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Absorption and Translocation of Metalaxyl in Cabbage, Red Raspberry, and Strawberry

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Roots of cabbage, red raspberry, and strawberry plants were exposed to ring-labeled [^{14}C]metalaxyl to study the uptake and translocation of this systemic fungicide. Radiolabeled metalaxyl was readily absorbed by roots and translocated into the aerial portion of test plants. More ^{14}C accumulated in older than younger leaves. Radioactivity was uniformly distributed in developing leaves but was concentrated at the margins of mature leaves. With pairs of stolon-connected, but separately potted, strawberry plants as donor–receptor units, ^{14}C was detected throughout the receptor when [^{14}C]metalaxyl was applied to the soil of the donor. Defoliating the donor or withholding water from the receptor increased lateral translocation through the stolon. Movement of ^{14}C into cabbage cotyledons and leaves decreased when transpiration of those plant parts was lowered, again indicating that uptake and translocation of metalaxyl are related to the movement of water through the plant.

The systemic fungicide metalaxyl effectively controls diseases on numerous crops caused by fungi in the order Peronosporales, including downy mildew of cabbage (Gabrielson and Getzin, 1979) and Phytophthora root rots of both red raspberry (Bristow, 1980) and strawberry (Bristow, 1981). Metalaxyl is rapidly absorbed by roots and then translocated throughout the plant when applied as a soil drench (Rowe, 1982; Zaki et al., 1981). Translocation of metalaxyl is mainly upward, and its distribution is fairly uniform throughout the plant, indicating movement with the transpiration stream (Cohen et al., 1979). Limited symplastic translocation has also been demonstrated as metalaxyl was detected in roots and tubers of potato (Bruin et al., 1982) and roots of avocado and tomato (Zaki et al., 1981) following foliar application.

The main objectives of this study were to examine the uptake of metalaxyl by roots of young cabbage, red raspberry, and strawberry plants and to follow its translocation within those plants. Levels of metalaxyl were quantified in various parts of the test plants, and in some experiments the transpiration flow was altered to determine what effect the change would have on the movement of the fungicide.

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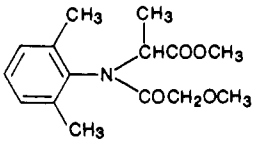
MATERIALS AND METHODS

Chemicals. Radioactive metalaxyl (U-ring-^{14}C; sp act. 24.6 μCi/mg; radiochemical purity >99%) was dissolved in 1.0 mL of methanol to provide a stock solution. Final test solutions were prepared by mixing [^{14}C]metalaxyl with unlabeled metalaxyl (Ridomil 2EC formulation; 0.24 kg of active ingredient/L) and diluting with distilled water to obtain the desired concentration and level of radioactivity.



Ridomil (metalaxyl) fungicide, Ciba-Geigy; downy mildew, soil-borne diseases (Phytophthora)

Plants. Cabbage plants (*Brassica oleracea* var. capitata L. cv. Market Prize) were grown from seed to the second true leaf stage in washed sand. Raspberry plants (*Rubus idaeus* L., from open-pollinated seed of the cvs. Chief and Meeker) were grown from stratified seed in plastic pots (5.5 × 5.5 × 4.8 cm), each containing ca. 0.12 L of a pasteurized soil–sand mixture (Puyallup sandy loam soil–sand,

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2:1, v/v). Runner plants of the cultivated strawberry (*Fragaria* × *ananassa* Duch. cv. Hood) and the wild beach strawberry [*Fragaria chiloensis* (L.) Duch.; Yaquina clone] were rooted and grown in plastic pots (7 × 7 × 7 cm) containing ca. 0.27 L of the soil–sand mixture. For some experiments pairs of stolon-connected runner plants were grown in separate pots. Plants were grown in a greenhouse (18–30 °C) without supplemental lighting. Cabbage seedlings were fertilized once with half-strength Hoaglund's solution. Raspberry and strawberry plants were fertilized once and as needed, respectively, with 10–20–20 fertilizer.

Fungicide Application and Test Conditions. Cabbage plants were lifted, and the adhering sand was thoroughly washed from the roots. The roots of individual plants were either (i) placed in vials containing 20.0 mL of [^{14}C]metalaxyl solution (100 μg of metalaxyl/mL; sp act. 1.725×10^{-3} μCi/mL) or (ii) transplanted into sand in a vial and then saturated with 5.0 mL of the fungicide solution. To reduce evaporation of the solution the stem of the plant was fitted through a foam plug that sealed the mouth of the vial; the vial and the plug were then wrapped in aluminum foil. The plants were maintained at ambient room temperature (20–25 °C) under continuous fluorescent light.

Transpiration and photosynthesis of cabbage plants were retarded by wrapping the leaves and/or cotyledons in clear plastic (Saran wrap) or aluminum foil. Roots of individual plants were in a vial containing 20.0 mL of [^{14}C]metalaxyl solution (50 μg of metalaxyl/mL; sp act. 1.725×10^{-3} μCi/mL). These plants were maintained in a growth chamber (Percival, Model MB-60B) with a 14-h day of fluorescent plus incandescent light (250 μE m^{-2} s^{-1}) at 24 °C, a 16 °C night, and relative humidity at ca. 13%.

Raspberry and strawberry plants were transferred to a growth chamber, set as above, at least 24 h before the fungicide was applied. A 10-mL sample of [^{14}C]metalaxyl solution (30.0 μg of metalaxyl/mL; sp act. 4.70×10^{-2} μCi/mL) was pipetted onto the surface of the soil for each raspberry plant. For each strawberry plant, 20.0 mL of [^{14}C]metalaxyl solution (30.0 μg of metalaxyl/mL; sp act. 3.95×10^{-2} μCi/mL) was similarly applied. When pairs of stolon-connected strawberry plants were used, the fungicide was applied to only the runner plant nearest the mother plant. This plant was designated the donor and the connected runner plant, the receptor, in the two-plant unit.

Duplicate plants or plant pairs were used for each treatment in all experiments. Untreated plants and plants treated with an equivalent amount of unlabeled metalaxyl were included as controls in each experiment.

Autoradiography. Soil was removed from roots with running tap water, and the entire plant was briefly rinsed. The plants were blotted dry, weighed, and dried in a plant press for 2–3 days. One of the duplicate plants was mounted on heavy paper and exposed directly to X-ray film (Kodak X-Omat AR film) for 30 days at −15 °C. Exposed film was developed according to the manufacturer's instructions.

Tissue Combustion. Following autoradiography, plants were dried at 56 °C for 24 h. Dry weights of intact plants and individual plant parts were taken. Dry plant parts were fragmented by a razor blade, the fragments were thoroughly mixed, and a sample (up to 150 mg) was combusted in a semiautomatic combustion system (Nuclear Chicago, Model 3151). Radioactive carbon dioxide was absorbed in an ethanolamine solution (ethanol–ethanolamine, 2:1, v/v), and an aliquot was added to scintillation cocktail (Beckman, Ready Solve HP) before counting with a scintillation spectrometer (Beckman, Model LS-7000).

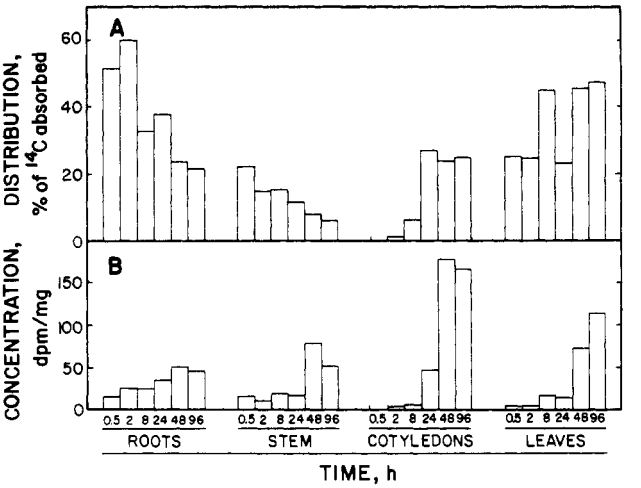

Figure 1. Radioactivity in different parts of young cabbage plants 0.5–96 h after [^{14}C]metalaxyl was applied to the roots (sand saturated with 5.0 mL of a solution containing 500 μg of metalaxyl and 0.009 μCi per plant). Data expressed as percentage of the amount of radioactivity absorbed at a given time (A) and dpm per milligram oven-dry weight (B).

Extraction of Metalaxyl and Derivatives. Air-dried tissues from duplicate plants were finely chopped with a razor blade and the fragments ground in a mortar. Ground tissue was extracted with 100 mL of methanol in a Soxhlet apparatus for 3 h. Extracts were reduced in volume to 3 mL in a flash evaporator at 40 °C. An aliquot (0.1 mL) was spotted on a silica gel thin-layer chromatography sheet (EM Laboratories, silica gel 60, precoated plastic sheets without fluorescent indicator, 0.2 mm). Sheets were developed for 15 cm in ethyl acetate and then in the perpendicular direction for 15 cm in ethyl acetate–acetic acid (90:10, v/v). The location and quantity of radioactivity were determined by (i) exposing the sheets to X-ray film for 1–4 weeks and (ii) scraping the silica gel from 5 × 5 mm quadrants into scintillation vials and counting, respectively.

Efficiency of the Soxhlet extraction was assessed by two methods. One, a known amount of [^{14}C]metalaxyl was added to ground root and leaf tissues from untreated control plants, the amended tissues were extracted, and the recovery efficiencies were calculated. Two, samples of tissue from plants exposed to [^{14}C]metalaxyl remaining in the Soxhlet thimbles after methanol extraction were analyzed by combustion. Both methods showed that ^{14}C recovery exceeded 97% for root and leaf tissues of all three crops.

RESULTS

Uptake by Cabbage. Uptake of metalaxyl by young cabbage plants was linear over the 96-h exposure period for plants with roots in saturated sand (r = 0.91) and solution (r = 0.99). Plants in saturated sand took up 217 μg of metalaxyl-radiolabeled equivalents or slightly over 40% of the applied dose while those in solution absorbed 392 μg per plant, but only 20% of the total amount available. Radioactivity was detectable in all plant parts within 2 h after the roots were exposed to [^{14}C]metalaxyl.

The pattern of distribution within cabbage plants was the same regardless of whether roots were in sand saturated with a metalaxyl solution or in the solution alone. The distribution for plants in saturated sand is shown in Figure 1A. The proportion of radioactivity in the roots was initially high, but by 8 h it had decreased as more material moved into the cotyledons and leaves. The con-

Table I. Uptake, Distribution, and Concentration of Radioactivity in Young Red Raspberry Plants (Open-Pollinated Seed from the Cv. Meeker) 1, 4, and 8 Days after [^{14}C]Metalaxyl Was Applied to the Soil[a]

time after applicn, days	plant part	^{14}C in plant part, dpm × 10^{-3}	% ^{14}C appl	distribn, %	concn,[b] dpm/mg
1	leaf blades	1.63	0.16	51.6	13.4
	petioles	0.05	0.00	1.6	3.2
	stem[c]	0.13	0.01	4.1	7.5
	roots	1.35	0.14	42.7	17.1
	total	3.16	0.31	100.0	14.0
4	leaf blades	13.74	1.33	91.1	115.4
	petioles	0.06	0.01	0.4	3.2
	stem	0.46	0.04	3.0	21.1
	roots	0.83	0.08	5.5	16.4
	total	15.09	1.46	100.0	71.9
8	leaf blades	15.46	1.49	83.0	119.6
	petioles	0.28	0.03	1.5	12.7
	stem	0.72	0.07	3.9	28.7
	roots	2.16	0.31	11.6	39.0
	total	18.62	1.80	100.0	80.3

[a] 300 μg of metalaxyl (0.47 μCi = 1.04 × 10^6 dpm) applied in 10 mL of water per plant to surface of soil. [b] Oven-dry weight (56 °C for 24 h). [c] Stem and root divided at soil line.

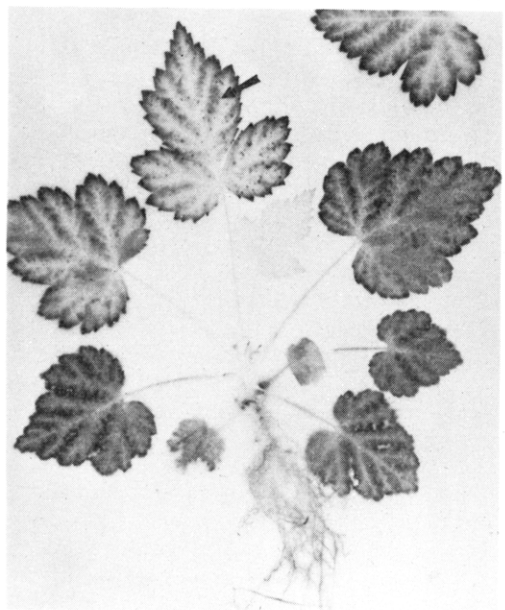

Figure 2. Autoradiograph of a young red raspberry plant (from open-pollinated seed of cv. Meeker) 8 days after [^{14}C]metalaxyl was applied to the surface of the soil. Arrow points to area damaged by mites.

centration of radioactivity in the roots and stems increased more slowly than it did in the cotyledons and leaves (Figure 1B). By 24 h it was apparent that radioactivity was concentrating in the margin of the leaves. Radioactivity was 3.4 times more concentrated in the margin of the leaves than in the center portion. The margin of some leaves became necrotic (after 24 h) when roots were exposed to a solution (solution alone not saturated sand) of either radioactive or unlabeled metalaxyl (2000 μg/plant). No necrosis was observed on leaves of plants in the water control. The concentration of radioactivity in the cotyledons reached a plateau by 48 h; after that time cotyledons began to senesce. After 96 h 98 and 87% of the radioactivity present in the roots and leaves, respectively, was identified as parent metalaxyl.

Uptake by Red Raspberry. There was no difference in the uptake and translocation of [^{14}C]metalaxyl by plants grown from open-pollinated seed of the two cultivars; hence, only the data for Meeker are presented. The young plants absorbed 0.31, 1.46, and 1.80% of the radioactivity applied to the surface of the soil–sand mixture after 1, 4, and 8 days, respectively. Data on the distribution of radioactivity within the plant and the concentration in various plant parts are presented in Table I. An autoradiograph (Figure 2) shows the distribution of radioactivity in a plant after 8 days. Radioactivity was uniformly distributed across young leaves. Petioles and veins of mature leaves contained little radioactivity. Radioactivity accumulated at the margin of mature leaves and areas where there was tissue damage, such as sites of mite feeding.

Metalaxyl accounted for 77% of the radioactivity extracted from raspberry roots, but only 5% of that from leaves. In both cases, the balance of the radioactivity failed to move from the origin spot on the TLC sheets.

Table II. Uptake, Distribution, and Concentration of Radioactivity in Young Strawberry Plants (Cv. Hood) 8 Days after [^{14}C]Metalaxyl Was Applied to the Soil[a]

plant part	^{14}C in plant part, dpm × 10^{-3}	% ^{14}C appl	distribn, %	concn,[b] dpm/mg
leaf blades	67.73	3.86	84.5	53.8
petioles	1.92	0.11	2.4	12.3
crown	3.61	0.21	4.2	13.8
roots	6.89	0.39	8.6	12.3
total	80.15	4.57	100.0	35.8

[a] 600 μg of metalaxyl (0.79 μCi = 1.75 × 10^6 dpm) applied in 20 mL of water per plant to surface of the soil. [b] Oven-dry weight (56 °C for 24 h).

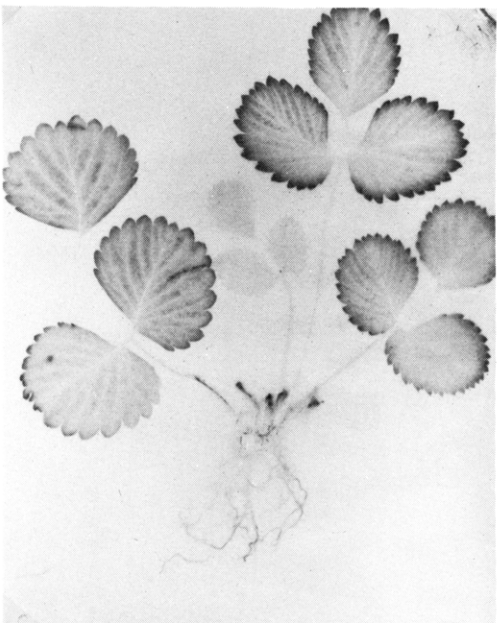

Figure 3. Autoradiograph of a young strawberry plant (cv. Hood) 8 days after [^{14}C]metalaxyl was applied to the surface of the soil.

Uptake by Strawberry. Uptake and distribution of radioactivity in the Yaquina clone of the beach strawberry and the Hood cultivar of the cultivated strawberry were nearly identical; both absorbed between 4 and 5% of the ^{14}C applied to the soil after 8 days. Because of the similarity only data for the cultivated strawberry are presented (Table II). Of the radioactivity in the plant, 85% was present in the leaves. Radioactivity in developing leaves was evenly distributed and at a lower concentration than in older more mature leaves (Figure 3). Again, radioactivity accumulated at the margins of the older leaves: 123.3 dpm/mg in the leaf margin compared to 35.5 dpm/mg for

Table III. Concentration of Radioactivity in Unwrapped Cotyledons and Leaves of Young Cabbage Plants and Those Wrapped in Either Clear Plastic or Aluminum Foil 24 and 48 h after [^{14}C]Metalaxyl Was Applied to the Roots[a]

plant part	wrapping	concn,[b] dpm/mg 24 h	48 h
cotyledon	none	46	135
	clear plastic	27	84
	foil	13	36
leaves	none	40	85
	clear plastic	36	60
	foil	7	11

[a] 1000 μg of metalaxyl (0.0345 μCi = 7.66 × 10^4 dpm) in 20 mL of water per plant. [b] Oven-dry weight (56 °C for 24 h).

Table IV. Uptake and Distribution of Radioactivity in Pairs of Stolon-Connected Strawberry Plants (Cv. Hood) 8 Days after [^{14}C]Metalaxyl Was Applied to the Soil of One Plant (Donor) and Transpiration Flow Was Altered by Withholding Water or Removing Leaves from One of the Plants[a]

treatment	% ^{14}C applied donor plant	stolon	receptor plant	total
none (control)	4.40	0.26	0.10	4.76
moisture withheld from *receptor* plant	6.86	0.19	2.91	9.96
moisture withheld from *donor* plant	0.90	0.00	0.11	1.01
leaves removed from *receptor* plant	3.55	0.06	0.19	3.80
leaves removed from *donor* plant	1.71	0.20	1.99	3.90

[a] 600 μg of metalaxyl (0.79 μCi = 1.75 × 10^6 dpm) applied in 20 mL of water to surface of soil of donor plant.

the remainder of the leaflet. The concentrations of radioactivity in the roots, crown, and petioles were essentially the same and severalfold less than that in the leaflets.

Of the radioactivity extracted 67% from the roots and 48% from the leaves was metalaxyl. Of the radioactivity extracted from leaves 4.5% represented an unidentified derivative while the remainder of the radioactivity from both roots and leaves did not migrate from the origin spot on the TLC sheets.

Altering Transpiration. Wrapping the leaves and cotyledons of cabbage plants in clear plastic to lower transpiration rates reduced the concentration of radioactivity in both plant parts compared to the unwrapped controls (Table III). The concentration was further reduced when these plant parts were wrapped in aluminum foil to slow the transpiration rate even more by also retarding photosynthesis.

In the paired strawberry plant test transpiration was modified by (i) withholding water from either the donor or receptor plant after [^{14}C]metalaxyl was applied to the soil of the donor plant or (ii) removing the leaves of either plant just prior to fungicide application. For the control pair (both watered as necessary and no leaves removed) about 5% of the applied radioactivity was absorbed by the pair after 8 days (Table IV). About 2% of the material taken up by the donor plant passed through the stolon to the receptor plant. Withholding water from the receptor plant doubled the total amount of radioactivity taken up by the plant pair; 40% of this amount moved into the receptor plant. Not watering the donor plant lowered the total amount of radioactivity absorbed but did not prevent some of it from moving through the stolon. Removing the leaves from either the donor or receptor plant reduced the radioactivity taken up by the pair compared to the control pair. Leaf removal from the donor plant markedly increased the amount and proportion of radioactivity in the receptor plant.

Table V. Distribution of Radioactivity in Leaf Blades, Petioles, Crowns, and Roots of Donor and Receptor Strawberry Plants in Table IV.

treatment	plant part	% ^{14}C applied[a] donor plant	receptor plant
none (control)	leaf blades	3.71	0.03
	petioles	0.11	0.00
	crown	0.20	0.01
	roots	0.38	0.06
water withheld from *receptor* plant	leaf blades	5.93	2.38
	petioles	0.15	0.11
	crown	0.32	0.18
	roots	0.46	0.24
water withheld from *donor* plant	leaf blades	0.62	0.05
	petioles	0.01	0.01
	crown	0.09	0.01
	roots	0.18	0.04
leaves removed from *receptor* plant	leaf blades	2.71	0.03
	petioles	0.15	0.00
	crown	0.45	0.16
	roots	0.24	0.00
leaves removed from *donor* plant	leaf blades	0.16	1.93
	petioles	0.02	0.02
	crown	1.04	0.04
	roots	0.49	0.00

[a] 600 μg of metalaxyl (0.79 μCi = 1.75 × 10^6 dpm) applied in 20 mL of water to the surface of the soil of donor plant.

Analysis of the various parts of both donor and receptor strawberry plants (Table V) showed that most of the radioactivity moved into the leaves. No radioactivity was detected in the roots of the receptor plant when the foliage was initially removed from either of the plants. Data reported for leaves on plants listed as having had leaves removed represent movement into leaves formed after the onset of the experiment.

DISCUSSION

Metalaxyl was rapidly absorbed by roots and translocated throughout young cabbage, red raspberry, and strawberry plants in this study and is in agreement with the general findings of other studies where different test plants were used (Cohen et al., 1979; Rowe, 1982; Zaki et al., 1981). Movement in the transpiration stream appears to account for the distribution of metalaxyl within the plants. This is indicated by the accumulation of radioactivity in older leaves and leaf margins (this being the first report of the latter), the increased uptake in strawberry plant pairs when water was withheld from the receptor (effectively doubling the leaf area dependent on the roots of the donor for water), and the accumulation of radioactivity at sites of injury to leaves. The concentration of systemic pesticides at sites where the epidermis has been broken is attributed to enhanced evaporation at that location (Prusky, 1979). Conversely, decreasing the rate at which water moves through the plant reduced uptake of metalaxyl as happened when the leaves and cotyledons of cabbage plants were wrapped in clear plastic and aluminum foil or when water was withheld from the donor strawberry plant.

Lateral translocation of radioactivity was readily demonstrated in experiments with stolon-connected strawberry plants. These results agree with reports on the movement of benomyl through stolons of strawberry (Nicholson et al., 1972) and creeping bentgrass (Meyer et al., 1971). Neither benomyl study, however, showed any basipetal

movement of that compound into the roots of the untreated plants as was demonstrated here. Basipetal translocation of foliar applied metalaxyl has been previously demonstrated in *Persea indica* (Zaki et al., 1981) and potato (Bruin et al., 1982).

Radioactivity was detected in the roots of the receptor strawberry plant only when the leaves of both plants in the donor–receptor unit were undisturbed (Table III), suggesting that basipetal movement is in some way related to the presence of leaves. Removal of leaves from either plant could have disrupted the source–sink relationship between the foliage and roots. This disruption may have resulted in the cessation of root growth, minimizing the movement of phloem-transported material into the roots.

Alternatively, newly forming leaves (on the defoliated plant) might act as a sink for materials transported in the phloem. During early growth, a leaf shows a net import of assimilates until the laminar surface is one-third to one-half full size (Leopold and Kriedman, 1975). Phloem transport between stolon-connected strawberry plants has been demonstrated (Guttridge, 1969).

Although the results of this study are reported as radioactivity and not as metalaxyl, the parent compound was present in the roots and leaves of all three test plants. The two-dimensional thin-layer chromatography procedure employed (provided by Ciba-Geigy Corp.) satisfactorily separates metalaxyl from known metabolites including metalaxyl conjugates. Most conjugates remain at or near the origin using this procedure. Reports on the systemic activity of soil-applied metalaxyl against foliar pathogens (Cohen et al., 1979; Gabrielson and Getzin, 1979; Johnson et al., 1979; Rowe, 1982) indicate that some of the material moving within the plant is biologically active and represents parent material as known metabolites have little or no activity (LeBaron, H. M., Ciba-Geigy Corp., personal communication). Similarly, metalaxyl is transported basipetally as *Phytophthora infestans* was controlled in tubers from potato plants where only the foliage was treated (Bruin et al., 1982).

Roots, stems, and petioles appear to be primarily organs of metalaxyl transport rather than organs of accumulation as are the cotyledons and leaf blades. Accumulation in cotyledons only continues until this plant organ ceases to function. Even though roots absorb and translocate metalaxyl to the aerial portion of the plant, the concentration remaining in the root at any given time is sufficient to control root diseases.

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Registry No. Metalaxyl, 57837-19-1.

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