

Factors Influencing the Uptake of Fenarimol and Flusilazol by Apple Leaves

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ABSTRACT

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The influence of temperature, light, leaf age, leaf surface, and fungicide concentration on the uptake of fenarimol and flusilazol by apple leaf disks was studied under controlled conditions using ^{14}C -labeled fungicides. Penetration of both fungicides through adaxial and abaxial leaf surfaces was temperature-dependent. After 24 hr, approximately 12, 40, and 60% of the ^{14}C -fenarimol and 15, 32, and 54% of the ^{14}C -flusilazol applied to the abaxial surface of leaf disks from fully expanded leaves were absorbed at 10, 20, and 30 C, respectively. Uptake through the adaxial surface was also temperature-dependent, but the rate of penetration was slower than for the abaxial surface. Penetration into young, partially unfolded leaves was

significantly higher than into fully expanded leaves. Although uptake of the ^{14}C -label increased with increases in temperature from 5 to 30 C, there was a shift to a higher rate of uptake between 15 and 20 C. Uptake of ^{14}C -fenarimol and ^{14}C -flusilazol increased linearly as the concentration of fungicide in the solution applied to the adaxial leaf surface was increased. Light did not affect uptake of the fungicides through either leaf surface. The results indicate that ergosterol biosynthesis-inhibiting fungicides are readily absorbed by the leaf and that absorption is controlled more by physical properties of the cuticle than by metabolism of the leaf.

Additional key words: *Malus domestica*.

An important characteristic of the ergosterol biosynthesis-inhibiting (EBI) fungicides is their systemic activity in plants. Direct evidence for the ability of EBI fungicides to penetrate leaf tissue has come from studies with radiolabeled fungicides. Fenapanil was absorbed by bean leaves and etaconazole and fenapanil were absorbed by apple leaves, but the majority of fungicide that was taken up remained in the treated leaves (10,12). Triadimefon was absorbed by grape leaves (11) but was translocated in a lateral direction in treated leaves and acropetally when applied to petioles and young stem tissue. Solel and Edgington (18) reported high transcuticular movement of triarimol through cuticles isolated from apple leaves. These studies suggest that the excellent postinfection control achieved from foliar sprays of EBI fungicides (20) is due to uptake of the fungicides by leaves and possibly other tissues of the plant.

In this study, we evaluate the effects of temperature, leaf age, leaf surface, fungicide concentration, and light on the uptake by apple leaves of the EBI fungicides fenarimol and flusilazol. These environmental and physical factors have been shown to influence the absorption by foliage of several growth regulators (1,3,6,13), antibiotics (5), and fungicides (18). Identification of the factors that influence the absorption of systemic EBI fungicides by apple leaves was motivated in part by an interest in the most effective ways to use these fungicides in scab control programs.

MATERIALS AND METHODS

Fungicides. The fungicide formulations evaluated in this study were either fully registered or being tested under an experimental use permit for the control of diseases of apple in the United States.

An emulsifiable concentrate formulation similar to Rubigan IE and containing 120 g/L of fenarimol was prepared by adding technical-grade fenarimol (Elanco Products Co., Indianapolis, IN) and [^{14}C -carbonil]fenarimol (specific activity 20.4 $\mu\text{Ci}/\text{mg}$), both dissolved in ethanol, to a formulation blank. The formulated fungicide was diluted with distilled water to obtain stock solutions containing 14.2 (0.15), 28.1 (0.29), 42.6 (0.44), and 56.2 $\mu\text{g}/\text{ml}$ of

fenarimol (0.57 $\mu\text{Ci}/\text{ml}$ of ^{14}C -fenarimol).

A dispersible granule formulation containing 20% flusilazol by weight was prepared by adding [triazole-3- ^{14}C]flusilazol (specific activity 17.9 $\mu\text{Ci}/\text{mg}$), dissolved in ethanol, to an aqueous solution of formulated flusilazol (DPX-H6573, Nustar; du Pont de Nemours & Co., Wilmington, DE) containing 2% active ingredient. Final concentrations of flusilazol (^{14}C -flusilazol) in the stock solutions were: 9.4 (0.17), 18.7 (0.33), 28.2 (0.50), and 37.4 $\mu\text{g}/\text{ml}$ (0.66 $\mu\text{Ci}/\text{ml}$).

Determination of absorption of ^{14}C -fenarimol and ^{14}C -flusilazol. All leaves for this study were obtained from greenhouse-grown trees of McIntosh apple (*Malus domestica* Borkh.). Unless indicated otherwise, the fungicides were applied to disks (13-mm diameter) taken from the fourth or fifth unfolded leaves that were fully expanded. Leaf disks were excised with a cork borer and placed either abaxial or adaxial side up in a nylon-screen basket. The disks were maintained at a high relative humidity by suspending each basket 6 cm above 200 ml of distilled water in 1-L glass jars. The system was allowed to equilibrate for 1.5 hr before fungicide was applied. A 10- μl drop of stock solution was placed on the surface with a syringe introduced through a rubber serum cap in each jar lid. Unless indicated otherwise, jars containing the leaf disks were incubated in the dark at 10 or 30 C, stock solutions containing 0.28 $\mu\text{g}/10 \mu\text{l}$ of fenarimol and 0.09 $\mu\text{g}/10 \mu\text{l}$ of flusilazol were applied to the leaf surfaces, three disks were assayed at each sampling interval, and each experiment was conducted twice. In experiments at 10 C, stock solutions were cooled to 10 C before application to the leaf disks.

At designated time intervals, the surface of each disk was rinsed with two 200- μl aliquots of 95% ethanol into scintillation cocktail (Safety-Solve, Research Products International Corp., Mount Prospect, IL). After rinsing, disks were oxidized by combustion in a model 306 Packard Oxidizer, and the $^{14}\text{CO}_2$ was trapped in Carbo-Sorb II carbon dioxide absorber and combined with Permafluor V scintillant (Packard Instrument Co., Downer's Grove, IL). Radioactivity in the ethanol rinses and in the disks was determined by counting for 5 min in a BetaTrac 6895 liquid scintillation counter (TM Analytic, Inc., Elk Grove Village, IL). Corrections were made for background, efficiency, and quenching. Recovery of the applied dosage was 93.8% for fenarimol and 96.3%

for flusilazol. Results were expressed as the percentage of the radioactivity applied to the surface of the leaf disks that was detected in the rinsed disks. In the study involving various concentrations of the fungicide, the disintegrations per minute in the fungicide solutions and in the rinsed leaf disks were converted to micrograms of fungicide.

Temperature. Jars containing leaf disks were incubated at 10, 20, and 30 C. After equilibration, a 10- μ l droplet of stock solution containing 28.1 μ g/ml of fenarimol or 9.4 μ g/ml of flusilazol was placed on each disk and the jars were returned to the appropriate temperature. Leaf disks treated on the adaxial surface were assayed at 1, 2, 4, 6, 8, 10, and 24 hr after application of the fungicides, and disks treated on the abaxial surface were assayed at 2, 4, 6, 10, and 24 hr.

Concentration. After leaf disks were equilibrated at 10 and 30 C, stock solutions containing 14.2, 28.1, 42.6, and 56.2 μ g/ml of fenarimol and 9.4, 18.7, 28.2, and 37.4 μ g/ml of flusilazol were applied to the adaxial leaf surface. Leaf disks were assayed at 8 hr.

Leaf age. Leaf disks were excised from the youngest (unfolded) leaf and from the fourth and eighth (fully expanded) leaves. Fungicides were applied to the adaxial surface, and the leaf disks were assayed at 8 hr after application

Light. Leaf disks, adaxial and abaxial side up, were placed on a metal screen suspended above moistened filter paper in a 90-mm glass petri dish. The petri dishes were placed at 28 C under a fluorescent light (200 μ mol m⁻²s⁻¹) for the light treatment or wrapped in aluminum foil for the dark treatment. Six leaf disks per treatment were assayed at 8 hr after application.

RESULTS

Temperature. The amount of ¹⁴C-fenarimol and ¹⁴C-flusilazol recovered from washed leaf disks was temperature-dependent (Fig. 1). An initial rapid increase in rate of ¹⁴C-label absorption was followed by a gradual decrease in the rate of absorption. With increasing temperatures from 10 to 30 C, the recovery of ¹⁴C-label

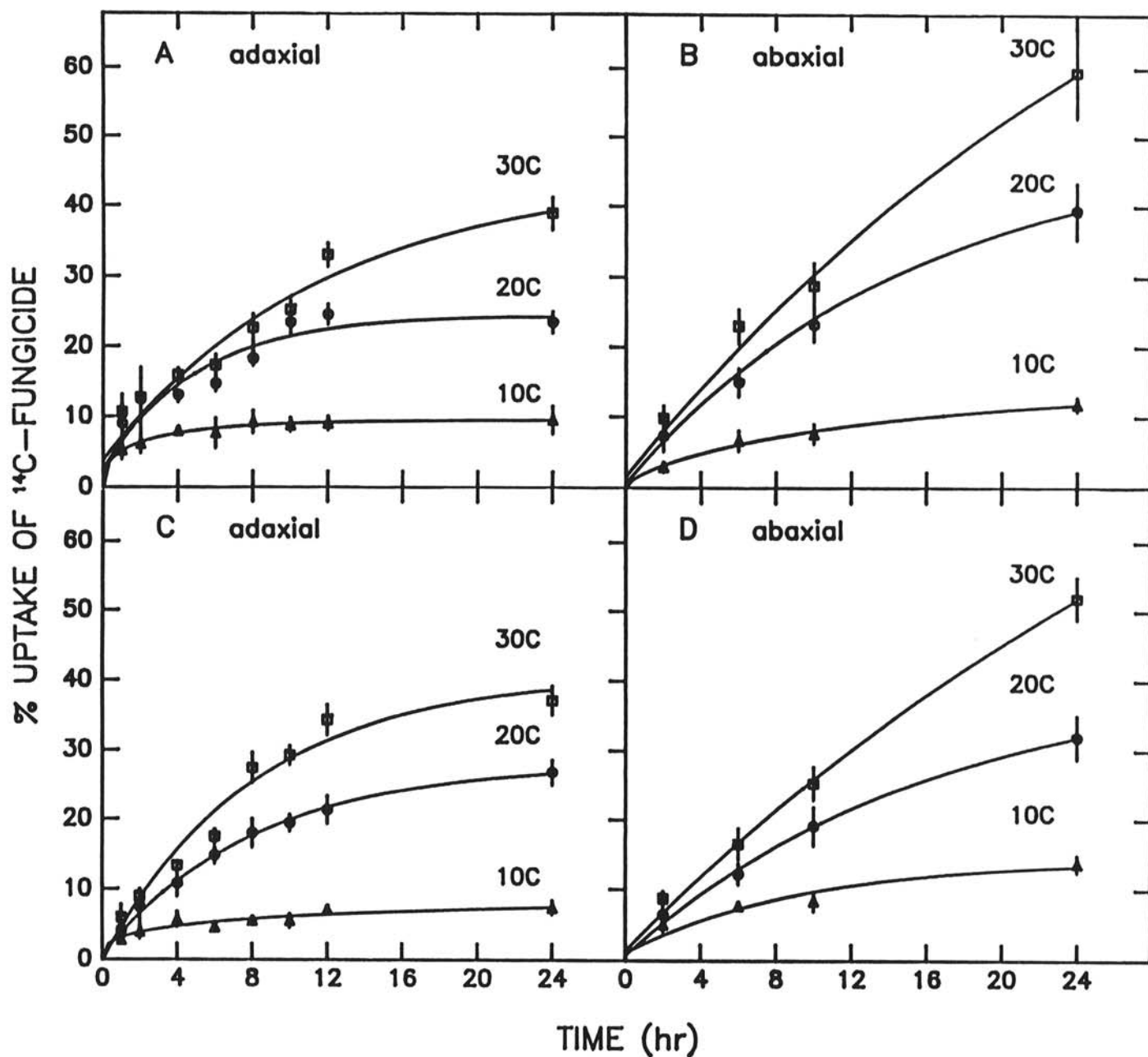


Fig. 1. Time course for penetration of ¹⁴C-fenarimol and ¹⁴C-flusilazol through the adaxial and abaxial surfaces of apple leaf disks incubated at three temperatures. The amount of fungicide applied to each 13-mm-diameter leaf disk was: **A and B**, 0.28 μ g of fenarimol and **C and D**, 0.09 μ g of flusilazol. Fungicides were applied in 10- μ l drops. Mean of six replicate leaf disks with standard error indicated by vertical bar.

also increased. Increasing the incubation temperature from 10 to 20 C or from 10 to 30 C resulted in a 2.5-fold and a fourfold increase, respectively, in the amount of fungicide taken up after 24 hr. Recovery of ^{14}C -label was greater from disks treated on the abaxial than on the adaxial surface, with a 1.3- to 1.6-fold increase in the amount of fungicide in disks treated on the abaxial surface after 24 hr.

To obtain additional information on the relationship of temperature to uptake of the fungicides, fungicide-treated leaf disks were held at 5, 10, 15, 20, 25, and 30 C and analyzed for ^{14}C -label after 8 hr (Fig. 2). As in the first experiment, the amount of ^{14}C -label detected in the washed leaf disks depended on the temperature at which the disks were maintained. The level of ^{14}C -label detected in the disks increased sharply between 15 and 20 C and continued to increase as temperatures were raised to 30 C. Temperature coefficients (Q_{10}) were calculated for each 10-C rise in temperature by dividing the amount of fungicide in the leaf disks at the higher temperature by the amount in the leaf disks at the lower temperature. Q_{10} values for rises in temperature from 5 to 15 C were 1.80 and 2.35 for fenarimol and flusilazol, respectively, and for rises from 15 to 25 C, 2.34 and 3.02, respectively.

Concentration. At 8 hr after droplets were applied to the adaxial surface of apple leaf disks, the amount of ^{14}C -label recovered from each disk increased linearly over a fourfold increase in the concentration of each fungicide applied (Fig. 3). To determine if the rates of uptake of ^{14}C -fenarimol and ^{14}C -flusilazol were similar at all concentrations of fungicide, regression analyses were performed. Slopes for the regression lines for both fungicides at 30 C, or for both at 10 C, did not differ significantly ($P = 0.01$), indicating that uptake was not affected differentially by concentration. However, the rate of uptake at 10 C was significantly slower ($P = 0.01$) than the rate of uptake at 30 C. Uptake at 10 C of the highest concentration of fungicide was less than uptake at 30 C of the lowest concentration of fungicide. At 30 C, about 20% more ^{14}C -flusilazol than ^{14}C -fenarimol was detected in the leaves 8 hr after treatment.

Leaf age. The amount of ^{14}C -label detected in leaf disks decreased as the age of the leaves from which the disks were excised increased (Fig. 4). At 30 C, about 80% of the ^{14}C -flusilazol and 70% of the ^{14}C -fenarimol were detected in the youngest leaf, compared

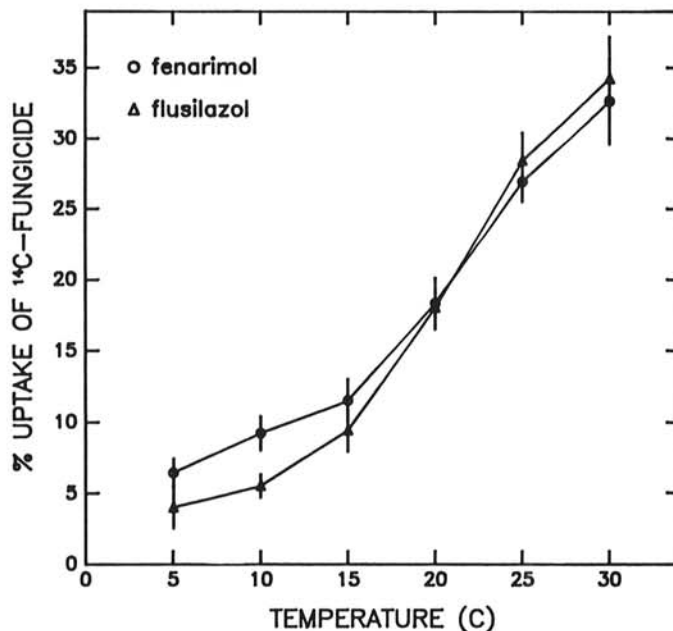


Fig. 2. Effect of temperature on penetration during 8 hr of ^{14}C -fenarimol (0.28 μg of fenarimol per 10- μl drop) and ^{14}C -flusilazol (0.09 μg of flusilazol per 10- μl drop) through the adaxial surface of 13-mm-diameter apple leaf disks. Mean of six replicate disks with standard error indicated by vertical bar.

with less than 30% in the fourth and eighth leaves. At each leaf age, about half as much ^{14}C -fungicide was detected in the leaf disks at 10 C as at 30 C.

Light. Regardless of which surface of the leaf was treated with fungicide, the amounts of ^{14}C -fenarimol and ^{14}C -flusilazol detected in leaf disks after 8 hr in the light were not significantly different ($P = 0.01$) from the amounts detected in leaf disks kept in darkness (Table 1).

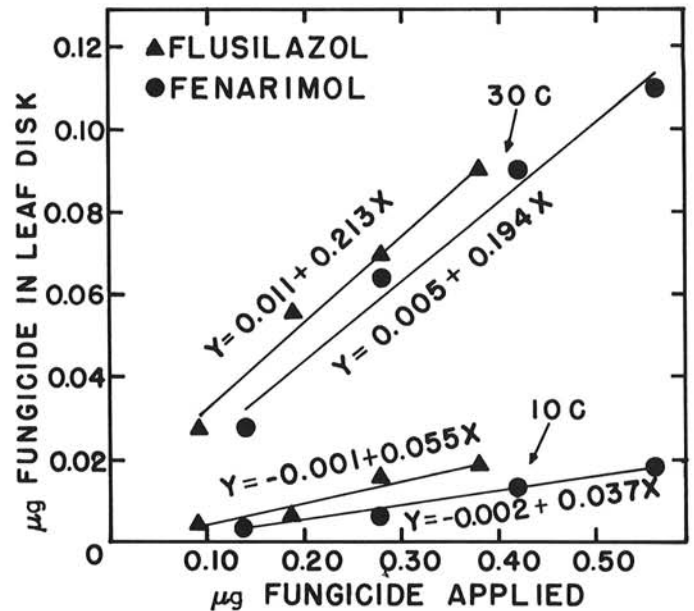


Fig. 3. Influence of concentration on penetration of ^{14}C -fenarimol and ^{14}C -flusilazol through the adaxial surface of 13-mm-diameter apple leaf disks incubated for 8 hr at 10 and 30 C. Fungicides were applied in 10- μl drops. Mean of six replicate disks. R^2 values for flusilazol 30 C, fenarimol 30 C, flusilazol 10 C, and fenarimol 10 C were 0.92, 0.95, 0.90, and 0.93, respectively.

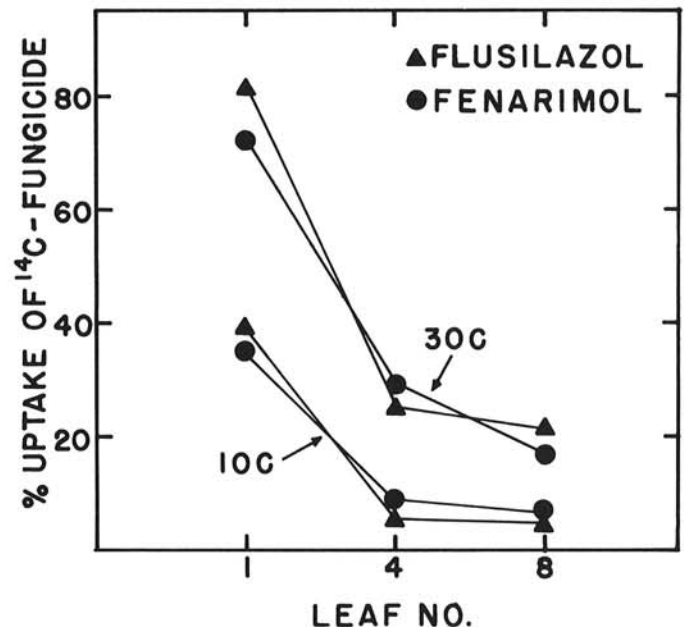


Fig. 4. Effect of leaf age on penetration of ^{14}C -fenarimol (0.28 μg of fenarimol per 10- μl drop) and ^{14}C -flusilazol (0.09 μg of flusilazol per 10- μl drop) through the adaxial surface of 13-mm-diameter apple leaf disks incubated for 8 hr at 10 and 30 C. Mean of six replicate disks.

TABLE 1. Uptake of ¹⁴C-fenarimol and ¹⁴C-flusilazol by apple leaf disks incubated in light or in dark for 8 hr at 28 C

Fungicide	Leaf surface	Light treatment	Uptake of ¹⁴ C-label (%)
Fenarimol	Adaxial	Dark	9.4 ^a
		Light	11.6
	Abaxial	Dark	21.0
		Light	25.1
Flusilazol	Adaxial	Dark	14.1
		Light	13.2
	Abaxial	Dark	22.9
		Light	26.6

^aLight treatments within each leaf surface and fungicide treatment were not significantly different ($P = 0.01$).

DISCUSSION

The concentrations of fungicides used in this study were based on rates used in practice for the control of apple scab. We evaluated solutions containing 14.2–56.2 µg/ml of fenarimol or 9.4–37.4 µg/ml of flusilazol. Fenarimol is registered in the United States for use on apples in dilute sprays containing 14.2–28.1 µg/ml of fenarimol. The base rate of 9.4 µg/ml of flusilazol gave consistent control of apple scab in greenhouse and field trials (15).

After application to the leaf surface, the amounts of fenarimol and flusilazol removable by washing with ethanol decreased with time. Because the apple leaf disks were maintained at about 100% relative humidity, droplets applied to the surface did not dry during the course of the experiments; thus, the fungicides were not fixed on the surface of the cuticle by drying.

Although long absorption times are sometimes encountered in the field, it is more usual for field sprays to be applied to dry trees, where they dry within minutes or, in the case of highly concentrated sprays, within seconds. This would not provide anywhere near the absorption time allowed in this study. Once deposits dry, absorption stops until there is rewetting (17). Therefore, we would expect less absorption to occur in the field than demonstrated in this study.

Improved absorption in the light has been interpreted to mean that energy derived from photosynthesis is involved in the penetration process (4,9). In our study, uptake of both fungicides was independent of light. Although temperature coefficients greater than 2 sometimes indicate an active uptake mechanism (16), Norris and Bukovac (14) suggested that the shift to a higher rate of foliar uptake between 15 and 20 C is the result of changes in the physical properties of the cuticular waxes initiated by the increase in temperature. High temperatures may also increase the kinetic energy of the diffusing fungicide molecules needed to overcome potential energy barriers between the liquid phase of the droplet and the wax layers in the cuticle (19).

We found penetration of both fungicides was greater through the abaxial leaf surface than through the adaxial surface. Guard cells and trichomes are located on the abaxial surface of apple leaves, and these structures have been shown to be preferred sites of entry (4,7). Cells at the base of trichomes have numerous ectodesmata and may have thinner cuticles (2,4). These cells have been shown to preferentially absorb compounds placed on the leaf (7). Also, the thickness and orientation of waxes in the cuticle of the adaxial surface may present a more formidable barrier than the cuticle of the abaxial surface (14).

The amount of fenarimol and flusilazol absorbed by leaf disks increased as the concentration of fungicide in the droplet applied to the leaf surface was increased to four times the lowest concentration. A similar relationship between the concentration in the solution applied to the leaf and uptake has been shown for 2,4-D in bean (16) and for NAA in pear (6) and may indicate that uptake depends on a concentration gradient established across the cuticle. The effect of concentration could explain the decreasing rate of absorption observed at the highest temperature. The initial rapid uptake of fungicide when the concentration gradient was

high was followed by a reduced rate of uptake as fungicide moved across the cuticle and lowered the concentration gradient.

It is fortuitous that the rate of absorption by expanding leaves is high because young leaves are more susceptible than old leaves to infection by the fungi that incite scab, powdery mildew, and cedar-apple rust. Greater uptake through the abaxial surface is also important because when apple leaves first emerge and before they unfold, only the abaxial surface is exposed to infection. Conversely, the poor postsymptom control of apple scab with EBI fungicides (20) may be related in part to poor uptake in older leaves.

The influence of temperature on absorption of EBI fungicides by apple leaves can provide insight into the fungicidal performance of these fungicides. When fenarimol and flusilazol are evaluated for control of apple scab, chlorotic lesions are more common on leaves in spring when it is cool than in early summer when it is warm. Development of chlorotic lesions rather than no development of lesions suggests less fungicide may be entering the leaves. In 1983, early-season scab control was poor with both fungicides after spray treatments at about 5 C (8). Reduced uptake of these fungicides owing to low temperatures may explain such occasional incidents of poor scab control from early-season spray applications, particularly where single applications are not followed by repeat applications of EBI fungicides. Where EBI fungicides are used under poor conditions for absorption, applications should be continued at regular intervals to maintain suppression of latent lesions (15). An alternative control strategy would be to use conventional protectant fungicides early in the season, followed by EBI fungicides, preferably in combination with a conventional fungicide, starting at pink or early bloom.

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