores is very complex, and many requirements are needed to complete infection.

The possibility exists that fungicide droplets may have been redistributed by spraying the spore suspension, over the leaf surface and the fungicide may have migrated more extensively. Hislop and Cox (1970) reported that two cycles of wetting followed by drying increased protection. If redistribution was accomplished by the spray application of spore suspension, in my tests it was not possible to differentiate between redistribution following the initial weathering and that following application of spore suspension. Under field conditions, however effective protection should be anticipated by distribution on either or both occasions.

The protected area in the infection test was not always circular around the point of application. This was presumably due to leaf orientation and to drainage patterns distributing the toxicant irregularly over the surface. Irregular leaf surface topography associated with the leaf hairs, stomata, vascular networks,and wax deposits etc. may have been involved.

The initial effluents from fungicide sprayed leaves markedly reduced germination of sporangiospores. After a few hours, however, the effluents were no longer toxic. Thus, readily soluble portions of these two fungicides were washed-off the leaves during the first few hours. The fact that effluent from untreated leaves showed some toxicity suggests that a toxic substance is present on the leaf surface of the potato. Others have also reported toxic materials in leaf exudates. High concentrations of leaf exudate sometimes found

in rain and dew were inhibitory to spores of Botrytis, Ascochyta, and Puccinia (Kovács and Szeöke, 1956); and germination of Alternaria tenuis conidia was slightly inhibited by the presence of leaf exudates (Hislop, 1966a).

In addition to toxicity of the effluent from fungicide-treated leaves, complex interactions between leaf exudates and the fungicide should be anticipated. In the study of sporangiospore germination at distances from the fungicide drop, the percent spore germination on untreated leaves without weathering was less than those weathered for 5 hours. These results also suggest the presence of a toxic substance on the potato leaf surface.

With the spreaders, Biofilm and Pinolene, technical compounds of tin and copper showed less redistribution than did the commercial fungicide, Kocide 101 or Du-ter at 5 mm distance from the fungicide drop following 5 and 24 hours weathering (Tables 2, 3). Electron microprobe analysis did not show marked differences in redistribution comparing the tin and copper compounds with commercial Kocide 101 and Du-ter. The effluent from the leaf sprayed with tin plus Pinolene showed high germination following 5 hours weathering. These observations suggest that the spreaders Pinolene or Biofilm were not more efficient than the surfactants formulated in the commerical fungicides.

As Hislop and Cox (1970) has pointed out, redistribution of copper was improved after washing. In my trials tin was also redistributed as was copper. The results also confirm Goossen's suggestion (1958) that tenacity of most fungicides is sufficient for maximum redistribution.

SUMMARY

Redistribution of copper and tin on the potato leaf surface was investigated by electron microprobe analysis, direct microscope observation, and bioassay with Phytophthora infestans.

Electron microprobe analysis of fungicide droplets containing copper or tin fungicides on the potato leaf showed that droplet margins were clearly defined and often had rim of heavy fungicide concentration. These margins were clearly defined and fungicide had not migrated in detectable quantities with or without weathering. Drops of either fungicide with Biofilm or Pinolene had clearly defined margins but the marginal rim was not as evident. Bioassay demonstrated that active toxicant existed immediatly outside the droplet, but the levels of copper or tin were apparently below the level of sensitivity of the electron probe.

Microscope examination of measured droplets and sprayed deposits showed clearly defined marginal rims, but the rim of heavy spray concentration at the margin of the drops of Kocide 101 and Du-ter was not completely continuous. Technical compounds of copper or tin with either Biofilm and Pinolene lacked the clearly defined rims.

Sporangial germination on leaves close to the fungicide drop was very low (0 mm distance from drop). Toxicity of Kocide 101 increased with 5 to 24 hours of weathering.

Reduced suppression of germination was obtained with cupric hydroxide plus either spreader.

Spore germination was suppressed by Du-ter up to 15 mm from the point of application immediately after application. After weathering spore germination was reduced only at the site of the fungicide drop. Results were essentially similar with either spreader.

Inhibition of infection around the fungicide drops after 30 min of weathering was evident up to 20 mm from the point of fungicide application. The area of fungicide distribution from the point of application was much larger when determined by frequency of infection sites than it was when estimated by prevention of spore germination. Perhaps the toxicants were more effective against zoospores, or germ tubes, or infection hyphae than against infection per se.

Spore germination was effectively prevented by 10 ppm of Kocide 101 but not by 1 ppm. Du-ter at 47.5 ppm prevented spore germination of most spores but not all spores.

The first effluents from fungicide sprayed leaves prevented sporangiospore germination. After 5 hours of gentle weathering, there was some evidence of low toxicity. Toxicity was not evident in effluents after 24 hrs or longer.

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