

### A Simple Method for Determining Efficacy and Weatherability of Fungicides on Foliage

W. H. Ko, Hsin-hsiung Lin, and R. K. Kunimoto

Associate Professor and Research Associates, respectively, Department of Plant Pathology, University of Hawaii, Beaumont Agricultural Research Center, Hilo 96720. Present address of second author: Taiwan Federation of Fruit Marketing Cooperatives, Taipei.

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#### ABSTRACT

Efficacy of fungicides was determined directly by observing with a vertical-illumination microscope the germination of fungal spores on detached leaves sprayed with various concentrations of fungicides. Weatherability of a fungicide under field conditions was studied by measuring spore germination on leaves at various time intervals after fungicide application. This simple and rapid technique should prove useful for determining fungitoxicity of chemicals on host tissues in a primary screening program, and for studying retention of fungicide deposits under field conditions.

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*Additional key words:* apparent half-retention time, *Alternaria alternata*.

Testing germination of fungus spores in the presence of chemicals is the classic technique for determining fungitoxicity in a primary screening program. This principle led to the development of the standardized slide-germination method (1) and test tube dilution technique (2) by The American Phytopathological Society. Although the laboratory methods have been extensively used in the initial evaluation of fungicides, the recent trend is toward the use of plants or plant parts which approximate natural conditions (6, 9). Previous foliage tests are based on the development of disease symptoms on inoculated leaves, and the procedure is very time-consuming. We report herein a simple and rapid method for determining efficacy of fungicides on foliage, and the application of this method to determination of weatherability of fungicide deposits under field conditions.

**MATERIALS AND METHODS.**—Conidia of *Alternaria alternata* (Fries) Keissler, *Helminthosporium maydis* Nisik. & Miyake, *Glomerella cingulata* (Ston.) Spauld. & Schrenk, *Botrytis cinerea* Pers. ex Fr., and *Penicillium frequentans* Westling, and sporangiospores of *Mucor ramannianus* Möller were produced by growing each under continuous fluorescent light for 10 days at 24 C on V-8 juice agar. *Helminthosporium maydis* was supplied by M. Aragaki. Zoospores of *Phytophthora palmivora* Butler were obtained as described previously (5). Spore concentration was determined by the microsyringe method (5).

Fungicides used were: *cis-N*[(1,1,2,2-tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide 30% (Difolatan 4F); tetrachloroisophthalonitrile 50% (Bravo

6F); and a coordination product of zinc ion and manganous ethylenebisdithiocarbamate 80% (Dithane M-45). The effect of fungicides on spore germination in water was studied by adding one drop (approximately 0.04 ml) of spore suspension to 2 ml of fungicide suspension in a small petri dish (60 × 15 mm). Spore germination was counted with a standard compound microscope after 24 hours of incubation at 24 C. The vertical-illumination microscope technique (4) was used to study the effect of fungicides on spore germination on leaves. Young detached leaves were sprayed with different rates of fungicides with an atomizer. After being air-dried for about 1 hour, spore suspensions were sprayed onto treated leaves which were then incubated in a moist chamber. The moist chamber consisted of a large inflated polyethylene bag with six pin-size vent holes. Moist air was continuously pumped into the bag. After 24 hours of incubation, a cut portion of leaf (approximately 30 × 10 mm) was mounted on a glass slide by placing cellophane adhesive tape on both ends of the leaf piece. Spore germination on leaves was counted directly with a Zeiss Universal Microscope equipped with a Model II C vertical illuminator and a heat reflection filter to reduce the heat generated. Large spores, like conidia of *H. maydis* and *A. alternata*, were observed without staining with a ×40 Epiplan objective. Smaller spores (< 15 × 5 μm) of the other fungi were stained with rose bengal (1% rose bengal, 5% phenol, and 0.01% CaCl<sub>2</sub>) before observation with an ×80 Epiplan objective. The experiments were repeated twice in triplicate.

Weatherability tests were done at Malama-Ki Field Station on the island of Hawaii. Four young, but fully expanded, leaves from each of six passion fruit vines for each treatment were sprayed with 100 μg/ml of Difolatan 4F or Bravo 6F until run-off occurred. Percentage germination of *A. alternata* on passion fruit leaves with 100 μg/ml of either compound was approximately zero. Each week, the oldest leaf from each of the six vines of each treatment was harvested, and spore germination of *A. alternata* on leaves was tested in the laboratory as described above. A dosage-response curve for each fungicide was obtained by testing spore germination of *A. alternata* on passion fruit leaves sprayed with various concentrations of the fungicide. Germination on the treated leaves was calculated as a percentage of that on untreated controls which averaged 84%. The concentration of each fungicide on leaves under field conditions at various time intervals was obtained by converting percentage germination to fungicide concentration using the dosage-response curve. Apparent half-retention time was defined as the period during which half of a fungicide on leaves was lost as determined by the germination test.

**RESULTS AND DISCUSSIONS.**—*Alternaria alternata* is the prevalent *Alternaria* sp. which causes leaf brown spot, the most important disease of passion fruit (*Passiflora edulis* f. *flavicarpa* Degener) in Hawaii (3). Therefore, this combination of host and pathogen was selected for each study unless otherwise stated. Conidia of *A. alternata* on passion fruit leaves were clearly observed, and germination was counted directly under the vertical-illumination microscope. The optimum concentration of conidia for observation was  $8 \times 10^3$ /ml for *A. alternata*. The percentage germination of spores on leaves was

inversely correlated with the concentration of fungicides applied. For example, the *A. alternata* conidia germinated 100, 75, 63, 30, 22, 4, and 3% on passion fruit leaves sprayed with 0, 5, 10, 25, 50, 100, and 200  $\mu\text{g}/\text{ml}$  of Difolatan 4F, respectively. All three fungicides tested were less effective against spore germination of *A. alternata* on passion fruit leaves than in water. The  $\text{ED}_{50}$  of Difolatan 4F, Bravo 6F, and Dithane M-45 was 0.38, 0.3, and 0.23  $\mu\text{g}/\text{ml}$ , respectively, in water; and 15, 30, and 180  $\mu\text{g}/\text{ml}$ , respectively, on leaves.

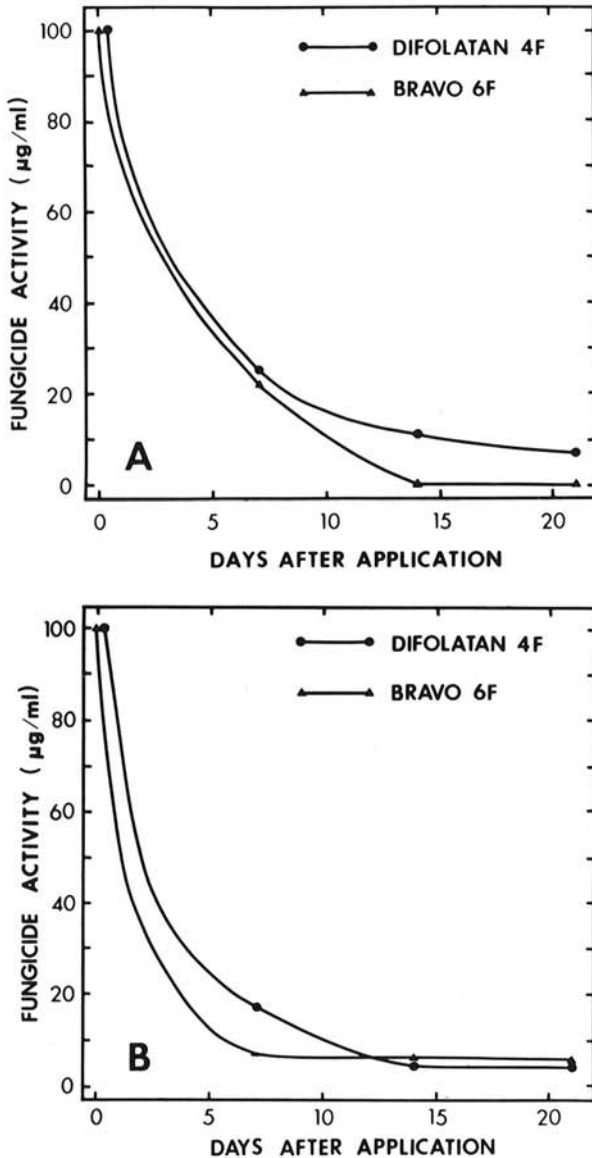


Fig. 1-(A, B). Activity of Difolatan 4F and Bravo 6F on passion fruit leaves in the field after different periods of application. A) The total rainfall during the test period was 8 cm. B) Total rainfall during the test period was 16 cm. Concentration of each fungicide was obtained by converting percent germination of *Alternaria alternata* conidia to fungicide concentration using the dosage-response curve.

Leaves of the following crops were collected from fields to determine whether the same technique could be applied to other plant species: string bean (*Phaseolus vulgaris* L.), soybean [*Glycine max* (L.) Merr.], pea (*Pisum sativum* L.), sweet potato [*Ipomoea batatas* (L.) Lam.], peanut (*Arachis hypogaea* L.), spinach (*Spinacia oleracea* L.), and lettuce (*Lactuca sativa* L.). Conidia of *A. alternata* were clearly observed on leaves of all test plants.

Spores of different sizes were also sprayed onto passion fruit leaves to determine whether the technique could be applied to other fungi. Conidia of *H. maydis* were clearly observed with the  $\times 40$  Epiplan objective without staining because they were relatively large. Conidia of *G. cingulata*, *B. cinerea*, and *P. frequentans* could be clearly observed with the  $\times 80$  Epiplan objective after staining with rose bengal. Staining with rose bengal also allowed sporangiospores of *M. ramannianus* to be observed easily even though the stain did not penetrate these spores.

To determine whether the technique was applicable to fruit, conidia of *G. cingulata* were sprayed onto banana fruit (8) and zoospores of *P. palmivora* were pipetted onto papaya fruit (7). Cut pieces of fruit rind ( $20 \times 10 \times 3$  mm) were observed after staining. Both types of spores were clearly observed with the  $\times 80$  Epiplan objective with staining.

The activity of both Difolatan 4F and Bravo 6F against germination of *A. alternata* spores decreased with time of exposure in the field and retention time was affected by rainfall (Fig. 1). The apparent half-retention time of Difolatan 4F and Bravo 6F on passion fruit leaves was 3 and 2.6 days, respectively, when the total rainfall during the 3-week test period was 8 cm. However, when the total rainfall was 16 cm, the apparent half-retention time decreased to 2 and 1.2 days for Difolatan 4F and Bravo 6F, respectively.

With the vertical-illumination microscope it was possible to count spore germination directly on host leaves. This simple and rapid technique should prove useful for determining fungitoxicity on host tissues in a primary screening program. It also provides an easier and less cumbersome method than the chemical analysis method for determining the weatherability of fungicide deposits under field conditions.

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