Effects of Sprayer Configuration on Efficacy for the Control of Scab on Crabapple Using Electron Beam Analysis

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Summary: Foliar diseases like apple scab result in significant economic losses to growers each year. Assessment in past studies involved only macroscopic disease ratings. More complete knowledge of the fate and behavior of fungicide has been needed to reduce pesticide use with less off-target contamination. Field studies were conducted in a production nursery for over 4 years. A moderately susceptible cultivar of ornamental crabapple, Malus spp. cv “Candied Apple”, was sprayed with a fungicide using two sprayer/nozzle configurations. The fungicide used in this study was Mankocide, combination of Cu(OH)₂ and mancozeb that permitted electron beam analysis (EBA) identification based on the presence of Cu, MN and Zn in the molecule and formulation. EBA was conducted using a cold field emission scanning electron microscopy and energy dispersive x-ray microanalyzer. Fresh leaf samples were placed on sticky stubs after each fungicide treatment. The presence or absence of fungal conidia and fungicide residue were measured. EBA permitted direct visualization and identification of the pathogens, morphologically, and chemical characterization of fungicide present. EBA was useful to quantify disease control related to fungicide coverage, sprayer configuration and treatment efficacy. SCANNING 31: 24–27, 2009. ¹Published 2009 by Wiley Periodicals, Inc.

Key words: fungicide, apple scab disease management, cold field emission scanning electron microscopy, X-ray microanalysis, spray deposition, nursery crops

Introduction

Production of woody environmental and floral crops represents over 12% of American agricultural receipts. Unfortunately, fungus diseases like apple scab caused by Venturia inaequalis result in millions of dollars of nursery crop losses each year. Effective fungicides must be applied to produce esthetically pleasing plants. New guidelines for registering, using and maintaining pesticides through the U.S. Environmental Protection Agency were created by the Food Quality Protection Act of 1996 and require information on how pesticides are used. Research is crucial on fungicide spray methods related to efficacy. Assessment in past studies involved only macroscopic disease ratings (Chatfield et al. 1996). More complete knowledge of the fate and behavior of fungicide will lead to reduced pesticide use with less off-target deposition (Krause and Derksen 2000; Krause 2000; Krause et al. 2003). The purposes of this study were: devise a technique to directly evaluate and correlate the fungicide coverage with the amount of apple scab disease; to assess the effects of sprayer/nozzle type on efficacy and disease management in production nurseries.

Materials and Methods

Plant Materials

Dedicated research plots at a production tree nursery consisting of six crabapple replication rows with three treatments/replications each were planted. A replication consisted of seven, 2-year-old
crabapple whips, *Malus* spp., using a moderately susceptible cultivar, ‘Candy Apple’. Barrier rows of other landscape tree species were also planted between crabapple treatment rows to prevent fungicide spray drift.

**Treatments**

Treatments were randomized in each of six rows as follows: (a) DW, an axial flow, airblast sprayer with conventional-high volume nozzle delivering 300 psi, traveling at 4 mph; (b) CF, experimental air curtain sprayer or cross-flow fan sprayer with air induction nozzles delivering 120 psi at 4 mph; (c) unsprayed control. Electron beam analysis (EBA): a combination of scanning electron microscope and energy dispersive x-ray analysis (EDXA). A cold field emission scanning electron microscope, Model 4700, Hitachi High Technologies America, Pleasanton, CA equipped with EDXA, Model Voyager II, (Thermo-Noran, Madison, WI) with a high-angle detector was used. EBA equipment was located at the Molecular and Cellular Imaging Center in the Ohio Agricultural Research and Development Center, Wooster. Operational conditions were: 10 kV acceleration voltage at 12 mm working distance.

**Bioassay and leaf analysis.** Leaves were collected from each treatment following each spray, mounted on stubs with conductive sticky tabs (Ted Pella, Tustin, CA) for bioassay and EBA. EBA permitted direct visualization of 3 fields per sample, and morphological identification of the pathogens, and chemical characterization of any fungicide present. The fungicide used in this study was Mankocide, a combination of the fungicides, Cu(OH)2 and mancozeb, that permitted EBA identification based on the presence of Cu, Mn and Zn in the molecule. Disease incidence and fungicide deposition were noted and recorded during spray treatments.

**Weather Stations**

A portable meteorological station (Campbell Scientific Instruments) was installed within the experimental plot to remotely monitor air temperature, leaf wetness, relative humidity, wind speed and direction, rainfall and solar radiation, according to guidelines of the United States National Oceanic and Atmospheric Administration (NOAA).

**Results and Discussion**

During the three of the four growing seasons, little apple scab disease symptoms were macroscopically observed. Free moisture on leaf surfaces was lacking, as recorded by the meteorological station using leaf wetness sensors, indicating that conditions were not conducive for disease development. Subsequent years of the study yielded an increase in disease as noted macroscopically with typical symptoms of olive drab lesions and defoliation (Table 1).

EBA of leaf samples observed during the early portion of the year confirmed the lack of the fungal pathogen (conidia) (Fig. 1(a)). Bar marker indicates 10 μm. Flame-shapes conidia (CON) verified the presence of the pathogen on the leaf surfaces *Malus* spp. cultivar. EDXA confirmed the absence of Cu, Mg and Zn, the constituents of the fungicide spray residue (Fig. 1(b)). Even when limited disease occurred on the crabapples in the spring of the first 2 years, EBA confirmed the presence of the pathogen despite the lack of visible symptoms. The lack of free moisture did not produce significant fungal infection. While fungicide coverage on sprayed leaves was observed with EBA, low disease pressure did not permit quantification of differences in efficacy in subsequent years.

EBA confirmed the presence of the pathogen (CON) and fungicide residue (arrows) was noted as in Figures 2(a) and 3(a) on leaves sprayed with CF and DW. Bar markers indicate 1 μm. EDXA confirmed the presence of Cu, Mg and Zn on leaves sprayed with fungicide as shown in Figures 2(b) and 3(b).

**Conidial Presence on Leaf Tissue (Percent of Leaves Infected)**

Application of fungicide had mixed results. The CF (cross-flow sprayer) appeared to yield decreased percentage of conidia found on leaf samples whereas the DW had no effect as the season lengthened (Fig. 3).

<table>
<thead>
<tr>
<th>Sprayer</th>
<th>Fungal Conidia Mean1</th>
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<tbody>
<tr>
<td>Control(4)</td>
<td>46.86a</td>
</tr>
<tr>
<td>CF(1)</td>
<td>11.36b</td>
</tr>
<tr>
<td>DW normal(2)</td>
<td>30.71ab</td>
</tr>
<tr>
<td>LSD = 21.65</td>
<td></td>
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Analysis of numbers of conidia on plants treated with various sprayers as were detected with CFE-SEM. Numbers of fungal conidia on leaves of plants treated with the cross-flow fan (CF) sprayer were significantly less than plants treated with either air blast (DW) sprayer.

1Numbers with the same letters do not differ significantly.
The percent of leaves infected with fungal conidia observed with EBA on the control treatment continued to climb after a leaf wetness period in May. The percentage of leaves infected with conidia fluctuated with the CF sprayer revealing the lowest percentage of infected leaves (Fig. 4). In other words, the percent of leaves infected with fungal conidia as detected with EBA on crabapple cv “Candy Apple” leaf samples, the cross-flow fan sprayer (CF) treatment appeared to reduce the
Fig 4. The percent of leaves infected with fungal conidia as detected with EBA on crabapple “Candy Apple” leaf samples, the cross-flow fan sprayer (CF) treatment appeared to reduce the amount of conidia found on leaf samples while the airblast sprayers (DW) had no effect as the season lengthened compared with unsprayed controls.

amount of conidia found on leaf samples whereas the airblast sprayers (DW) had no effect as the season lengthened compared with unsprayed controls. Statistical analysis of the samples showed the cross-flow samples had significantly less fungal conidia present than the control, but not significantly different from the other treatments as shown in Table 1.

This is a technique paper to be used in the development of experimental methods essential for studies of apple scab disease. The use of fungicides as tracers for EBA was developed as part of the analytical protocol for studying spray efficacy. Specimen handling technology was also developed for subsequent studies. Techniques developed in this study will be adapted for assessment of other disease management methods. EBA was useful to microscopically quantify and observe disease control related to fungicide coverage, sprayer configuration and treatment efficacy. Improved knowledge of the basis of efficacy and coverage will improve grower profitability; protect farm workers, enhance consumer confidence in crop safety and reduce the impact of fungicides on the environment.

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