Chlorothalonil Residues on Field Tomatoes and Protection Against Alternaria solani

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ABSTRACT

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Leaf disk samples (1 cm in diameter) from sprayed plants of tomato cultivar New Yorker were analyzed for chlorothalonil residue and bioassayed for appressorial and lesion formation with spores of *Alternaria solani*. Reduction of residue levels of chlorothalonil and percent inhibition of appressorial formation were linear with time. Protection, defined as inhibition of appressorial formation greater than 50 percent, persisted 11 days for top and middle leaves and 13 days for bottom leaves. The loss of residue and protection was linear with time and failed to coincide with rainfall, plant growth, or crop age. The ED_{50} concentration for inhibition of appressorial formation by chlorothalonil at all leaf levels averaged 1.2 $\mu g/cm^2$, and against lesion formation, 0.6 $\mu g/cm^2$. On plants sprayed at weekly intervals, chlorothalonil exceeded the initial deposit on bottom leaves 2-5 weeks after treatments were commenced, whereas no accumulation was noted on upper and middle leaves. Evidently, a 10-day spray schedule with chlorothalonil at the standard rate for BRAVO 6F would provide protection against early blight disease of tomato.

Additional key words: appressoria, lesions, early blight.

Concern for the environment and the high cost of pesticides necessitate detailed information on efficacy of fungicides as foliar residues for control of plant pathogens. Effective concentrations of fungitoxic residues can be determined by chemical analyses of the fungicide and by bioassay. With the aid of simple analytical procedures, the effects of environment on the protectant efficacy of fungicides can be measured independently. An integration of such information can predict field performance and, hence, permit the use of minimal amounts of fungicide to protect plants from diseases.

Residual dosages of copper and zineb received considerable attention in relation to deposition and retention on foliage (1, 5). With the introduction of the organic fungicides, dosage of fungicide in sprays was emphasized because of a lack of sensitive analyses of residues. However, quick and accurate analyses with the use of gas chromatography enables one to equate disease control to the levels of fungicide in foliar residues.

Although essential to the usefulness of such studies, data gathered from natural infection are variable because of uncontrollable parameters of inoculum density and weather. Artificial inoculation under controlled conditions removes much of this variability (3, 4, 8).

The protectant action of chlorothalonil (2, 4, 5, 6-tetrachloroisophthalonitrile) in a conventional spray program on a tomato crop was investigated by analyzing foliar residues for active ingredient and a laboratory bioassay of leaf disks with spores of *Alternaria solani*.

MATERIALS AND METHODS

Tomato plants (*Lycopersicon esculentum* Mill. 'New Yorker'), a short-season determinate cultivar, were placed at 0.9-meter intervals in rows of 20 plants each. Rows were spaced 1.8 meters apart. Each row served as a spray block.

BRAVO 6F, a 54 percent active flowable formulation of chlorothalonil (manufactured by Diamond Shamrock Corporation, P.O. Box 348, Painesville, Ohio 44077) was sprayed on tomatoes at a rate of 2.2 liters in 931 liters of water per hectare at a pressure of 14 Kg/cm^2 (2 pints per 100 gal at 200 psi). Sprays were started 5 weeks after the tomatoes were transplanted into the field. One treatment was sprayed weekly for 8 weeks, and other individual treatments were sprayed once, with applications being made during each of the next 8 weeks. An unsprayed treatment was included, and other tomatoes, planted 15 meters away from the spray plots, served as checks on the unsprayed treatments for possible residue from spray drift. Treatments were replicated three times in a randomized block design.

Leaf tissue was taken from plants in the middle half of all three rows of each treatment and pooled for analyses. Three leaf levels were samples: A, the topmost expanded leaf; B, leaf at midplant height; and C, the lowest green leaf. For each leaf level, 30 disks were punched one per plant from the mid-section to either side of the mid-vein of one of the three terminal leaflets. Samples were taken in the morning when leaf surfaces were dry and before

spray application. Those of initial spray deposition were taken after the spray had dried. Leaf samples for chemical analysis were stored at −24 C until analyzed, and those for bioassay were analyzed on the day of sampling.

Chemical analysis.—Chlorothalonil was chosen because it is a standard fungicide for the control of early blight of tomatoes and because it can be easily analyzed by gas chromatography. Ten disks were extracted with 10 ml of benzene and the chlorothalonil was measured directly from 1-3 μliters of the benzene fraction or a 1/10 dilution of the fraction with a Bendix 2600 gas chromatograph. The benzene fraction was dehydrated with 1 g magnesium sulfate before analysis. For analyzing chlorothalonil, the chromatograph was equipped with a 0.6 cm × 1.8 meter (1/4 inch × 6 ft) column packed with 10% DC 200 on Anakrom ABS, 140- to 131-μm (110- to 120-mesh); the carrier gas flow rate was 80 ml per second; the column temperature was 220 C, the inlet and transfer temperature 240 C, and the detector (^{35}Ni: electron capture) 245 C. Under these conditions, chlorothalonil was detected 2.7 - 3.0 minutes after injection of solution. The amount of chlorothalonil was computed from peak height compared with that of standards which were run for each day's analyses. Sensitivity was less than 10^{-4} μg, the lowest range of interest.

Bioassay.—Foliar residues of chlorothalonil were assayed for protectant action using spores of *Alternaria solani* (Ell. & G. Martin) L. R. Jones & Growt., the cause of early blight of tomato. Spores of a uniform age, produced by the filter paper method (2) and stored in a desiccator at 4 C were brushed from the paper to leaf disks that had been placed in petri dishes over moist filter paper. A new camel's-hair brush was used each time for removing the spores to avoid contamination by old spores on used brushes. A spore density of 10-20 per 3 mm^2 of leaf surface was approximated for convenience of assay. Culture dishes were sealed with Parafilm (Marathon Products, Neersh, Wisconsin) and incubated at 25 C for 17 hours.

Ten leaf disks were examined per treatment and the number of spores and lesions per 3 mm^2 of leaf surface were counted at ×50 magnification. Noninoculated disks from the various treatments were examined for lesions from other sources and these were subtracted from the data of the lesion assay. Five disks were then stained with trypan blue in lactophenol and viewed at ×100 magnification to determine spore germination and the number that had formed appressoria. Fifty spores per disk were examined.

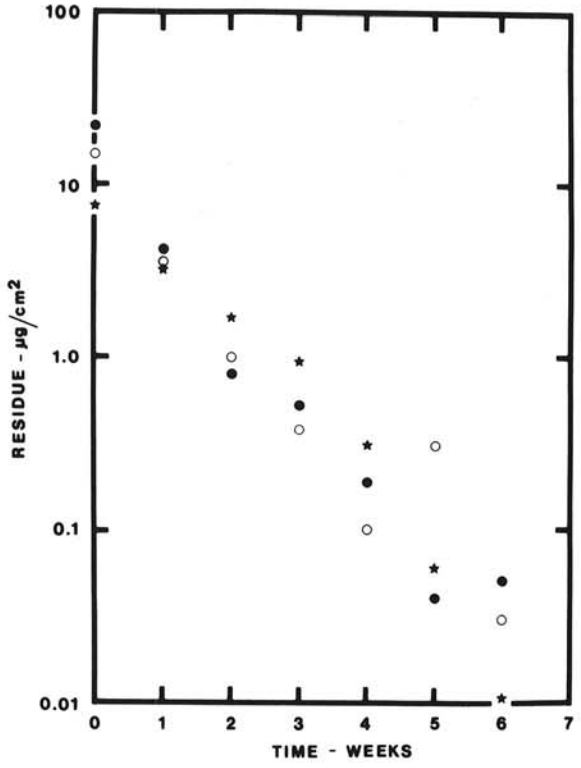

Fig. 1. Loss of chlorothalonil from tomato foliage with time. (•) = A, or newest expanded leaf, (○) = B, or mid-leaf, (*) = C, or lowest green leaf.

RESULTS

Chlorothalonil residues.—Initial deposition of chlorothalonil at the start of the experiment was 20, 15, and 7 μg/cm^2 for leaves A, B, and C, respectively. The seasonal averages of residue values on plants sprayed repeatedly at weekly intervals just before reapplication were 4, 3, and 14 μg/cm^2 for leaves A, B, and C, respectively. Individual values for leaves A and B never approached those of initial deposition whereas those for leaf C reached a maximum of 30 μg/cm^2 prior to the third spray and gradually declined to 5 μg/cm^2 at the time of the eighth spray. Occasional deposits of chlorothalonil in the range of 0.01 to 0.1 μg/cm^2 on plants in unsprayed rows indicated spray drift.

The loss of residue during the 6 weeks following a single spray application is illustrated in Fig. 1. The log concentration of residue is linear with time. When data are subjected to linear regression of log residue on time ($y = a + bX$), b values of −0.45, −0.40, and −0.43 ($P = 0.05$ for all b values) were obtained for leaf levels A, B, and C, respectively. From values of the regression coefficient b, residue was lost most rapidly on top leaves (A) and slowest on mid-level (B) leaves.

Residue levels during weeks 4-6 fell below those required for protection (see Results of the Bioassay). In addition, residue levels less than 1 ng/cm^2 are highly influenced by spray drift and redistribution. If attention is restricted to data of biological significance (weeks 0-3, Fig. 1), the loss of residue following a single spray was most rapid for the top leaves and slowest for the bottom leaves (b = 0.56, 0.53, and 0.47 for leaf levels A, B, and C, respectively). The mean residue levels (μg/cm^2) of the

TABLE 1. Correlation coefficients between variables of infection by conidia on unsprayed field-grown tomato leaves

	LP[a]	Wk	LS	A
Week of crop (Wk)	0.000			
Lesions per 100 spores (LS)	0.332	0.225		
Appressoria formed (A)	0.314	−0.201	0.564*[b]	
Spore density (SD)	−0.103	−0.466	−0.254	−0.075

[a]Leaf position on plant.
[b]Asterisk (*) indicates statistical significance ($P = 0.05$).

seven single treatments for leaves A, B, and C, respectively, were as follows: 4.3, 3.6, 3.3, one week after application; 0.8, 1.8, 1.6, two weeks after application; and 0.5, 0.4, 1.0, three weeks after application. Deviation of individual treatments from the mean did not correlate with any one factor such as rainfall, frequency of leaf wetness, plant growth, or crop age, all of which were recorded during the season.

Bioassay.—Within 17 hours of incubation at 25 C, spores had germinated, the germ tubes had formed appressoria, and the fungus had penetrated the host. At 17 hours, the epidermal cells became brown at penetration sites. Areas of one or more brown cells were counted as lesions. Effective residues of the flowable formulation of chlorothalonil did not stop spore germination, but did inhibit appressorium formation and subsequent host penetration. On glass slides, unformulated chlorothalonil inhibited spore germination at one-fifteenth the chlorothalonil dosage in the flowable formulation that inhibited appressorium formation only. Apparently, toxicity of chlorothalonil is reduced by formulation of the compound.

Appressorium formation data and lesion data were recorded for the first 3 weeks of the experiment; then collection of lesion data was discontinued because of difficulty in distinguishing between lesions and aphid feeding scars. However, a correlation of lesion and appressorium formation data on unsprayed leaves was significant (Table 1). All data from sprayed and unsprayed leaves for the first 3 weeks (60 assays) were subjected to linear regression analysis of lesion on appressorium formation to give the formula $y = -2.1 + 0.37X$ where b was significant at $P = 0.01$. Thus, lesions were produced at a rate of 1 per 2.7 spores forming appressoria, and spores forming appressoria are indicative of infection. Data on appressorium formation were recorded for the remainder of the season. As indicated by Table 1, appressorium formation was not affected by position of leaf, age or crop, or the density of spores applied to the leaf during the assay.

The percentage of spores forming appressoria on unsprayed leaves varied from 30 to 80 percent with a 10-week mean of 62 ± 11 for the three levels of leaves. Plants sprayed weekly gave seasonal means of 62, 59, and 71 percent inhibition of appressorium formation for leaves A, B, and C, respectively. Data for C were significantly different from A and B ($P = 0.05$) on the basis of the t-test.

Immediately following application of chlorothalonil, appressorium formation was inhibited 79-87 percent. This inhibition declined to 17-30 percent in 3 weeks for plants that had received one application (Fig. 2). Linear regression curves for inhibiting appressorium formation with time were: y A 86−3.2 X for A, y = 86−2.99 X for B, and y = 79−2.29 X for C. The ability to inhibit appressorium formation declined faster on the upper leaves than on those at the bottom level. Values of ED_{50}

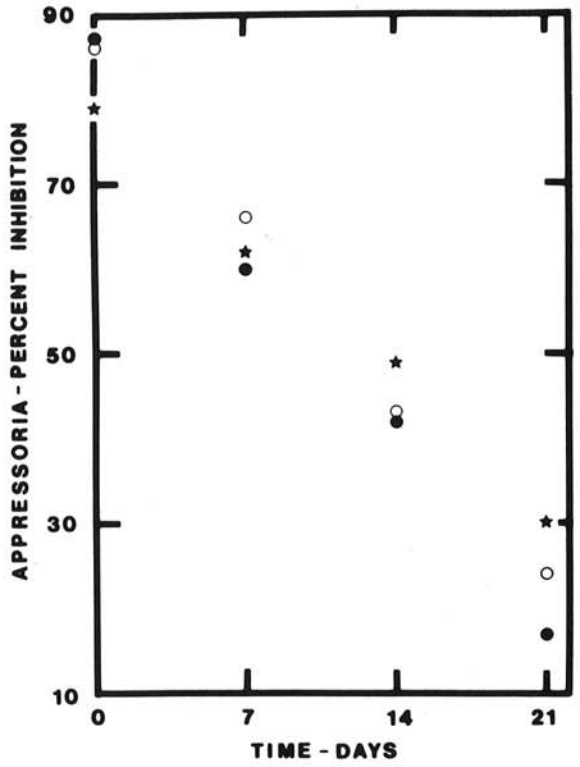

Fig. 2. Decline of protection of tomato foliage from one spray of chlorothalonil with time. (•) = A, or newest expanded leaf, (○) = B, or mid-leaf, (*) = C, or lowest green leaf. ED_{50}: A and B = 11 days, C = 13 days.

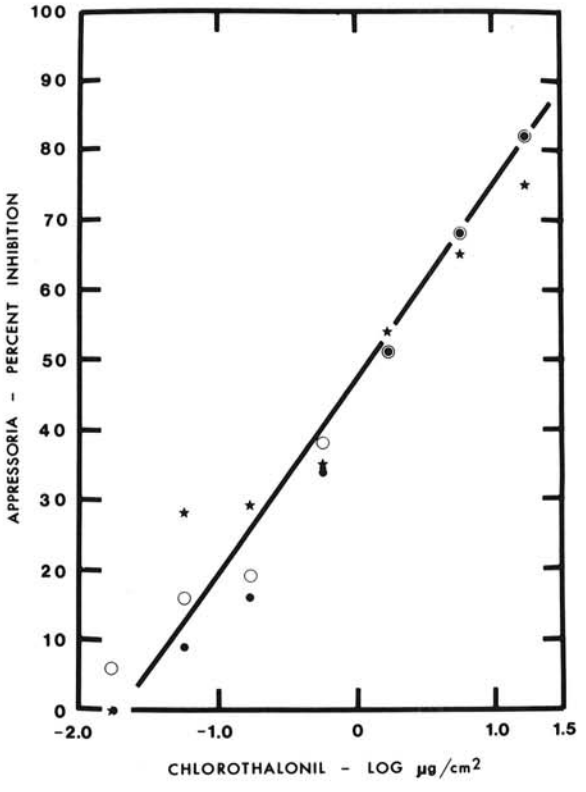

Fig. 3. Relation between chlorothalonil residue and inhibition of appressorial formation by spores of *Alternaria solani* on tomato foliage. (•) = A, or newest expanded leaf, (○) = B, or mid-leaf, (*) = C, or lowest green leaf. ED_{50}: A, B, C = 1.2 $\mu g/cm^2$.

for residues as a function of time were 11, 12, and 13 days for leaves A, B, and C, respectively.

Protection by chlorothalonil.—Residue data of 168 analyses during the season were grouped logarithmically and the means of percent inhibition of appressorium formation were computed. From these data (Fig. 3), the dosage-response curves, the ED_{50} value for inhibiting appressorium formation is 1.2 μg of chlorothalonil per square centimeter of tomato foliage.

Residue levels of chlorothalonil necessary to prevent lesions can be determined from data of the first 60 assays in which lesions were determined. Disregarding leaf position, linear regression analysis of lesions per 100 spores on log residue ($100 \times \mu g$ per cm^2) gave the curve: $y = 25-7.1\ X$. On leaves lacking residue ($X = 0$), 25 percent of the spores formed a lesion. The amount of residue to reduce this infection level in half (ED_{50}) as obtained from the regression curve is 0.56 μg of chlorothalonil per square centimeter of tomato foliage. As a direct comparison of lesion and appressorium formation by *A. solani* in the presence of chlorothalonil on the same leaf samples, linear regression of percentage of spores forming appressoria on log residue ($100 \times \mu g/cm^2$) gave the curve: $y = 68-16.2\ X$ and an ED_{50} value of 1.19 μg of chlorothalonil per square centimeter of tomato foliage.

Visual estimates of defoliation began on the 4th week of the experiment following the appearance of a few lesions of early blight on bottom leaves. Defoliation was about 2, 12, 65, and 94 percent on weeks 5, 6, 8, and 9, respectively, regardless of treatment. Because defoliation followed days of high ozone levels and blighted leaves failed to produce spores of the pathogen on incubation, we concluded that the defoliation that we saw was caused mainly by injury from air pollution rather than from early blight.

DISCUSSION

The amount of chlorothalonil residue needed to protect plants can be determined with the use of the dosage-response curve (Fig. 3). Because these data have been determined under high inoculum potential and conditions favoring rapid spore germination and infection (9), this dosage-response curve may indicate a conservative estimate of protection for a given amount of residue.

Apparently, the flowable formulation of chlorothalonil in foliar residues protects by inhibiting appressorium formation and host penetration, not spore germination, even though the unformulated fungicide can inhibit germination (7). On glass slides and formulations without plastic polymers, chlorothalonil inhibits spore germination at very low levels of residue. This apparent selective action of the flowable formulation probably occurs because the fungicide is slowly released from the residue matrix. The plastic polymers of the flowable formulation, although conveying excellent coverage and retention properties, retard the movement of chlorothalonil out of residues. Evidently, the spores germinated before accumulating lethal dosages of chlorothalonil.

The apparent low availability to the fungus of chlorothalonil in residues can explain its greater protectant action against host penetration than against appressorium formation. Considering the relative ED_{50} values in the sequence: spore germination, appressorium formation, and lesion formation, > 20, 1.2, and 0.6 μg of chlorothalonil per square centimeter of leaf surface and the comparative incubation times for half-response of 1, 3, and 8 hours (9, 10), respectively, the time for mobilization of fungicide to an active phase is longest for the last step of host penetration. Since uptake and toxic reactions of the fungicide proceed in minutes and not hours, slight differences in fungal responses with development would have little influence on the actions of chlorothalonil on the leaf surface.

Morphological considerations may explain, also, the apparent greater sensitivity of host penetration (than spore germination) to chlorothalonil residue. Proximity to the residues on the leaf surface increases with fungal development from spore, to germ tube, to appressorium. The appressorium with the greatest proximity to the plant surface may accumulate more of the available chlorothalonil from the residue matrix than the germ tube or spore and, hence, appears to be the most sensitive. The lesser amount of chlorothalonil in residues required to inhibit host penetration than appressorium formation provides an added margin of safety to appressorium formation as a bioassay of protection.

Several plant and environmental factors may cause the loss of chlorothalonil deposits. In the early season rapid leaf growth appears important for leaf A. Samples 2 weeks after application were taken from leaves that were unexpanded at time of application. Although rainfall can wash chlorothalonil from foliage (8), the effect of rain or frequency of wetting of residue cannot be identified in the present data. Log decay of residues and protection appear proportional with time.

Some build-up of fungicide was apparent during the early season on the lowest leaves of plants that received repeated sprays. Whether the increased residue on lower leaves is due to build-up from repeated sprays (6) or redistribution (8) has not been determined. Further studies are needed to measure independently the effects of leaf expansion, rewetting of residues, and washoff from the rain.

Our data that indicated a loss of protection from a spray within 11-13 days (ED_{50} values, Fig. 2) substantiate the recommendations on the label of BRAVO 6F for spray intervals of 7-10 days on tomatoes. This corresponds to residue levels decaying to 1 $\mu g/cm^2$ during the interval. The persistence data agree with those of Neely (4) who found that protection persisted an average of 2.8 weeks for chlorothalonil on foliage of 12 woody plants. The information provided here can be used to simulate a fungicide sub-program for EPIDEM (9). The simulator then can be used to determine spray timing and number of applications for delaying possible epidemics.

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