Evaluation of a Microemulsion Formulation of Fenarimol for the Control of Apple Scab Caused by *Venturia inaequalis*

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ABSTRACT

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Microemulsion (ME) and emulsifiable concentrate (EC) formulations of fenarimol were equally effective in greenhouse trials in controlling apple scab (caused by *Venturia inaequalis*) when applied 48, 72, 96, or 120 hr after inoculation. The percent of surface area affected with chlorotic and sporulating lesions did not differ significantly between the two formulations at 10, 20, or 30 μg a.i./ml. There was a significant positive linear relationship between time of fungicide application after inoculation and percent of leaf surface covered with sporulating lesions or chlorotic flecks. Uptake of ^{14}C-label from radiolabeled fenarimol from leaves treated with the EC formulation was significantly greater than uptake of the label from leaves treated with the ME formulation at 10 and 30 μg a.i./ml. Autoradiographs indicated that leaves on seedlings treated with ^{14}C-labeled fenarimol in the EC formulation had greater radioactivity than those treated with the ME formulation. Fenarimol movement was acropetal and some ^{14}C-label of both formulations could be detected in the four or five leaves immediately above the treated leaf.

Formulations can greatly influence the biological activity of a fungicide by directly affecting activity of the active ingredient, or the formulation ingredients may be biologically active (1). Most fungicides used for foliar disease control are formulated as wettable powders, flowables, dry flowables, and emulsifiable concentrates (EC).

Microemulsions (ME) are thermodynamically stable, translucent systems of oil, water, and surfactants (4). They are widely used in commerce for such products as mouthwash, shaving lotion, dry cleaning fluid, deodorizers, cosmetics, cleaners, waxes, etc. (4). However, they have not been widely used for agricultural purposes. ME have the advantage of being thermodynamically stable systems with an emulsion droplet size of 0.01 μm or less. Problems encountered with other formulations such as sedimentation, flocculation, and creaming do not occur. Furthermore, Urton (5) demonstrated that an insecticide formulated as an ME is more active than other formulations of the same insecticide.

Presently, the only pesticide formulated as an ME and widely used in commercial agriculture is the herbicide butylate + atrazine (Sutan+, ICI Americas, Inc., Agricultural Products, Wilmington, DE). The insecticides dursban and diazinon are also formulated as an ME, but are registered only for use by pest control operators. No fungicide currently registered for use in the United States is formulated as an ME.

Fenarimol (Rubigan, Eli Lilly Corp., Indianapolis, IN), a demethylation-inhibiting fungicide, is sold as a 1 EC (1 lb a.i./gal [120 mg a.i./ml]) for the control of such diseases as apple scab (caused by *Venturia inaequalis* (Cke.) Wint.) and apple powdery mildew (caused by *Podosphaera leucotricha* (Ell. & Ev.) Salm.). The objective of this research was to compare EC and ME formulations of fenarimol in regard to plant uptake and biological activity. Apple scab was used as the model system for this study.

MATERIALS AND METHODS

Postinfection study. Seedlings of *Malus* × *domestica* Borkh. 'Delicious' were inoculated by atomizing them with a suspension of conidia of *V. inaequalis* (5×10^5 conidia/ml) prepared by washing naturally infected leaves with distilled water. The youngest expanded leaf on each seedling was tagged and identified as the reference leaf. Immediately after inoculation, seedlings were enclosed in a plastic bag containing two moist laboratory towels. Seedlings were kept in the dark at 20 C for 48 hr. They were then unbagged and moved to a humidity chamber in the greenhouse. The humidity chamber consisted of a wooden frame 3 m long × 2 m wide × 2 m tall covered with burlap on each side and cheesecloth on top. High relative humidity (>90%) was maintained in the chamber by wetting the burlap periodically. In addition, two humidifiers were placed at each end of the chamber and operated for 5 min every 30 min. Temperature and relative humidity were measured in the chamber with a hygrothermograph.

At 48, 72, 96, and 120 hr after inoculation, groups of four seedlings were sprayed to runoff with 10, 20, or 30 μg a.i. fenarimol/ml formulated as an EC or ME. Data were recorded 16 days after inoculation by estimating the percent of leaf surface covered with chlorotic flecks and sporulating lesions. These parameters were estimated for the reference leaf and two leaves immediately below and above the reference leaf.

The experimental design was a randomized complete block and the experiment was repeated once. Treatments were arranged as a two (fungicide formulations) × three (fungicide rates) × four (times after inoculation) factorial plus an untreated control. Data were analyzed with an analysis of variance that included appropriate subdivisions of the treatment variation into main effects and interaction sources. Polynomial curve-fitting was employed for the rate and time factors. To critically examine the fungicidal effects, data were also analyzed with the exclusion of the nonsprayed check, which had a different variance from the other treatments.

Uptake of ^{14}C-fenarimol. Technical grade fenarimol and ^{14}C-fenarimol (specific activity, 20.4 μCi/mg) were formulated as an EC (121.1 g of fenarimol per liter) or an ME (131.0 g of fenarimol per liter) stock solution according to instructions provided by the manufacturer. The stock solutions were diluted with water to obtain working solutions of 10 (0.1 μCi/ml) and 30 (0.3 μCi/ml) μg of fenarimol per milliliter.

All studies were conducted on approximately 6-wk-old vigorously growing Delicious seedlings. Seedlings

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were sprayed twice with triadimefon (0.3 mg a.i./ml) for powdery mildew control. However, no application was made within 7 days of the study. Trees were maintained in the laboratory at 18–20 C following application of the ^{14}C-fenarimol.

Fungicides were applied with a micropipette in 10-μl droplets to the adaxial surface of the youngest expanded leaf on each plant. Five droplets were applied to each leaf. Each drop of the 10-μg/ml solution contained 0.001 μCi, and each drop of the 30-μg/ml solution contained 0.003 μCi of ^{14}C-fenarimol. At 0, 5, 15, 30, and 60 min and 24 hr after application, treated leaves were cut from the plants and washed in 10 ml of 95% ethanol for 1 min to remove the fungicide residue. Preliminary tests indicated this procedure removed 95–98% of the fungicide applied to the leaf surface. Two milliliters of the ethanol wash were then added to 10 ml of scintillation fluid (Scinti Verse II, Fisher Scientific Co., Fair Lawn, NJ) and counted for 10 min in a Beckman LS7500 liquid scintillation counter (Beckman Scientific Instrument Div., Irvine, CA). After washing, the fungicide-treated leaves were oxidized by combustion in the presence of oxygen in a biological oxidizer. The $^{14}CO_2$ was trapped in 20 ml of Oxifluor-CO_2 (New England Nuclear, Boston, MA), and the radioactivity was counted. Radioactivity was corrected for the efficiency of the oxidizer and quenching. Uptake of fenarimol was expressed as percentage of radioactivity applied to the surface that was detected in the washed leaves.

A completely random experimental design was used. Treatments were arranged as a two (formulations) × two (rates) × six (sample times) factorial. Each treatment was replicated three times and the experiment was repeated once.

Autoradiography. Five 10-μl drops, each containing 0.3 μg (3 nCi) of fenarimol in either EC or ME formulation, were applied to the adaxial surface of the youngest expanded leaf of each seedling as previously described. The treated seedlings were maintained at 25–28 C in a greenhouse for 7 days, and the upper 20-cm sections of the seedlings were excised and pressed for autoradiography. Autoradiography was made with Kodak XAR diagnostic film (Eastman Kodak Co., Rochester, NY) at −80 C for 1 mo.

RESULTS

Postinfection study. Scab infection was generally most severe on the reference leaf and the leaf immediately above it. Therefore, data from these leaves were used in all analyses. Mean temperature in the humidity chamber averaged 22.3 and 24.2 C for runs 1 and 2, respectively.

There was no significant difference between the two formulations of fenarimol when averaged over all rates and application times. Both formulations significantly reduced the amount of infection and sporulation in comparison with the unsprayed check (Table 1).

There was no significant difference between the three rates of each fungicide formulation when the analysis was performed without the unsprayed check (Table 2). The relationship between the percent sporulation and the rate of the ME formulation was linear and negative ($P = 0.01$). There was no significant linear or quadratic relationship between the rate of the EC formulation and the percent sporulation or between the rates of the EC and ME formulations and the percent of surface area affected. Time of application, when averaged over formulations and rates, was not significant for the percent sporulation (Table 3). However, time was significant for the percent of leaf area affected; the relationship between the percent of leaf area affected and the time of application was linear and positive ($P = 0.01$). The time × formulation interaction was nonsignificant for both variables, indicating that both formulations provided similar postinfection activity over time of application after inoculation.

Although run was not a treatment factor, the run variation was examined as an indication of possible environmental effects. Run as a main effect was not significant. However, the run × formulation interaction was significant for both variables. In run 1, the percent of leaf area infected was less with the EC formulation when averaged over all other factors (4.71 vs. 7.22% for the EC and ME, respectively); in run 2, the ME formulation was more active (7.38 vs. 4.41% for the EC and ME, respectively). Sporulation was suppressed to a greater extent by the EC formulation in run 1 (0.30 vs. 1.09% for the EC and ME formulations, respectively); in run 2, the ME formulation was more effective (0.095 vs. 0.41% for the ME and EC formulations, respectively). No other interaction terms with run were significant.

Uptake of ^{14}C-fenarimol. ^{14}C-label recovered from leaves treated with the EC formulation was significantly greater ($P = 0.01$) than the ME formulation at both 10- and 30-μg/ml rates. Very little uptake of fenarimol in either formulation was detected in the leaves within 15 min (Fig. 1A,B). At 30 min, approximately 1–2% of the ^{14}C-label was recovered from leaves treated with either material. However, after 1 hr, significantly more ^{14}C-label was recovered from leaves treated with the EC formulation than with the ME formulation ($P = 0.05$) (Figs. 1 and 2). The amount of ^{14}C-label recovered from leaves treated with the ME after 24 hr did not differ from the amount recovered after 1 hr. There was no difference ($P = 0.05$) in the amount of ^{14}C-label recovered after 1 or 24 hr with the EC formulation applied at 10 μg of fenarimol/ml, but significantly more ^{14}C-label was recovered after 24 hr

Table 1. Effect of the formulation of fenarimol on the percent of surface affected with sporulating lesions or chlorotic flecks of *Venturia inaequalis* and the percent sporulating lesions

Formulation	Surface affected (%)[x,y]	Sporulating lesions (%)[y,z]
Emulsifiable concentrate	6.18	0.36
Microemulsion	5.95	0.62
Control	52.75	38.37
LSD_{05} comparing control mean with formulation mean	6.08	3.06
LSD_{05} comparing two formulation means	3.25	1.63

[x] Percent of leaf surface covered with chlorotic and sporulating lesions.
[y] Mean percent averaged over all rates (10, 20, and 30 μg/ml) and times after inoculation (48, 72, 96, and 120 hr).
[z] Percent of leaf surface covered with sporulating lesions.

Table 2. Effect of the rate of fenarimol on the percent of surface area affected by *Venturia inaequalis* and the percent sporulating lesions

Rate (μg/ml)	Surface affected (%)[x]	Sporulating lesions (%)[y]
10	6.79[z]	0.55[z]
20	5.62	0.37
30	6.09	0.35

[x] Percent of leaf surface covered with chlorotic and sporulating lesions.
[y] Percent of leaf surface covered with sporulating lesions.
[z] Mean percent averaged over all times after inoculation (48, 72, 96, and 120 hr) and both formulations.

Table 3. Effect of the time after inoculation on the percent of leaf surface affected or percent sporulating lesions of *Venturia inaequalis* on apple leaves treated with fenarimol

Time after inoculation (hr)	Surface affected (%)[w]	Sporulating lesions (%)[x]
48	3.39[y,z]	0.53
72	4.54	0.47
96	6.39	0.26
120	9.76	0.69

[w] Percent of leaf surface covered with chlorotic and sporulating lesions.
[x] Percent of leaf surface covered with sporulating lesions.
[y] Mean percent averaged over all formulations and rates.
[z] Significant ($P = 0.05$) linear relationship between the time after application and the percent of surface area affected.

in leaves treated with 30 μg of fenarimol/ml.

Translocation of ^{14}C-fenarimol. Autoradiographs showed that highest intensities of radioactivity were detected at the sites of application. Although translocation of fenarimol from the treated leaf to other parts of the seedling was minimum, radioactivity was detected in the petioles and main veins of younger, but not older, leaves (Fig. 2). Autoradiographs further indicated that leaves on seedlings treated with ^{14}C-fenarimol in the EC formulation (Fig. 2B) had higher radioactivity than those treated with the same compound in the ME formulation (Fig. 2D).

DISCUSSION

Although uptake of the ME formulation of fenarimol was significantly less than the EC formulation, the postinfection activity of the two formulations was similar. Both formulations significantly reduced the percent of surface area affected with either chlorotic flecks or sporulating lesions when compared with the unsprayed control. The postinfection activity of the ME formulation may be related to greater biological activity. Urton (5) reported that an ME formulation of pyrethrin was 10–100 times more active than that of conventional emulsions. He suggests that the increased efficacy was due to smaller emulsion droplet size, "greater penetrability, increased stability, greater specificity as a result of protection from reaction with the environment and greater adhesion to plants." However, in our study, the penetrability of apple leaves with the ME formulation was less than with the EC formulation.

The three rates of the two formulations of fenarimol were effective as postinfection treatments. The current labeled rate for dilute orchard applications of the EC formulation is 10.63 μg/ml, which is slightly above the lowest rate that we tested. Environmental factors such as temperature have been shown to influence the activity of the EC formulation (2) and should be studied in relation to the ME formulation. In addition, the protectant activity of the two formulations needs to be compared.

Both formulations of fenarimol demonstrated similar postinfection activity with time after inoculation. However, there was an increase in the percent of surface area affected by time after inoculation. Acceptable control of apple scab was achieved up to 120 hr after inoculation. However, previous studies have indicated that postinfection control is poor after 72 hr (3). Differences found in the degree of postinfection control between studies may be related to the environmental conditions under which the tests were conducted, the cultivar used, or the method of fungicide application.

There were some differences between the two runs of the greenhouse experiment. In one run, the EC formulation appeared more active when the percent of surface area affected was assessed, and the ME formulation was more active on the percent sporulating lesions. Although differences in the two runs were not great, they may be due to temperature differences or differences in the physiological condition of the seedlings. O'Leary and Jones (2) have shown that uptake of the EC formulation is affected by temperature.

The general pattern of uptake of the ^{14}C-label with time that we observed was similar to that reported by O'Leary and Jones (2) for the EC formulation. The rapid uptake of the commercially available EC formulation suggests that activity in the orchard would be retained if rainfall occurred after only 1–2 hr of drying. There was also, proportionally, a greater amount of ^{14}C-label absorbed when applied at 30 μg/ml as opposed to 10 μg/ml. This also confirms the report of O'Leary and Jones (2) who found increased uptake with increased

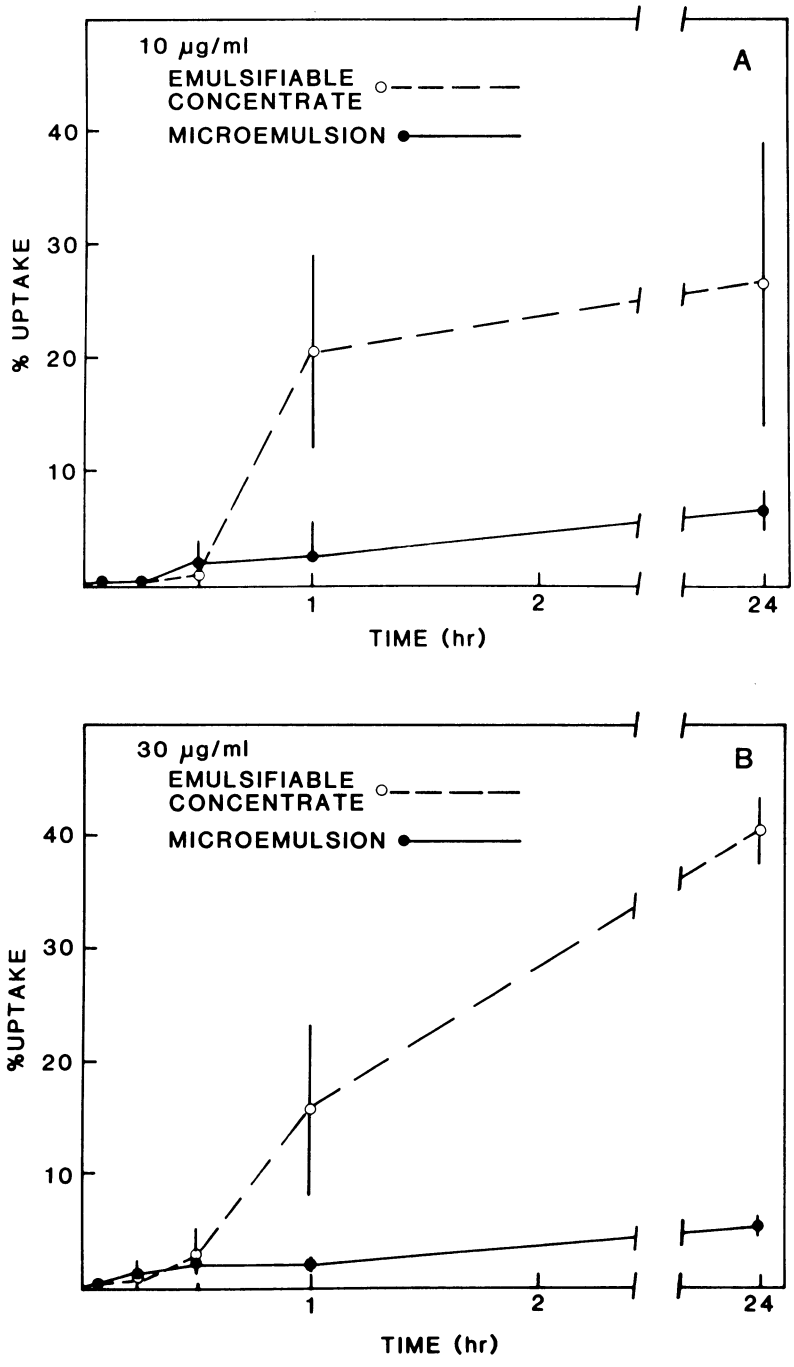

Fig. 1. Time course for penetration of ^{14}C-fenarimol formulated as an emulsifiable concentrate or microemulsion through adaxial leaf surface. Determinations were made 0, 5, 15, 30, and 60 min and 24 hr after application at **(A)** 10 μg/ml and **(B)** 30 μg/ml. Mean of six leaves with standard error indicated by vertical bar.

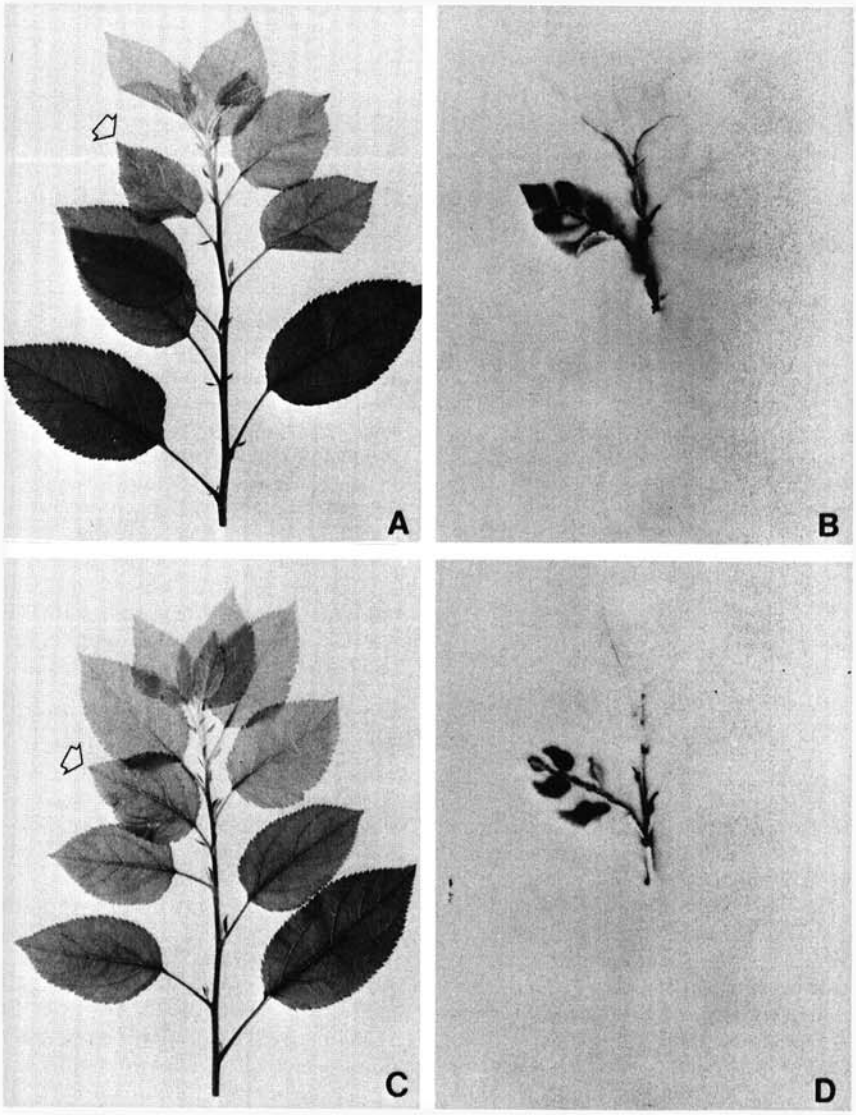

Fig. 2. Translocation of ^{14}C-label in apple seedlings. Five 10-μl droplets of ^{14}C-fenarimol containing 0.3 μg (3 nCi) active ingredient **(A)** in an emulsifiable concentrate and **(C)** in a microemulsion were applied to the youngest expanded leaves, as indicated by arrows. **(B and D)** Autoradiographs were made from A and C, respectively, 7 days after fungicide application.

concentration.

Results of the autoradiography were in agreement with the uptake study; significantly more ^{14}C-label in the EC formulation was taken up by the apple leaves than in the ME formulation. Movement of the ^{14}C-label was primarily acropetal. Some ^{14}C-label of each formulation was detected in the four to five leaves above the treated leaf. However, it is not known if fenarimol accumulated in sufficient concentrations to provide any activity against *V. inaequalis*.

Our study suggests that microemulsions may be useful formulations for fungicides and should be evaluated under field conditions for the control of apple scab and certain other diseases. Efficacy of the ME formulation of fenarimol was equivalent to the commercial EC formulation under greenhouse conditions. Improved stability in storage, improved mixing properties, and possible enhanced activity (4,5) are incentives for further investigation of ME formulations of fungicides.

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LITERATURE CITED

1. Backman, P. A. 1978. Fungicide formulation: Relationship to biological activity. Annu. Rev. Phytopathol. 16:211-237.
2. O'Leary, A. L., and Jones, A. L. 1987. Factors influencing the uptake of fenarimol and flusilazol by apple leaves. Phytopathology 77:1564-1568.
3. O'Leary, A. L., Jones, A. L., and Ehret, G. R. 1987. Application rates and spray intervals for apple scab control with flusilazol and pyrifenox. Plant Dis. 71:623-626.
4. Prince, L. M. 1977. Microemulsions. Theory and Practice. Academic Press, Inc., New York. 179 pp.
5. Urton, J. T., inventor; Vanguard Chemical Co., Inc., assignee. Method of producing microcolloidal aqueous emulsions of unsaturated organic insecticidal compounds. U.S. patent 3,954,967. 1976