Persistence of Foliar Protective Fungicides

Dan Neely

Plant Pathologist, Illinois Natural History Survey, Urbana, Illinois 61801.
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ABSTRACT

A cellophane transfer bioassay technique was used to determine the persistence of 14 commercial fungicides on the leaves of 12 woody plant species. Residues sufficient to inhibit germination of 99% of *Monilinia fructicola* conidia were detected 3 months after application. Field persistence was not correlated with in vitro fungitoxicity. Phytopathology 60:1583-1586.

Protective fungicides applied to plant foliage are subjected to a number of factors that tend to reduce the amount of fungicide remaining on the leaf. The fungicide may be removed by rain, wind, or mechanical abrasion, or it may be decomposed or degraded by various chemical, physical, or biological agents.

The processes of deposition and degradation of fungicides on plant parts are associated with fungicide persistence. Ebeling (2) reviewed the literature on equipment, adjuvants, formulations, adsorption, penetration, and translocation as they relate to pesticide deposition. In addition, he reviewed papers on the plant surface, plant growth, formulations, rain, humidity, volatilization, wind, temp, and light as they relate to disappearance of pesticide deposits. Linskens et al. (3) reviewed work on the relationship between the plant cuticle and pesticide residues. The physical and chemical interactions of fungicides and plants were also discussed by Burchfield (1).

Investigators seeking to control plant diseases by chemical means frequently use in vitro tests or laboratory tests with plant parts to aid in selection of fungicides to be included in field trials (4, 6). A purpose of the present study was to determine the ability of the cellophane transfer bioassay to detect fungicide residues on plants with differing leaf surface characteristics. No effort was made to establish beyond doubt that one fungicide was more persistent than another or that leaves of one plant retain fungicides more effectively than leaves of another plant.

MATERIALS AND METHODS.—The inherent fungitoxicity of the fungicide determines to a large extent the detectability of residues by bioassay techniques. The measurement of fungitoxicity used in this test was the lowest aq concn of each fungicide that would inhibit 99% or more conidial germination of *Monilinia fructicola* (Wint.) Honey. In laboratory trials, the cellophane transfer method (5) was used at concn intervals of 0.04, 0.2, 1, 5, 20, 100, and 500 ppm active ingredient of fungicide.

The bioassay to detect residues of 14 fungicides on the foliage of 12 plants was accomplished in the following manner: A concn of fungicide commonly used commercially, usually 2.4 g/liter (2 lb./100 gal), was prepared by mixing the fungicide in tap water. One twig possessing mature leaves was immersed in the fungicide, agitated slightly, and slowly removed. One day after treatment, then at weekly intervals, three discs of leaf tissue 9 mm in diam were taken from a mature leaf with a paper punch. Insofar as possible, discs were taken from the same leaf week after week. The leaf discs were taken to the laboratory in capped bottles. In the laboratory the leaf discs were placed on Coors porcelain spot plates containing filter paper pads moistened with distilled water. On each leaf disc was placed a nonmoistureproof disc of cellophane (DuPont 215 PD-62) which was then seeded with a conidial suspension of *M. fructicola* using a micropipette. After 24-hr incubation in a moist chamber at 24 C, the cellophane discs were transferred to microscope slides and conidial germination determinations were made microscopically (Fig. 1). The bioassay was terminated when the residual fungicide failed to inhibit 99% or more conidial germination for 2 consecutive weeks.

The fungicide-persistence field trials were conducted on plants in the Natural History Survey arboretum 1 mile south of Urbana, Illinois. The plants were 1.5 m apart in nursery-type rows 2.1 m apart. All species were planted in 1960-1962 with the exceptions of willow planted in 1964 and dogwood planted in 1966. The field study was subdivided into 3 tests. All fungicides were applied in 1967 to four species on 20 June (Test A), to four other species on 28 June (Test B), and to the four remaining species on 1 August (Test C). The meteorological conditions present during each test are given in Fig. 2. Meteorological data were obtained from the Urbana weather station located 1.5 miles north of the test plot.

The following fungicides were assayed: a mixture of 5.2 parts by wt (83.9%) of ammoniate of [ethylenebis(dithiocarbamate)] zinc with 1 part by wt (16.1%) ethylenebis(dithiocarbamic acid) bimolecular and trimolecular cyclic anhydrosulfides and disulfides (Polyram 80WP), captan (Captan 50-W), dichlone (Phygon), 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine (Dyrene), dodine (Cyprex 65-W), ferbam (Fermate), folpet (Phaltan 50 Wettable), maneb (Manzate), phenylmercurimonoethanolammonium acetate (Puratized Apple Spray), *N*-(1,1,2,2-tetrachloroethyl)sulfenyl-*cis*-4-cyclohexene-1,2-dicarboximide (Difolatan 80 Wettable), tetrachloroisophthalonitrile (Daconil 2787), thiram (Thylate), triphenyltin hydroxide (Duter), and ziram (Zerlate).

The 14 fungicides were assayed on leaves of each of the following 12 woody plant species: ash (*Fraxinus pennsylvanica* Marsh.), catalpa (*Catalpa speciosa* Warder), dogwood (*Cornus alba* L.), euonymus (*Euonymus fortunei* [Turcz.] Hand.-Maz.), hackberry (*Celtis occidentalis* L.), maple (*Acer saccharum* Marsh.), oak (*Quercus rubra* L.), redbud (*Cercis canadensis* L.), sycamore (*Platanus occidentalis* L.), tulip tree (*Lirio-*

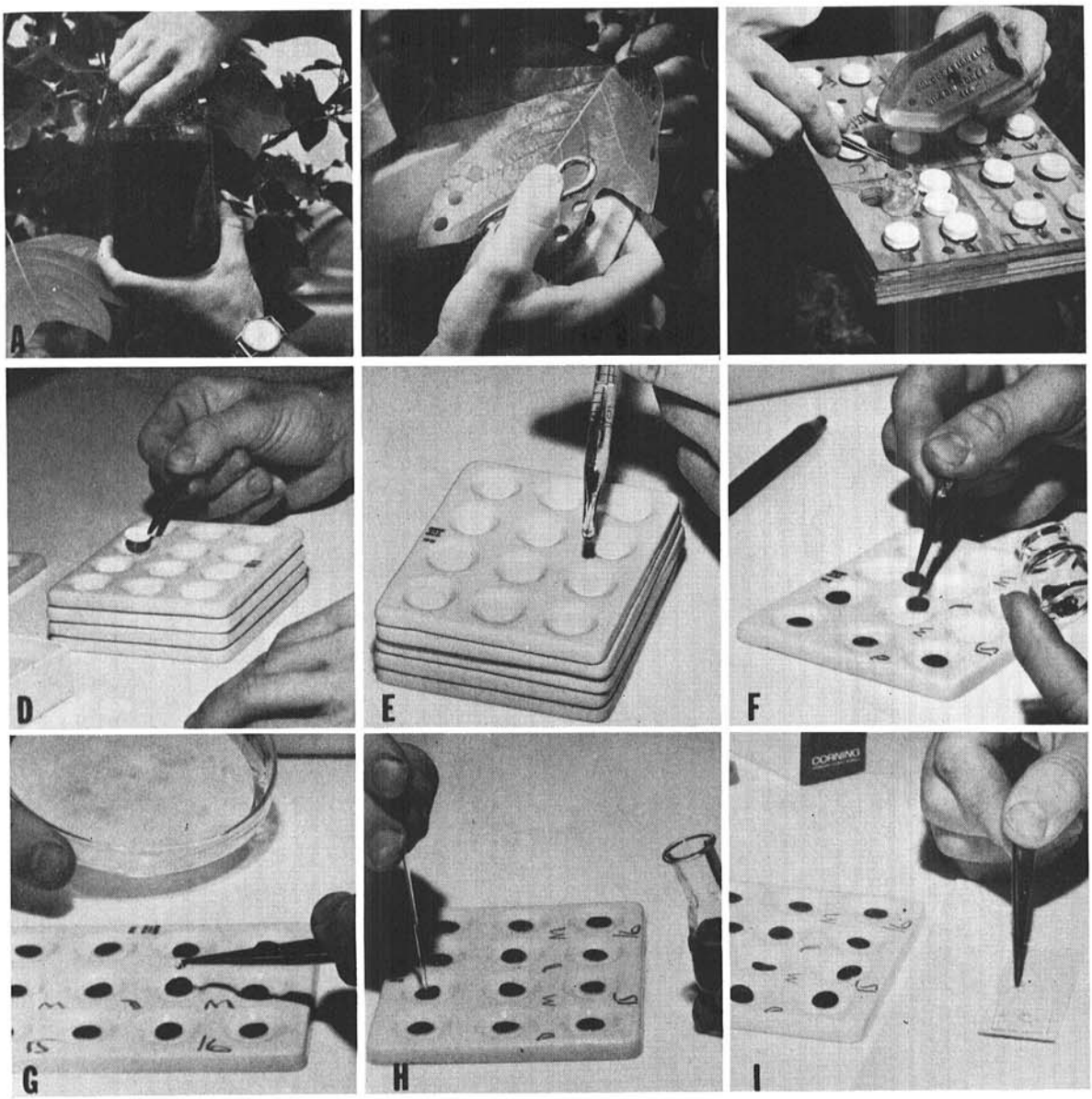

Fig. 1. Procedure for bioassay of persistence of fungicide on foliage. **A)** A twig terminal with mature leaves was dipped in the aqueous fungicide concn. **B)** At weekly intervals, three discs of leaf tissue were taken from the leaf, and **C)** carried to the laboratory in capped bottles. **D)** Coors porcelain spot plates were prepared by addition of filter paper pads **E)** moistened with distilled water. **F)** A leaf disc was placed on each pad. **G)** A moist nonmoistureproof disc of cellophane was placed on each leaf disc and **H)** seeded with a conidial suspension using a micropipette. **I)** After 24-hr incubation in a moist chamber, the cellophane discs were transferred to microscope slides for conidial germination determinations.

dendron tulipifera L.), viburnum (*Viburnum carlesii* Hemsl.), and willow (*Salix discolor* Muhl.).

Since morphological characteristics of the plants can be expected to influence deposition, distribution, and retention of fungicides, certain structural features of leaves were determined from mature leaves of the 12 plant species. Leaf inclination was determined in August 1968 by using a protractor to measure the deviation from perpendicular of 50 leaves of each plant species. Water retention was measured by placing five leaves of each species on an inclined plane 30 degrees from horizonal and adding 0.05 ml drops of water from a height of 6 cm to one spot on the leaf surface until runoff occurred. The abundance and type of pubescence on the upper leaf surface of the 12 plant species were determined through observation with a stereo microscope.

Results.—The cellophane transfer bioassay detected residues of fungicides 3 months after application in the field. The residues were sufficient to prevent germination of 99% or more of the conidia of the test fungus. Using these criteria for persistence, 11 of the 14 com-

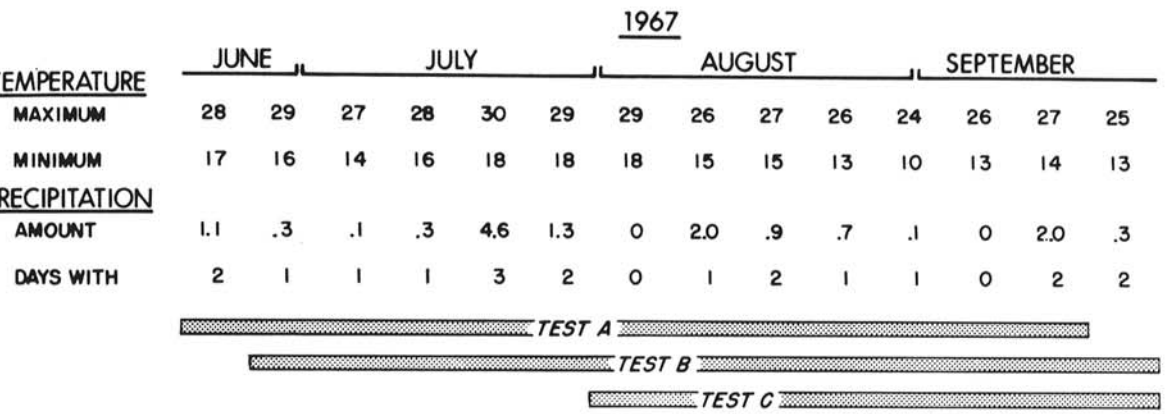

Fig. 2. Meteorological data for Urbana, Ill. Temperature as weekly mean max and min in C. Precipitation as weekly total in cm and days with measurable rainfall.

TABLE 1. The persistence in weeks of 11 foliar protectant fungicides on leaves of 12 woody plant species as determined by cellophane transfer bioassay

	Test A				Test B				Test C			
Fungicide	Maple	Redbud	Ash	Catalpa	Euonymus	Dogwood	Willow	Viburnum	Oak	Sycamore	Tulip tree	Hackberry
Captan	2	2	2	2	3	3	3	4	1	1	1	2
Daconil 2787	4	1	3	2	3	5	3	3	4	2	2	2
Dichlone	1	2	2	0	1	1	1	3	1	2	2	2
Difolatan	7	5	5	7	5	5	4	11	4	4	4	6
Dodine	2	1	3	1	4	3	3	9	2	2	3	2
Duter	1	0	1	1	2	4	2	3	2	2	1	2
Dyrene	2	0	1	1	3	2	3	3	2	1	1	3
Ferbam	5	3	4	4	5	5	4	12	1	3	5	3
Folpet	2	1	2	1	3	2	1	3	1	2	2	2
Thiram	5	3	2	1	1	1	2	3	4	2	6	6
Ziram	2	1	1	1	3	3	4	10	1	2	2	2

mercial fungicides persisted for 1 or more weeks. The residues of maneb, Polyram, and Puratized Apple Spray did not prevent conidial germination 1 week after application. Most of the persistent fungicides on most plant species were detected at least 2 or 3 weeks after application regardless of meteorological conditions (Table 1).

Field persistence was not directly correlated with in vitro fungitoxicity. Although all fungicides prevented spore germination in in vitro tests at concn of 5 ppm or less (exception: Duter, 20 ppm) and all fungicides were applied to plants at concn of 1,200 to 2,000 ppm (exception: Puratized Apple Spray, 300 ppm), average persistence on the 12 hosts varied from less than 1 day to over 5 weeks (Table 2).

With a few notable exceptions, the leaf structures of the 12 woody plant species did not differ greatly. All are broadleaved deciduous plants with the exception of euonymus, which is a rather small-leaved evergreen. The leaves were mostly parallel to the ground with the exceptions of euonymus, which has upright leaves, and oak and viburnum, which have pendulous leaves. Sycamore and maple retained the most water prior to runoff; euonymus retained the least. The upper leaf surface on most species was glabrous or almost glabrous, but willow, dogwood, hackberry, and viburnum were definitely pubescent. In the present study, the leaf characteristic best correlated with fungicide persistence was the amount of pubescence (Table 3). The very pubescent viburnum retained the fungicides for a much

TABLE 2. The aqueous concn of materials fungistatic to *Monilinia fructicola* conidia in in vitro trials and the rates of usage and persistence on foliage of 14 fungicides in field trials

Fungicide	In vitro fungistatic concn	Rate of field usage	Avg persistence 12 hosts
	ppm[a]	*ppm*[a]	*weeks*
Maneb	5	1,920	0
Polyram	5	1,920	0.1
Puratized A. S.	0.2	300	0.2
Dichlone	1	1,200	1.5
Duter	20	1,200	1.8
Dyrene	1	1,200	1.8
Folpet	5	1,200	1.8
Captan	5	1,200	2.2
Ziram	5	1,820	2.7
Daconil 2787	0.2	1,800	2.8
Dodine	5	1,560	2.9
Thiram	1	1,560	3.0
Ferbam	5	1,820	4.5
Difolatan	1	1,920	5.6

[a] Active ingredient.

TABLE 3. Structural characteristics of leaves of 12 woody plant species and average persistence of 11 fungicides

Plant	Leaf inclination degree from perpendicular		Runoff at 30 degrees, drops	Pubescence, upper surface		Avg persistence 11 fungicides, weeks
	Avg	Range		Amount	Description	
Redbud	103	80-120	4	+	Sericeous	1.7
Catalpa	104	80-120	5	—	Glabrous	1.9
Sycamore	109	80-120	8	—	Glabrous	2.1
Oak	131	100-160	4	—	Glabrous	2.1
Ash	98	70-120	4	—	Glabrous	2.4
Tulip Tree	96	60-140	4	+	Tomentulose	2.6
Willow	96	70-120	5	++	Sericeous	2.7
Hackberry	92	80-110	4	++	Hispid	2.9
Maple	91	60-120	7	+	Tomentulose	3.0
Euonymus	73	40-100	3	—	Glabrous	3.0
Dogwood	107	80-130	4	++	Sericeous	3.1
Viburnum	112	60-150	6	+++	Tomentose	5.8

longer period than did any of the other plant species. Euonymus, however, which is glabrous, retained the fungicides as long as many of the pubescent species.

Rain was not demonstrated to be a primary agent causing disappearance of the fungicide residue. Although in 1967 Urbana experienced a dry summer, there was substantial rainfall during the 3rd week of June, the 3rd and 4th weeks of July, and the 2nd week of August (Fig. 2). The periods of rainfall were not correlated with loss of persistence in Tests A, B, or C.

DISCUSSION.—The value of persistence of a fungicide will vary with host and disease. Knowledge of persistence is important in determining the min number of chemical applications that will control a disease. Persistence at concn above legally established tolerances, however, is unwelcome when the fungicide is applied to edible crops.

Undoubtedly, persistence data obtained from chemical assays and bioassays are available from the research units of fungicide manufacturers. These data are not readily available to investigators seeking practical and effective control of specific fungus diseases. Even though data on fungicidal persistence were obtained from various sources, there is little likelihood that it would be comparable due to the varying methods used to obtain it.

The cellophane transfer bioassay can be readily adapted to determine the toxicity of a fungicide to a specific fungus pathogen and to determine the length of time the fungicide will persist in the field at an efficacious concn on the host plant. Comparable data from a number of fungicides can be obtained in a limited period of time. Data of this type should assist investigators in selecting fungicides most likely to control diseases in the field.

The data in the present study indicate a great difference of persistence among commercial fungicides, all of which are quite toxic to the test fungus. The ratio of rate applied to fungistatic concn does not give a factor that can be readily converted to field persistence. Apparently the quantity of deposit on the leaf surface and the rate of degradation or removal of this deposit will differ for each fungicide.

The data in the present study also indicate a difference of persistence of fungicides on plants with varying leaf surface characteristics. It is not surprising that amount of pubescence or retention of water drops may show a correlation with persistence of a fungicide. It is surprising that the leaves of evergreen euonymus, which are upright and glabrous and retain water poorly, retain fungicides as long as many pubescent leaves. The demonstrated differences among hosts, fungi, and fungicides emphasize the need for additional experimental data specific to host, fungus, and fungicide.

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