Translocation of Metalaxyl and RE26745 in Potato and Comparison of Foliar and Soil Application for Control of *Phytophthora infestans*

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ABSTRACT

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The efficacy of metalaxyl and RE26745, systemic fungicides specific for fungi in the Peronosporales, was tested with an excised-leaf bioassay technique for control of *Phytophthora infestans* on potatoes grown under greenhouse conditions and in the field. In greenhouse tests, *P. infestans* was controlled up to 90 days with soil application of either material. Both were translocated from lower to upper foliage, but only RE26745 showed appreciable basipetal movement. In-furrow field treatments with both materials provided control up to 60 days after planting, but only the higher rates of RE26745 were effective up to 90 days. RE26745 provided control longer than metalaxyl when applied as a foliar spray at flowering. In field tests, using an in-furrow application followed by one or two foliar sprays, RE26745 provided season-long control but metalaxyl was less effective.

Additional key words: chemical control, potato late blight

The development of systemic fungicides specific for the Peronosporales (1,20,24) has presented many possibilities for new approaches to control of these pathogens on potatoes (*Solanum tuberosum* L.) (1,8,10,12,23,24) and many other crops (4,13,25). Recent reports on the occurrence of resistant strains of these fungi (2,3,5,6,11,14,23) have prompted considerable concern over proper use of these materials in the field. Although it is generally agreed that these fungicides should not be used alone, there is much disagreement as to whether they should be used as foliar or soil-applied protectants or be reserved as eradicants for established infestations.

Research on metalaxyl was initiated in Ohio in 1976 and on RE26745 in 1978. Initial field evaluations indicated that these materials had great potential for control of *Phytophthora infestans* (Mont.) d By. when applied either as foliar sprays or in soil (15–18). This report summarizes research on efficacy of these systemic fungicides when applied to soil as detected by an excised-leaf bioassay technique. A preliminary report has been published (19).

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MATERIALS AND METHODS

Fungicides tested. Systemic fungicides tested were metalaxyl and RE26745 [2-methoxy-*N*-(2,6-dimethylphenyl)-*N*-(tetrahydro-2-oxo-3-furanyl)acetamide; Chevron Chemical Co., Richmond, CA 94802]. These two compounds are extremely similar, the latter being a cyclized analogue of the methyl ester of the alanine moiety of metalaxyl. The standard, wide-spectrum foliar protectant fungicide mancozeb was used for comparison in the field studies. All rates listed are in terms of active ingredient.

Excised-leaf bioassay. A bioassay was developed to monitor the degree of systemic protection of foliage over time as a result of various fungicide treatments. Fully expanded, healthy, compound leaves were removed from treated potato plants and immersed for 30–40 min in a tap water suspension of 2,000–4,000 *P. infestans* zoospores per milliliter at ca. 12 C. After soaking, leaf petioles were inserted into moist vermiculite in 10-cm-diameter plastic pots. Pots were randomly arranged in a controlled environment chamber equipped with a fog generator and kept at 20 C in continuous fog under a 12-hr photoperiod for 7–10 days. At that time, disease development on each leaf was evaluated on the following scale: 0 = no visible symptoms, 1 = pinpoint lesions without sporulation, 2 = a few expanded lesions with light sporulation, and 3 = large lesions with heavy sporulation (Fig. 1).

Greenhouse soil application tests. Potted potato plants were used to evaluate the duration of foliar blight control obtainable with soil applications of these systemic fungicides. Potato tubers (cv. Kennebec) were cut into single-eye seed pieces and dipped for ca. 10 sec in a solution of gibberellic acid (10 μg/ml) to break dormancy. Two-liter plastic pots were half-filled with steam-disinfested soil, and four rates each of metalaxyl 2E and RE26745 50W were applied evenly on the soil surface. The highest rate of 68 mg was equivalent, per plant, to 3.4 kg/ha (3 lb/acre) based on 49,400 plants per hectare (20,000/acre). One seed piece was then placed in each pot and covered with 10 cm of soil. Controls were planted in the same manner without the addition of fungicides. Plants were grown in a greenhouse at 20–25 C with 16 hr/day of supplementary fluorescent light. Beginning 41 days after planting, healthy, fully expanded leaves were removed from each plant for the excised-leaf bioassay. Plants were sampled every 2–3 wk until the effects of soil treatments were no longer evident.

Systemic movement. Greenhouse tests were designed to evaluate movement of foliar-applied treatments upward and downward into untreated foliage. Two-month-old, rooted stem cuttings (cv. Superior), pruned to a single stem about 38 cm high, were used. The unsprayed upper or lower portion of each plant was covered with a plastic bag secured to the stem by a wire twist-tie. Plants were positioned horizontally on a greenhouse bench and sprayed to runoff with a hand atomizer at a rate of ca. 1.2 mg/ml (1 lb/100 gal) of water. Plants were air-dried for a few hours and then set upright, and the bags were removed. Irrigation water was applied directly to soil without wetting the foliage. At weekly intervals thereafter, leaves were removed from each plant, one from immediately above and one from immediately below the point separating the treated and untreated portions, and bioassayed.

Field evaluations. Field plots of complete randomized block design with four, 9.3-m, single-row replicates per treatment were established in mid-May of 1979, 1980, and 1981 at Wooster, OH.

Seed pieces cut from certified Kennebec seed tubers were planted 25 cm apart in rows spaced 1.8 m apart in Wooster silt loam soil. In-furrow treatments were applied by evenly distributing granular or liquid formulations into furrows prior to placing seed pieces. All plots were treated with an in-furrow application of the insecticide aldicarb at 3.4 kg/ha (3 lb/acre) and maintained throughout the season under standard commercial fertility and weed and insect control programs. Foliar applications of experimental fungicides were applied at 2.1–2.8 kg/cm^2 (30–40 psi) with a one-row, bicycle-tire, plot sprayer pressurized by carbon dioxide with a boom containing two over-the-row and two drop, flat-fan nozzles. Sprays applied at regular intervals were initiated in late June and continued until mid-September. Single flowering sprays were applied to some treatments in mid-July to late July when plants were in full bloom.

Data were taken throughout the 1979 and 1980 seasons using the excised-leaf bioassay. Leaf samples were collected from each plot beginning in mid-June and continuing at ca. 2-wk intervals. Four leaves were collected at random from plants of each replicate row for a total of 16 leaves per treatment. Initially, only fully expanded, green leaves with no evidence of foliar lesions of any kind were selected for bioassay; however, during the last two samples in late August, leaves with some early blight lesions caused by *Alternaria solani* (Ell. and G. Martin) Sor. had to be used.

Table 1. Assessment of control of *Phytophthora infestans* on excised leaves from greenhouse-grown potatoes treated with systemic fungicides applied to soil at planting

Fungicide	Rate[x]	Avg. disease index[y] at days after planting				
		41	52	73	90	104
Metalaxyl 2E	68	0 c[z]	0 c	0.9 bc	0.4 cd	1.9 b
	34	0 c	0.4 bc	1.1 b	0.9 bcd	2.1 b
	17	0.9 b	1.0 b	0.7 bc	1.6 b	2.7 a
	9	0.1 c	0.6 bc	1.1 b	0.3 d	3.0 a
RE26745 50W	68	0 c	0.1 c	0.4 bc	0.1 d	2.9 a
	34	0.3 c	0.1 c	0.4 bc	1.1 bc	3.0 a
	17	0 c	0.1 c	0.1 c	1.3 b	3.0 a
	9	0 c	1.0 b	0.9 bc	1.6 b	3.0 a
Untreated	...	3.0 a	3.0 a	2.7 a	3.0 a	3.0 a

[x] Milligrams of active ingredient per 2-L plastic pot. Rate of 68 mg is equal to 3.4 kg/ha at 49,400 plants per hectare.

[y] Each figure is average reaction of seven fully expanded, green leaves removed from separate replicate plants and rated 7–10 days after inoculation on the following scale: 0 = no visible symptoms, 1 = pinpoint lesions without sporulation, 2 = a few expanded lesions with light sporulation, and 3 = large lesions with heavy sporulation.

[z] Means within a column followed by the same letter do not differ at $P = 0.05$ according to Duncan's new multiple range test.

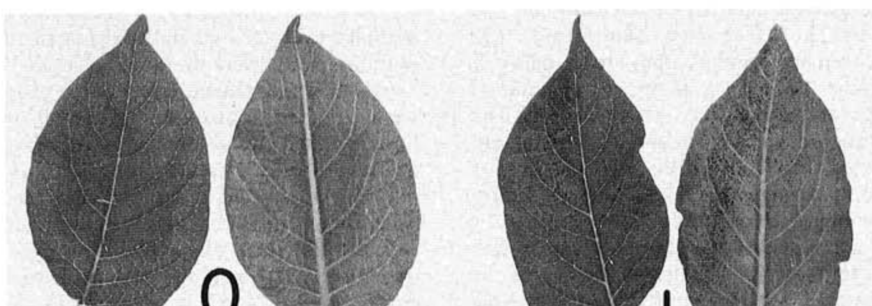

Fig. 1. Disease index values assigned to potato leaves excised from fungicide-treated plants and inoculated with *Phytophthora infestans* in a controlled environment chamber. Leaves were rated 7–10 days after inoculation on the following scale: 0 = no visible symptoms, 1 = pinpoint lesions without sporulation, 2 = a few expanded lesions with light sporulation, and 3 = large lesions with heavy sporulation.

RESULTS

In initial greenhouse tests, both metalaxyl and RE26745 applied to soil at planting provided control of *P. infestans* up to 90 days at all rates tested (Table 1).

Data from translocation studies (Table 2) were subjected to analysis of variance followed by linear contrasts to evaluate the significance of factor effects and their interactions (Table 3). In all cases, control was relatively constant across the 3-wk sampling period. When foliar samples were taken from directly treated portions of plants, little difference between the two materials was detected by the excised-leaf bioassay. Untreated foliage removed from upper portions of plants in which the lower foliage had been treated showed evidence of upward translocation of both materials. RE26745, however, provided significantly better protection of untreated upper foliage. Untreated lower foliage removed from plants in which the upper foliage had been treated was protected from *P. infestans* only with RE26745, indicating that metalaxyl was not basipetally translocated in efficacious amounts.

Field tests in 1979 monitored with the excised-leaf bioassay showed the potential duration of control attainable with soil or foliar applications of these fungicides (Fig. 2). Using a disease index of 1.5 (below which little or no sporulation occurs) as a threshold of acceptable control, in-furrow treatments all provided control up to 60 days after planting (Fig. 2B). Metalaxyl at 2.2 kg/ha (2 lb/acre) provided control up to 75 days, whereas RE26745 at the same rate provided control for ca. 90 days. Foliar treatments of RE26745 at flowering depressed disease indexes below the control threshold for 30–40 days following application. Metalaxyl applied in this manner failed to suppress the index below the control threshold (Fig. 2A).

Field tests in 1980 compared metalaxyl rates of 0.15 kg/ha (0.3 lb/acre) applied on a 14-day schedule with combinations of in-furrow and limited foliar applications of both materials. The latter treatments were designed to maintain the disease index below the designated control threshold throughout the season. In the 14-day metalaxyl treatments, the control threshold was exceeded 70–80 days after planting, and disease indexes for both these treatments remained above 2.8 for the remainder of the season. In-furrow treatments of both materials at 2.2 kg/ha (2 lb/acre) gave acceptable control up to 70 days after planting (Fig. 3). At this rate, RE26745 provided control below the threshold level for 10–20 days longer than metalaxyl, as was also noted in 1979 (Fig. 2B). Some activity was also detected throughout the remainder of the season. There was a dramatic difference in the response between the two fungicides when 1.1 kg/ha (1 lb/acre) applied in-furrow was followed by one or two foliar applications later in the season. Metalaxyl-treated samples taken 2 days after spray applications were protected from infection, but the effect did not last through the following sampling date 10–14 days later (Fig. 3A). In contrast, plants treated with RE26745 were protected from infection for the entire season (Fig. 3B).

Further tests in 1981 confirmed that low rates of metalaxyl (0.17 kg/ha) applied to foliage at 14-day intervals provided inadequate control (Table 4). Tank mixes of metalaxyl with a low rate of mancozeb were much more efficacious but no more so than standard rates of mancozeb alone applied on a weekly schedule. Applications of metalaxyl as a soil drench at flowering resulted in good control of late blight during the following 4-wk period.

DISCUSSION

Previous studies with these systemic fungicides have indicated that they are moderately to highly water soluble (9,24) and are readily absorbed by roots and translocated to upper foliage in many plant species (1,4,9,24,25). Our results confirm acropetal translocation in potato and demonstrate some basipetal movement of RE26745 but not of metalaxyl. Slight basipetal translocation of metalaxyl has been reported only in tomato and avocado (22,25).

Our tests with metalaxyl as a midseason to late-season foliar spray showed that it provided only limited systemic protection of foliage (Figs. 2A and 3A). This may indicate that metalaxyl is not absorbed as well by foliage as is RE26745. It has been reported that uptake of foliar-applied metalaxyl occurs primarily through stems and petioles (4,22,25) and that absoprtion through leaves is poor (8,25). Because of the much higher water solubility of metalaxyl (7,100 ppm) as compared with RE26745 (140 ppm), it may not as easily penetrate the leaf cuticle (9). Results in this study show that both fungicides are absorbed efficiently through roots and that much higher levels of protection are provided in foliage if they are applied to soil rather than to foliage. This was apparent in our 1980 and 1981 field studies.

The primary advantage of using these systemic fungicides for control of potato late blight is a reduction in the number of spray applications as compared with conventional foliar protectants while maintaining an equal or improved level of control. Our experience has indicated that soil application at planting or soon after emergence, coupled with one or two midseason to late-season sprays, can give season-long control, especially with RE26745 (Fig. 3). Another approach could be the application of a midseason soil drench, as in our 1981 tests, possibly followed by a second treatment 1 mo later.

Soil application has a number of distinct advantages. Because the use of in-furrow, systemic insecticides has become a standard practice with many potato growers, spray programs in July are primarily for late-blight control. The use of a soil-applied, systemic fungicide in conjuction with a systemic insecticide could eliminate all spraying until midsummer. At that time, tank mixes of various insecticides and standard wide-spectrum fungicides for control of early

Table 2. Assessment of systemic movement of metalaxyl and RE26745 within potato plants by inoculating leaves excised from treated and untreated portions of greenhouse-grown plants with *Phytophthora infestans*

Fungicide and rate (a.i.)	Weeks after treatment	Avg. disease index of sampled foliage[x] U/U[y]	U/L	L/U	L/L
Metalaxyl 2EC	1	1.4	2.4	1.7	1.1
(1.2 mg/ml)[z]	2	1.5	2.3	1.7	1.3
	3	1.3	2.4	1.8	0.2
RE26745 50W	1	1.0	1.3	0.6	0.8
(1.2 mg/ml)	2	0.7	1.5	0.8	0.4
	3	1.0	1.1	0.9	1.0
Untreated	1	2.9			
control	2	2.9			
	3	2.5			

[x]Each figure is the average of two replicates of four plants each. Leaves were rated 7–10 days after inoculation on the following scale: 0 = no visible symptoms, 1 = pinpoint lesions without sporulation, 2 = a few expanded lesions with light sporulation, and 3 = large lesions with heavy sporulation.

[y]Portion of plant treated (upper or lower)/portion of plant sampled (upper or lower).

[z]1 lb/100 gal of water.

Table 3. Significance levels of selected linear contrasts of average weekly disease indexes from Table 2

Contrast	Weekly intervals 1	2	3
Treatments vs. control	0.00	0.00	0.00
Metalaxyl vs. RE26745	0.00	0.00	0.01
Metalaxyl U/U[z] vs. RE26745 U/U	0.18	0.04	0.23
Metalaxyl U/L vs. RE26745 U/L	0.00	0.08	0.00
Metalaxyl U/L vs. control	0.05	0.15	0.69
Metalaxyl L/U vs. RE26745 L/U	0.00	0.03	0.01
Metalaxyl L/L vs. RE26745 L/L	0.37	0.04	0.01

[z]Portion of plant treated (upper or lower)/portion of plant sampled (upper or lower).

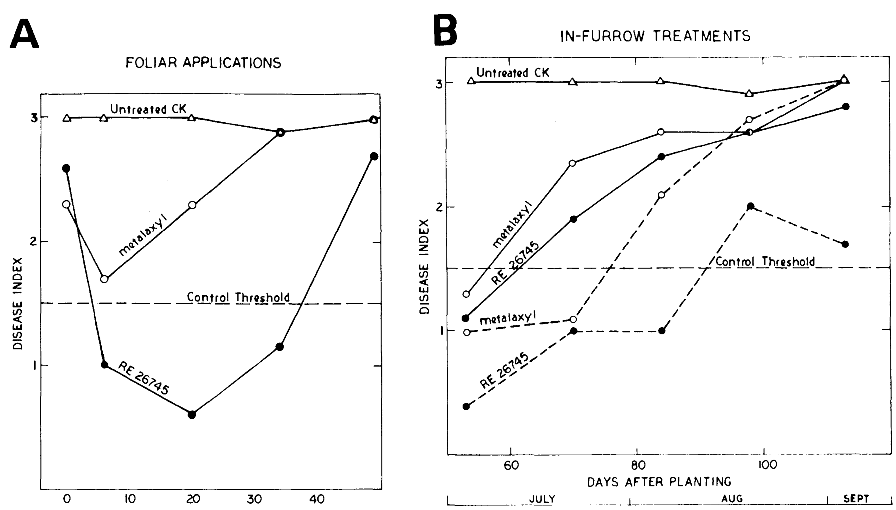

Fig. 2. Assessment of control of *Phytophthora infestans* in 1979 on excised leaves from field-grown potato plants: **(A)** Single foliar application at flowering of fungicides at 1.1 kg/ha. **(B)** In-furrow applications at planting at 1.1 kg/ha (solid lines) or 2.2 kg/ha (dashed lines). Disease severity evaluated as shown in Figure 1. Threshold of acceptable control arbitrarily established at 1.5, below which little or no sporulation occurs. $LSD_{0.05} = 0.53$.

Table 4. Control of *Phytophthora infestans* in 1981 in field-grown potatoes with metalaxyl applied to foliage and in soil

Treatment	Rate (kg a.i./ha)	Application method[x]	Disease index[y]			
			17 Aug.	24 Aug.	31 Aug.	9 Sept.
Metalaxyl 25W	0.17	14-day spray	2.7 b[z]	2.7 b	2.8 c	5.0 b
Metalaxyl 25W plus mancozeb 80W	0.17 0.78	14-day spray	0.8 a	0.9 a	0.8 ab	5.0 b
Mancozeb 80W	1.79	7-day spray	0.0 a	0.0 a	0.2 a	3.8 a
Metalaxyl 25W	1.68	Soil drench at flowering (3 Aug.)	0.0 a	0.6 a	1.6 b	3.9 a
Untreated control	···	···	3.3 b	3.7 b	4.8 d	5.0 b

[x] Sprays applied with plot sprayer pressurized by carbon dioxide at 2.8 kg/cm² or as soil drench in water sprinkled at bases of plants.

[y] Each figure is the average of four replicates rated as follows: 0 = no visible infection, 1 = <10 lesions per 10 m of row, 2 = ca. 3 lesions per meter of row, 3 = 6–15 lesions per meter, 4 = 20–60 lesions per meter, and 5 = >60 lesions per meter.

[z] Means within a column followed by the same letter do not differ at $P = 0.05$ according to Duncan's new multiple range test.

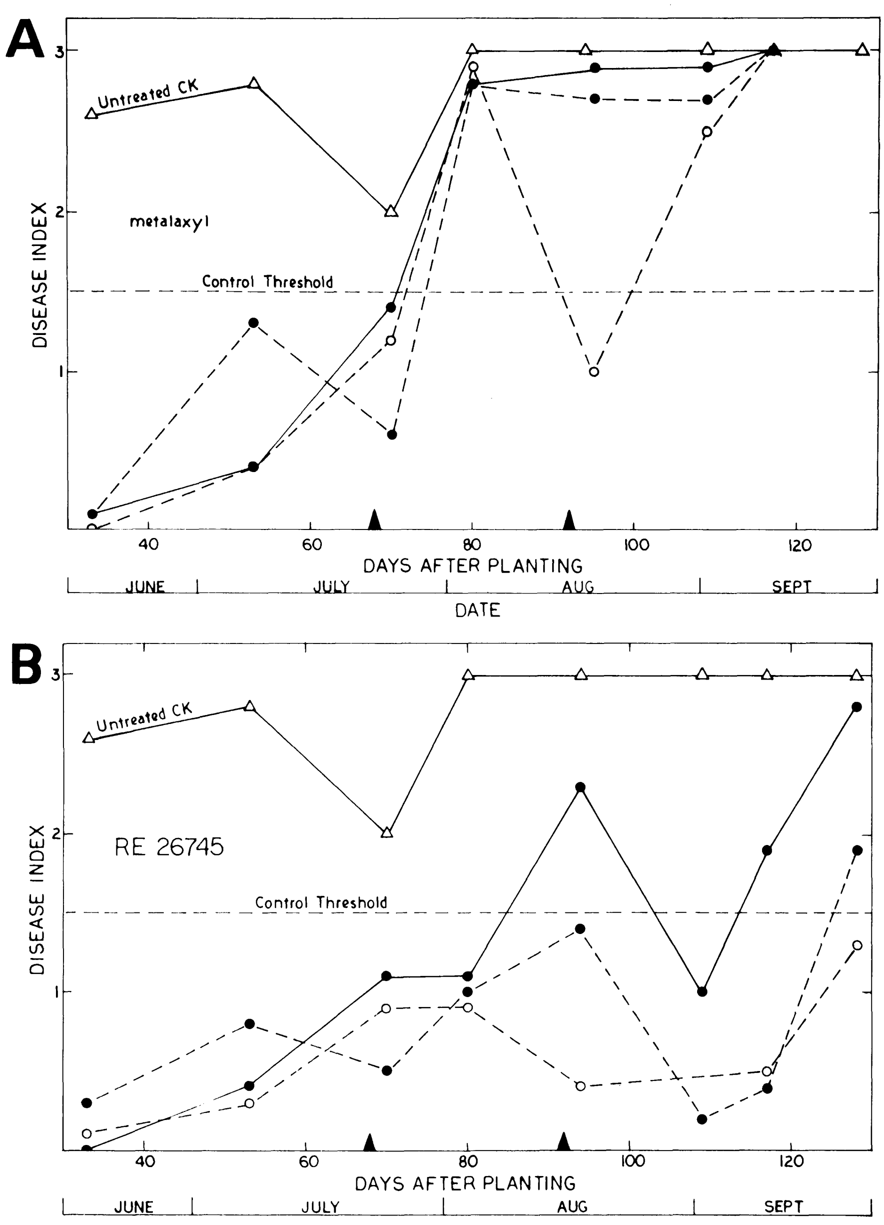

Fig. 3. Assessment of control of *Phytophthora infestans* in 1980 on excised leaves from field-grown potato plants treated with metalaxyl **(A)** or RE26745 **(B)**. Treatments were 2.2 kg/ha in-furrow at planting (solid lines) or 1.1 kg/ha in-furrow followed by 1.1 kg/ha at flowering (dashed line, solid circles) or the same treatment followed by 0.6 kg/ha in mid-August (dashed line, open circles). Black triangles on horizontal axes indicate dates of foliar applications. Disease severity evaluated as shown in Figure 1. Control threshold set at 1.5 as in Figure 2. $LSD_{0.05} = 0.64$.

blight (*A. solani*) could be applied in a few timely applications. These systemic fungicides could be applied in the tank mix or as a supplementary soil drench. The program would provide late-blight protection to the foliage throughout the season without the necessity of numerous, precisely timed foliar applications, while assuring that protection was continued even when weather patterns favoring blight development might preclude spray applications (12).

Recently, there has been much concern about the potential development of resistance to metalaxyl and similar fungicides. Although initial reports concerning *P. infestans* indicate that resistant strains selected in vitro are not pathogenic in vivo (23), more recent results with in vitro strains of *P. megasperma* Drechs., *P. capsici* Leon., and *Pythium graminicola* Subr. have shown in vivo pathogenicity as well as cross-resistance to RE26745 (3,5). Naturally occurring resistant strains of *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow have been found in greenhouse-grown cucumbers and outdoor melons in Israel (14) and Greece (11). Resistant strains were also collected from metalaxyl-treated tobacco infected with *Peronospora tabacina* Adam in North Carolina (2) and were associated with failures of metalaxyl to control *P. infestans* in Holland during 1980 (6).

Objections have been raised to soil application of systemic fungicides because continuous selective pressure may be exerted favoring resistant forms of pathogens, particularly when residues in plants become exhausted (7). Although caution is necessary in the introduction of these systemic fungicides into commercial late-blight control programs, continuous pressure from potato late blight is not a factor in Ohio. The pathogen is certainly not present at all times or in all fields, but it generally appears somewhere in the state every year, probably from introduction on infected seed stock. Although use of these fungicides in seed production areas may be unwise because of the potential for widespread dispersal of any resistant strains that might arise, the risk in commercial production areas where occurrence of the disease is sporadic would be much less. Use of these fungicides in soil could be followed by foliar applications of tank mixtures with wide-spectrum protectant fungicides that would minimize the development of resistant strains (9,21).

Two alternatives to soil application have been proposed. One is to formulate these materials with a nonsystemic, foliar protectant ethylenebisdithiocarbamate fungicide and make applications at low rates at 14-day intervals. The other is to reserve use of these compounds only for eradication of established late-blight infestations when traditional spray programs have failed (7,10). Both these approaches may appear more cautious than soil applications, but they also have many shortcomings. They completely forgo the advantages of a reduction in the total number of applications and do not solve the problem presented when weather patterns favoring late-blight development also preclude further spraying. They also disregard poor foliar absorption of metalaxyl and its lack of translocation to lower leaves within the canopy where late blight often develops.

In relation to the resistance problem, spraying with low rates may lead to sublethal doses of fungicide within much of the foliage (as was seen with 14-day applications in our 1980 and 1981 field plots) with the potential for selection of resistant strains (7,21). This may become a problem even if combinations with ethylenebisdithiocarbamate fungicides are applied at 14-day intervals because the latter will be dissipated in 5–7 days, leaving only the systemic material. The eradicant approach has the disadvantage of exposing billions of spores to the fungicide at one time, thus increasing the possibility of selecting a resistant mutant already existing within the population. In practice, future selection of one system or another will depend upon relative costs, available methods of application, expected occurrence of late blight in a given locality, and further experience with the development of resistant strains of *P. infestans*.

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