Retention of Fungicides for Control of Alternaria Leaf Blight of Muskmelon Under Greenhouse Conditions

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ABSTRACT

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Retention of chlorothalonil and mancozeb deposits on muskmelon leaf surfaces in the greenhouse was determined using a biological cellophane bioassay with *Alternaria cucumerina* as the test organism and inhibition of spore germination as the response variable. The bioassays were performed on plants exposed to wet and dry conditions. A linear relationship resulted between inhibition of spore germination and exposure time for all treatments. Differences in retention among treatments were negligible under dry conditions. However, significant differences in retention occurred after treated plants were exposed to a wet regime; the rate of loss of mancozeb was significantly greater than the loss rate of chlorothalonil. No improvement in retention occurred when a surfactant was added to the mancozeb fungicide.

Alternaria leaf blight, caused by *Alternaria cucumerina* (Ellis & Everh.) J. A. Elliot, is one of the most important foliar diseases of cucurbits in Indiana. The disease occurs primarily on muskmelon (*Cucumis melo* L. var. *reticulatus* Naudin) and has been responsible for yield reductions of nearly 50% in situations where proper management was not applied (R. X. Latin, *unpublished*). Because of the rapid spread of the disease, cultural practices such as crop rotation and fall plowing provide only partial control. Attempts to manage the disease with resistant muskmelon cultivars have not been implemented because genetic resistance to *A. cucumerina* is not available in commercially acceptable cultivars. Therefore, growers must rely on repeated applications of protective fungicides to prevent the development of serious Alternaria leaf blight epidemics.

Two of the most effective fungicides for control of Alternaria leaf blight are chlorothalonil and mancozeb. They are protective fungicides designed to provide a chemical barrier to infection by the pathogen. The barrier is subject to depletion by plant growth (2) and environmental factors such as precipitation, wind, and radiation (2,8,10). Because fungicides differ with respect to their retention on plant surfaces (6), the standard (7-day) application interval that is normally recommended may not be appropriate for certain fungicides under different environmental conditions. Application intervals that are too long result in severe epidemics, possible yield reductions, and increased pathogen populations in subsequent seasons. A season-long application program with intervals that are too short will result in unnecessary fungicide applications and increased costs to producers.

Information about the retention of fungicides on plant surfaces can be used to advise appropriate, environmentally sound application programs without risk to the crop. Therefore, the objectives of this research were to investigate the retention of chlorothalonil and mancozeb for Alternaria leaf blight control under wet and dry conditions and to suggest appropriate application schedules for each fungicide.

MATERIALS AND METHODS

Treatments were applied to seedlings of the muskmelon cultivar Superstar (Harris Moran Seed Company, Rochester, NY) that had two fully developed true leaves (4–5 wk after planting). Seedlings were raised in 7.64-cm-diameter plastic pots containing an approximate volume of 250 cm of a potting mixture consisting of sand, soil, and a peat, bark, and perlite substrate (2:1:1, v/v). Starter fertilizer was applied at transplanting and, thereafter, a compound fertilizer (Ra-Pid-Gro, Ra-Pid-Gro Corp., Dansville, NY) was added through irrigation at the rate of 2.5 g/L at 7-day intervals. Temperature in the greenhouse was maintained at 26 ± 2 C. After the first two true leaves had expanded, additional leaves were excised as they emerged. Plants were sprayed with three fungicide treatments at 10^4 ppm of active ingredient each or with deionized water as a check. The fungicide treatments included chlorothalonil (Bravo 720, Fermenta Plant Protection, Painesville, OH), mancozeb (Manzate 200 DF, E. I. du Pont de Nemours & Co., Wilmington, DE), and mancozeb plus 1.25 ml/L of spreader-sticker (Bond Surfactant, Loveland Industries, Inc., Loveland, CO). The spreader-sticker contained 45% synthetic latex, 10% primary oxyalkylated alcohol, and 45% inert ingredients.

Fungicides were applied in a custom-built spray chamber fitted with a single 80015 EVS Teejet flat fan nozzle (Spraying Systems, Co., Wheaton, IL) that delivered droplets with a volume median diameter of approximately 500 μm. An air compressor maintained a nozzle pressure of 2.11 kg/cm^2. An electromotor-driven conveyer belt moving at 3.22 km/hr moved the plants beneath the nozzle to ensure uniform coverage. The distance between the tip of the nozzle and the leaf surface was approximately 45 cm.

Each fungicide treatment was applied to three replicate plants at 1, 3, 5, 7, and 9 days before sampling. From the time the treatments were applied until the time they were sampled, plants were exposed to either a wet or dry regime. For the wet regime, plants were placed in a mist chamber that provided constant leaf wetness for 12 hr (1800–0600 hours) of each 24-hr period throughout the 1- to 9-day incubation period. Fungicides were allowed to dry for 1 hr before exposure to the wet regime. The amount of precipitation that resulted from the mist was approximately 4 cm per 12-hr wet period. Runoff occurred periodically during the 12-hr wet period after water droplets coalesced and ran off the leaf surface. For the dry regime, plants were placed on the greenhouse bench. Environmental conditions inside the mist chamber were monitored with a digital leaf wetness and temperature recorder (Datapod Model DP 223, Omnidata International, Inc., Logan, UT). The entire experiment was conducted twice.

Retention of fungicides on leaves was determined with a cellophane-transfer bioassay (5), with *A. cucumerina* (isolate 8721) as the test organism. The isolate was collected locally in 1987 from muskmelon foliage and preserved on silica gel. The inoculum was increased following the method adopted from Zhu et al (11) and adjusted to approximately 10^5 spores per milliliter for inoculation.

A cork borer (1 cm i.d.) was used to

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sample leaves from each treatment. All samples were taken 24 hr after the last treatment was applied. A sample consisted of four disks that were removed from the first two true leaves of each replicate plant. All four samples from each plant were placed in a 9-cm-diameter petri dish containing moistened Whatman No. 1 filter paper. Disks of 7-mm-diameter, nongreased, permeable cellophane 215 PD (Du Pont, Clinton, IA), prepared with a paper punch and steam-treated for 20 min, were placed individually on the leaf samples. Approximately 1 μl of a spore suspension containing 10^5 conidia of *A. cucumerina* per milliliter of deionized water was placed on each cellophane disk with a micropipet.

After 4–6 hr of incubation in the petri dishes at 26 ± 2 C, the cellophane disks were removed from the surface of the leaf samples, mounted onto glass microscope slides, and examined at ×400 magnification for spore germination. A spore was considered to have germinated if the length of the germ tube was equal to or greater than the spore diameter. Twenty spores on each cellophane disk were evaluated for germination. Percent inhibition was regressed against time (days after fungicide application) for each fungicide treatment and moisture regime. The slopes of the regression lines were used as a measure of retention for each fungicide under the different moisture regimes. Regression parameters were compared with a general test for linear equality (7).

Table 1. Regression parameters[a] for germination of *Alternaria cucumerina* conidia for three fungicide treatments after incubation under wet or dry regimes

Moisture regime	Fungicide treatment	Intercept	Slope	r^2 value
Dry	Chlorothalonil	99.9	−1.34	0.34
	Mancozeb	102.4	−1.80	0.54
	Mancozeb + sticker	100.2	−1.78	0.62
Wet	Chlorothalonil	98.2	−3.86	0.72
	Mancozeb	103.0	−9.81	0.88
	Mancozeb + sticker	101.2	−9.41	0.88

[a] Performed on combined data from two experiments.

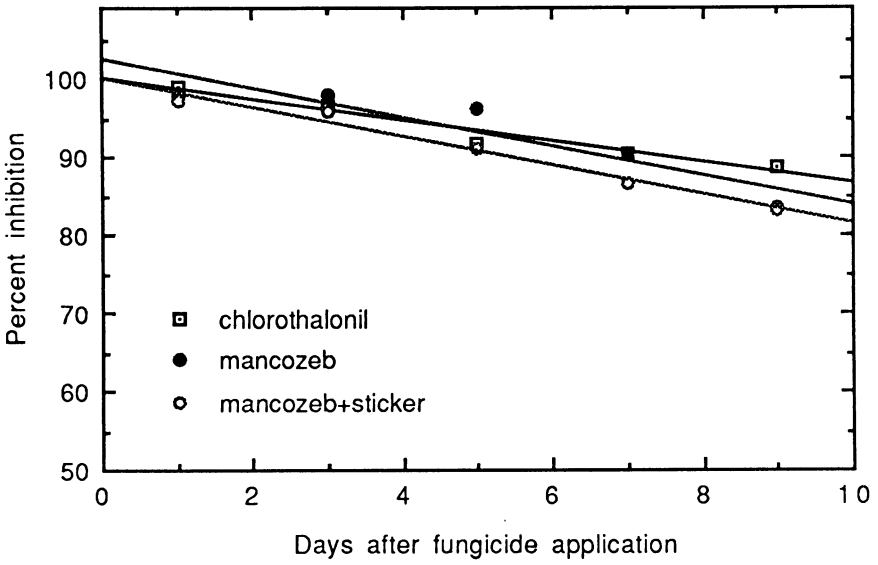

Fig. 1. Regression lines describing the amount of fungicide remaining on leaves sampled at 1, 3, 5, 7, and 9 days after application for three different fungicide treatments. Plants were maintained in a dry environment after fungicide application. Intercept and slope values for each treatment are given in Table 1.

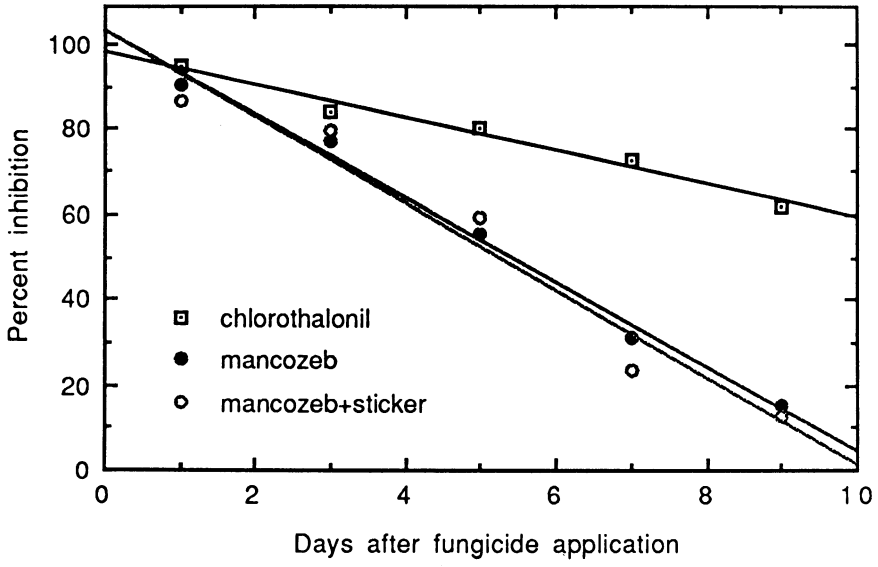

Fig. 2. Regression lines describing the amount of fungicide remaining on leaves sampled at 1, 3, 5, 7, and 9 days after application for three different fungicide treatments. Plants were exposed to 12 hr of continuous leaf wetness each day after the fungicide application. Intercept and slope values for each treatment are given in Table 1.

RESULTS

Data for both experiments were combined after a test for equality of regression lines showed that corresponding regression parameters for each experiment were not significantly different from one another ($P = 0.01$). Results of the residue bioassay demonstrated a linear relationship between increase in spore germination and time of exposure to either the dry or wet regime. The coefficients of determination for regressions of wet regime treatments ranged from 0.72 to 0.88 and indicated an acceptable fit to the linear model (Table 1). The coefficients of determination for the dry regime regressions were not as great (r^2 = 0.34–0.62), but the r^2 values were significant, and scatter and residual plots did not suggest anything other than a linear pattern. Tests for equality of regression parameters showed that the slope and intercept values for each fungicide treatment (Table 1) were not statistically different for the dry regime. Spore germination was inhibited 90% after 7 days and nearly 80% after 10 days (Fig. 1).

Under the wet regime, regression lines for both mancozeb treatments (slopes = −9.8 and −9.4 for mancozeb and mancozeb + sticker, respectively) (Table 1) were not significantly different. Mancozeb deposits remaining after 7 days of exposure inhibited spore germination only 35%; effective deposits were nearly nonexistent after 10 days (Fig. 2). The slope of the line representing the chlorothalonil treatment (slope = −3.86, Table 1) was significantly different from the mancozeb treatments. After 7- and 10-day exposures to the wet regime, the chlorothalonil deposit inhibited spore

germination nearly 72 and 60%, respectively (Fig. 2).

Tests for equality of regression lines resulted in significant differences in retention between wet and dry regimes. For each fungicide treatment, retention was significantly greater (slopes were less negative) under dry conditions than under wet conditions (Table 1).

DISCUSSION

The linear relationship between fungicide activity and time was initially unexpected and is not supported by other reports concerning fungicide retention (1,3). It is possible that the gentle misting of leaf surfaces does not remove the fungicide deposit to the same extent as rainfall, which is associated with a massive initial loss (9). The difference in the loss models also could be attributable to the nature of the response variable used to develop the model. Exponential loss models express fungicide activity in terms of micrograms per milliliter (3) or micrograms per square centimeter (1), whereas our linear model expressed activity in terms of percent inhibition determined by bioassay. By using percent inhibition to represent the fungicide deposit, there exists a maximum of 100% efficacy, regardless of the amount of fungicide on the leaf. Also, the greater sampling intervals (7 days) used by Ko et al (3) may have contributed to the curvilinear fungicide loss pattern that resulted from their research.

Given that the two fungicides are comparable in dry conditions, it is possible that the longer retention of chlorothalonil under wet conditions contributes substantially to its superior performance in the field (4). Although the data presented in this study show that the selected spreader-sticker had no measurable effect on fungicide performance, there are no published data that support the greenhouse study. However, personal observations of mancozeb treatments with and without the surfactant in commercial fields support the need for further investigation to determine whether growers actually realize the intended benefit.

Our research supports the need for quantitative field comparisons of fungicides and application intervals for control of Alternaria leaf blight. Greenhouse results suggest that to achieve a comparable level of protection, mancozeb fungicides should be applied at shorter intervals than chlorothalonil when weather favorable for disease persists. The shorter application interval for mancozeb may be necessary because the same conditions that remove fungicide deposits from leaves also favor infection by *A. cucumerina*.

Interpretation of the regression lines in Figure 2 shows that under the very favorable conditions of 12 hr of continuous leaf wetness, percent inhibition for mancozeb falls below 70% after 3 days, whereas chlorothalonil maintains a percent inhibition of 70% or greater for 7 days. Also, 50% inhibition occurs at 5 and 13 days for mancozeb and chlorothalonil, respectively. Under conditions favorable for disease, mancozeb fungicides probably should not be applied at intervals greater than 5 days. Increasing the application interval for chlorothalonil to 13 days would not be advisable because other factors, particularly host growth, will increase the proportion of unprotected foliage, leaving the crop more vulnerable to infection. Research is underway to incorporate fungicide retention into a weather-based decision rule for fungicide applications for Alternaria leaf blight control.

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