Quantification of Factors Influencing Potato Late Blight Suppression and Selection for Metalaxyl Resistance in *Phytophthora infestans*: A Simulation Approach

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ABSTRACT

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A computer simulation model was validated and used to evaluate strategies for management of potato late blight and resistance in *Phytophthora infestans* to the systemic fungicide metalaxyl. Subjects investigated included the frequency of metalaxyl applications (same total dosage per season), comparison of mixtures of metalaxyl and the protectant chlorothalonil versus alternations of the two fungicides, dosages of component fungicides in a mixture, timing for a limited number of metalaxyl applications, use of host resistance, and pathogen fitness. The study investigated disease management for foci initiated by single pathogen genotypes. Metalaxyl/chlorothalonil mixtures performed better than alternating metalaxyl and chlorothalonil sprays in suppressing both the metalaxyl-sensitive and -resistant pathogen when the same total dosages and number of sprays were applied during the season. More frequent sprays applied at lower rates (same total amount of fungicides per season) resulted in less disease caused by either metalaxyl-sensitive or -resistant strains for both mixtures or alternations. Fewer applications of the mixture (replaced by protectant sprays) achieved substantially improved control of the metalaxyl-resistant pathogen. An application of the mixture once in the middle of the season (about 8 wk after emergence in a 12-wk-long season) resulted in the least final disease for the metalaxyl-sensitive strain. Field experiments performed with a metalaxyl-sensitive strain indicated that a mixture application in the middle of the season (5–7 wk after emergence in a 11- or 12-wk season) was most effective in suppressing disease. However, simulations using the metalaxyl-resistant strain showed that application of the mixture once late in the season resulted in the least disease. Adjustments in fungicide rates or timings were less effective in suppressing the metalaxyl-resistant pathogen than use of moderately resistant cultivars. Small differences in aggressiveness between the strains had little influence on the selection for metalaxyl resistance. However, large reductions (such as 50%) in the aggressiveness of the metalaxyl-resistant strain could nullify selection for resistance. Identification of the "best" strategy for suppression of metalaxyl resistance depended somewhat on whether evaluation was based on the ratio of resistant to sensitive individuals or on the total number of metalaxyl-resistant individuals.

Resistance of fungal pathogens to fungicides has become increasingly common and has resulted in disease control problems (3,12). The threat of fungicide resistance has forced a reevaluation of disease management strategies using a more holistic analysis. Not only is short-term disease control important, but also care must be taken to avoid strategies which result in excessive selection for fungicide-resistant strains of the pathogen. Unfortunately, strategies effective in reducing selection for fungicide resistance may be less effective in overall disease suppression. Thus, the final strategy selected is likely to be a compromise between the potentially conflicting goals of disease suppression and of fungicide resistance suppression.

Several strategies have been proposed for the use of a fungicide with potential pathogen-resistance problems (12,31). These include using the fungicide in mixture with another effective fungicide, alternating applications of the fungicide with another fungicide, and integrating use of the at-risk fungicide with other methods of disease management (e.g., resistant cultivars) (12). It has also been proposed that a high dosage be used for the companion fungicide in the mixture, that application intervals not be excessive, and that the number of applications during the season of the at-risk fungicide be limited (31).

Modeling studies (8,12,19,20,22,24,29) have been crucial for developing strategies for managing fungicide resistance in plant pathogens because of the difficulty and expense of performing field experiments investigating fungicide resistance. However, these modeling studies (with one exception [24]) have used very simple and general models. Such simple models may not realistically simulate the dynamics of the pathogen population and of the fungicides in the field. There has been a lack of comprehensive evaluation of strategies due to the expense and difficulties of field experiments and lack of realistic models.

The systemic fungicide metalaxyl is very effective against *Phytophthora infestans* (Mont.) de Bary (4,16), the causal agent of late blight of potatoes (*Solanum tuberosum* L.). Although metalaxyl-resistant strains of *P. infestans* have led to failures in late blight control in some locations (11), no metalaxyl-resistant strains have yet been found in northeastern United States, and there is a desire to avoid problems due to resistance. Thus, strategies that minimize selection for fungicide resistance need to be evaluated and implemented.

The objective of our study was to quantify the relative contributions to late blight control of various fungicide-use strategies that may suppress the development of metalaxyl resistance in pathogen populations. A complex and realistic model simulating the life cycle of *P. infestans* and the complex dynamics of metalaxyl and the protectant fungicide chlorothalonil was used to evaluate several strategies. Our study expands and extends the work of Milgroom and Fry (23,24) in that their simulation model has been revised, and the simulations have considered more factors and a greater number of strategies and have quantified the relative contributions of the factors. Additionally, the present study investigated disease management under the assumption that an individual focus was initiated by a single pathogen genotype, whereas previous studies have assumed physical mixtures of resistant and sensitive genotypes in the same focus.

MATERIALS AND METHODS

Revision and validation of the simulation model. A late blight model had been created to simulate epidemics in experimental plots (16 m^2) in central New York State (5–7,23). The model, which has been developed over the last decade, simulates the life cycle of *P. infestans* (5) and the use of two fungicides, the protectant chlorothalonil (Bravo 500; Fermenta Plant Protection Co., Mentor, OH)(6,7), and the systemic metalaxyl (Ridomil 2E; Ciba-Geigy Corp., Greensboro, NC) (23,25). For chlorothalonil, the model considers spray deposition, spatial distribution of residues in the canopy, fungicide decay and weathering, and inhibition of fungal infection. For metalaxyl, the model considers the distribution of fungicide, decay over time, and inhibition of infection, lesion expansion, and sporulation by the fungus. The simulation model was written in the C programming language and run on IBM microcomputers.

The late blight simulation model was revised for use in this simulation study. A simple host growth submodel was added which represented typical changes in leaf area index similar to the patterns presented in Ivins and Bremner (18) but based on observations of potato foliage growth in central New York State. Some of the model parameters were adjusted using results from field epidemics (these data were not used for validation) to enable more accurate prediction of disease progress when the actual amount of initial inoculum was input into the model.

The resultant version of the model was subjected to a more comprehensive validation than the earlier version. Data for validation were obtained from field experiments performed in Freeville, NY. All treatments were applied to small plots (4 × 4 m) of potato cultivars which had been inoculated with *P. infestans*. Only metalaxyl-sensitive isolates of *P. infestans* were used as inoculum in the field experiments. Results of 34 different treatments from 6 yr were used in validation (none had been used for construction of the model). Fourteen of the treatments were not sprayed with fungicides, eleven were sprayed with metalaxyl, and nine were sprayed with chlorothalonil. Seventeen of the treatments used potato cultivars susceptible to late blight, and seventeen treatments used resistant cultivars. More specific details of cultural manipulations, experimental treatments, and disease assessment results have been reported elsewhere (14–16). Assessments of percentage of defoliation due to late blight were taken every 3–7 days. Weather data were monitored with hygrothermographs and rain gauges. The inputs for the model were the number of sporangia applied to foliage of the plot as initial inoculum and weather data. The area under the disease progress curve (AUDPC) (calculated as given by Shaner and Finney [27]) and the final percentage of foliage diseased predicted by the model were compared with those observed.

As a further test of the simulation model, we compared model predictions with the observed defoliation for the 1987 field experiment to determine whether the model performed well when the number of mixture sprays was limited. The inputs for the simulation model were the amount of initial inoculum, day of inoculation, days for spraying, fungicide dosages, and weather data (gathered from a hygrothermograph placed in the field during the epidemic for the 1987 field experiment). The method of inoculation for the 1987 field experiment was different from that assumed for the model. Therefore, to determine an equivalent amount of initial inoculum for use with the model, the total lesion area resulting from the field inoculation was measured for each plot 7 days after inoculation.

Field experiments. Field experiments were performed in 1987 and 1988 to determine when a single application of the metalaxyl/chlorothalonil mixture would most effectively suppress late blight. Experiments were performed in small plots separated by 5-m-wide fallow areas at the Homer C. Thompson Vegetable Research Farm of Cornell University near Freeville, NY. Certified potato seed tubers were planted 23 cm apart in four rows which were spaced 0.9 m apart and 3.7 m long. Experimental fungicides were applied with a tractor-mounted boom-sprayer at 470 L/ha and 880 kPa. There were three treatments with a single metalaxyl/chlorothalonil mixture spray (Ridomil/Bravo 81W) (0.15 and 1.21 kg a.i./ha of metalaxyl and chlorothalonil, respectively) applied at different times in the season. Each treatment also consisted of weekly applications of the protectant chlorothalonil (Bravo 720) (0.42 kg a.i./ha), except for the wk that the mixture was applied and the subsequent wk when no fungicide was applied. A reference treatment consisted exclusively of weekly applications of chlorothalonil (0.42 kg a.i./ha). Lower dosages were used so that differences in disease severity between treatments would be easier to assess. In 1987, the mixture applications for the different treatments were applied at 40, 54, or 68 days after emergence; and in 1988, they were applied at 34, 48, or 62 days after emergence.

Plots were inoculated with a suspension of sporangia obtained from cultures on plates of V-8 juice medium (26). Prior to inoculation with metalaxyl-sensitive isolates, plots were sprinkler irrigated to assure a favorable microclimate for infection. One leaf was inoculated in each quadrant of the plot. One drop (approximately 0.05 ml) of the suspension was applied per leaflet on half of a leaf one night (about 8 p.m.) and on the other half of the leaf the next night. Sprinkler irrigation (0.2 cm/hr) (0.5 hr in the morning and in the evening) was used throughout the epidemic to aid disease development. AUDPC (27) was used as a measure of disease suppression.

In 1987, the susceptible cultivar Hudson was planted on 2 June. There were four replications in a completely randomized design. Median emergence occurred on 19 June. The plots were inoculated with *P. infestans* (50,000 sporangia per ml) on 21 and 22 July. The fungicide sprays were initiated on 29 July. The amount of defoliation was assessed every 3 or 4 days until 11 September when the potato vines were mowed (vinekill). Tubers were dug from the field on 28 September and graded and weighed on 19 October.

In 1988, the moderately resistant cultivar Sebago was planted on 1 June. There were four replications in a randomized block design. Median emergence occurred on 22 June. The plots were inoculated with *P. infestans* (20,000 sporangia per ml) on 18 and 19 July. The fungicide sprays were initiated on 26 July. The amount of defoliation was assessed once a week until 6 September (vinekill). Although tubers were harvested, the results are not presented here because disease levels were very low.

General method for simulation studies. Simulations were run for 50 yr using a weather data set consisting of weather data derived from National Oceanic Atmospheric Administration weather records for Steuben County, NY (an important potato-producing county 100 km west of Freeville). The mean season length (time from median emergence of the potato foliage to vinekill) for the simulations was 80 days and ranged from 77 to 83 days.

Simulations were run separately for metalaxyl-resistant (MR) and -sensitive (MS) strains. In a small area of a field (like that considered by the model) commonly only a MS or MR strain has been found (10,11). The model was considered to simulate a disease focus of a single genotype. We assumed equal fitness of the MR and MS strains except in the simulation study on the effect of altered fitness. The MR strain was assumed to be completely unaffected by metalaxyl.

The general conditions for the simulation studies were as follows: 0.5 colonies per plant (unless otherwise stated) initiated 25 days after median emergence of the potato plants. The first fungicide spray was always on day 28. The potato cultivar was susceptible to late blight (unless otherwise stated). The two fungicides in the simulations were metalaxyl and chlorothalonil applied separately or in combination. During these experiments, metalaxyl was commercially available in the United States for use on potatoes only as prepacked metalaxyl/chlorothalonil mixtures. The dosages used varied according to the treatment in the study. However, the standard treatments (based on 1987 label recommendations) were chlorothalonil (Bravo 720) applied on a 7-day schedule at 1.26 kg a.i./ha and a metalaxyl/chlorothalonil mixture (Ridomil/Bravo 81 WP) applied on a 14-day spray schedule at 0.20 and 1.61 kg a.i./ha for metalaxyl and chlorothalonil, respectively.

Experimental treatments were evaluated using final disease and AUDPC. Final disease was used as an indicator of the pathogen population size. Increases in metalaxyl resistance in the pathogen population were estimated by two variables: the amount of final disease caused by the MR strain and the ratio of the final disease caused by the MR strain to the final disease caused by the MS strain. Since low AUDPCs are associated with small yield losses (15,16), AUDPC was considered a measurement of disease suppression as it relates to yield loss.

Comparison of mixtures, alternations, and dosages. The use of mixtures was compared with alternating applications of metalaxyl and protectant fungicides. Frequency of application and fungicide dosages were varied such that the total amounts of fungicides applied per season were the same for all treatments (0.80 and 6.44 kg a.i./ha of metalaxyl and chlorothalonil, respectively). These amounts of fungicides were equal to the amounts applied in four applications of the standard mixture formulation (spray dosages and intervals as recommended by the 1987 label). Four types of alternation schedules were considered: 1) The first spray was metalaxyl and the time intervals between sprays were equal; 2) the first spray was protectant and intervals between sprays were equal; 3) the first spray was metalaxyl and the time interval after a metalaxyl spray was double that after a protectant; and 4) the first spray was protectant and the time interval after a metalaxyl spray was doubled.

Adjustment of component dosages in the mixture. The influence of dosages of metalaxyl and chlorothalonil in the mixture on disease control and on selection for metalaxyl resistance was determined. All possible combinations of three dosages of metalaxyl (0.10, 0.20, and 0.30 kg a.i./ha) and five dosages of chlorothalonil (0.5, 1.0, 1.5, 2.0, and 2.5 kg a.i./ha) were evaluated. All treatments were applied on a 14-day spray schedule.

Limited number of mixture sprays. The effect of different numbers and different timings of metalaxyl/chlorothalonil sprays per season on suppression of late blight and metalaxyl resistance was investigated via simulation. Each mixture application strategy was integrated with the appropriate combination of protectant applications. The number of mixture sprays ranged from a maximum of four (no protectant applications) to a minimum of zero (only protectant). The interval between sprays after a mixture spray was always 14 days and after a protectant spray was 7 days. For one, two, or three mixture sprays per season, all possible timings of sprays were simulated (within the restriction that spray intervals were 7 or 14 days after protectant or metalaxyl spray, respectively).

Host resistance. The interaction between host resistance to late blight and dosage of the mixture as reflected by the suppression of disease and of metalaxyl resistance was determined. Six different spray treatments were evaluated on the susceptible and moderately resistant host. Each schedule consisted of a different fraction of the standard mixture dosage (ranging from 0.25 to 1.5 × the standard dosage) being applied on a 14-day schedule.

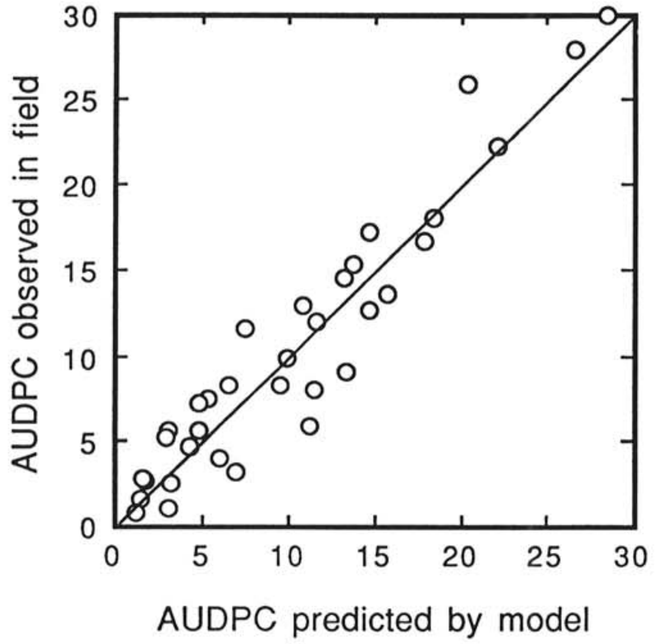

Fig. 1. Comparison of the area under the disease progress curve (AUDPC) for potato late blight epidemics predicted by the model with the AUDPCs observed in field experiments. The line is for perfect agreement between the predicted and the observed values and is not a regression line. Data points represent 34 different epidemics over 6 yr and were not used in model development.

Influence of fitness of metalaxyl-resistant strains. Because pathogen fitness may influence the effectiveness of management strategies, the impact of decreased or increased fitness of MR strains on disease control and selection for metalaxyl resistance was quantified. The fitness was altered by adjusting values for three components of aggressiveness in the model (relative lesion radial expansion rate, relative sporulation rate, and relative infection efficiency). Three fungicide treatments were used: the standard protectant; the standard mixture; and, for reference, a metalaxyl treatment (0.36 kg a.i./ha on a 14-day schedule).

RESULTS

Validation. The AUDPCs predicted by the simulation model were close to the AUDPCs actually observed in field experiments (Fig. 1). The predicted AUDPCs appear randomly dispersed about the line of perfect fit which indicates that the model was not consistently over- or underpredicting disease (Fig. 1). From regression analysis, we found that (observed AUDPC) = −0.14 + 1.02 (predicted AUDPC) ($r^2 = 91\%$). The model predicted AUDPC well since the intercept and slope were not significantly ($P > 0.05$) different from the 0.0 and 1.0, respectively, expected for a perfect fit. The model also predicted the final disease severity (percentage of foliage diseased) with reasonable accuracy. From regression analysis we found that (observed severity) = 3.2 + 0.92 (predicted severity) ($r^2 = 71\%$). The coefficients 3.2 and 0.92 were not significantly ($P > 0.05$) different from 0.0 and 1.00, respectively.

Using the weather data and amount of initial inoculum present for the 1987 field experiment, the simulation model predicted (Fig. 2B) that the late blight epidemics would progress similarly to those observed in the field (Fig. 2A). For example, the disease

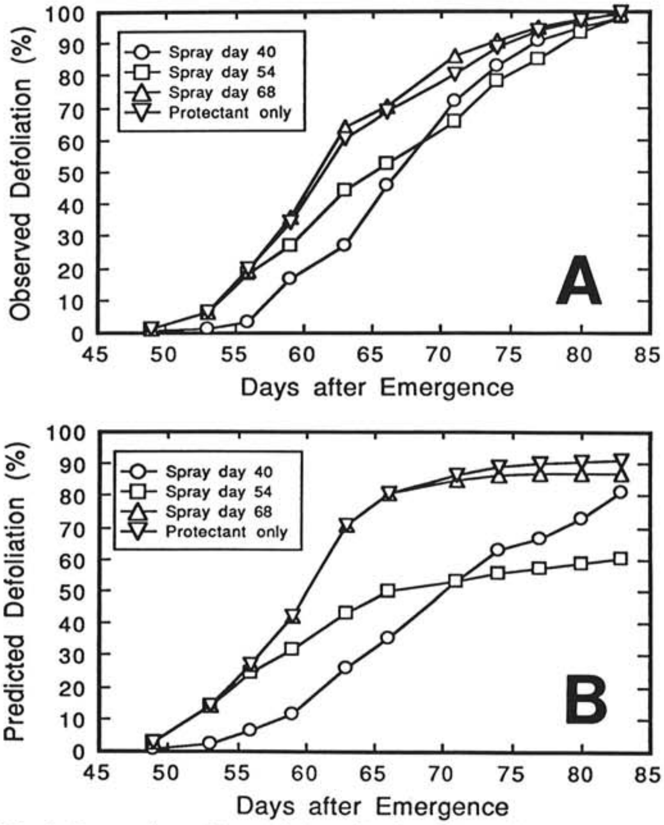

Fig. 2. Progression of late blight epidemics with a single metalaxyl/protectant mixture spray applied at various times during the season against a background of weekly protectant fungicide applications. **A,** Field observations from the 1987 experiment. **B,** Model prediction for disease progressions using the weather data and the initial disease observed in 1987.

progress curves resulting from the treatment with mixture spray applied on day 40 and the treatment with mixture applied on day 54 intersected about week 10 for both the predicted and the observed. In agreement with field observations, simulation analysis indicated that the timing which resulted in lowest AUDPC was application of the mixture on day 40, but the timing which resulted in lowest defoliation at the end of the season was application on day 54. Similar to the observed, there was little difference in the foliage blighted resulting from a late mixture spray and the protectant-only treatment. At the end of the season, the ranking of the treatments according to total defoliation was the same for observed and predicted, although the model predicted less defoliation than observed. The AUDPCs predicted by the model were 12.5, 14.1, 21.1, and 21.4 proportion-days for the treatments with the earliest, mid, latest, and no-mixture sprays, respectively. The observed AUDPC had a similar pattern (Table 1).

Comparison of mixtures and alternations. In general, mixtures were more effective than alternations in suppressing disease and limiting metalaxyl resistance. Use of mixture sprays (relative to alternations) resulted in smaller AUDPCs (not shown) and less final disease caused by either the MS strain (Fig. 3A) or MR strain (Fig. 3B) when the same number of sprays and same total amounts of dosages were applied during the season. More frequent applications (with the same total dosages for the season) increased the efficacy of all treatments (Fig. 3A and B). Use of frequent (eight) alternations was more effective than use of four applications of the standard mixture. For MS strains, eight alternations produced lower levels of AUDPC and final disease than did four mixture applications (Fig. 3A). For MR strains, use of eight alternations resulted in approximately the same levels of AUDPC and final disease as did the application of four mixture applications (Fig. 3B).

No one alternation treatment was clearly superior to the other alternation treatments in terms of decreasing the AUDPC or final disease severity (Fig. 3A and B). When the pathogen was sensitive, all treatments resulted in small AUDPCs and low levels of final disease (Fig. 3A). When the pathogen was resistant, all treatments resulted in large AUDPCs and high levels of final disease (Fig. 3B).

For almost all treatments involving metalaxyl, the resulting ratio of disease caused by the MR strain relative to disease caused by the MS strain was high (greater than 10) (Fig. 3C). When more than four sprays were applied per season, use of mixtures resulted in an increase of MR relative to MS that was substantially higher than those for any of the alternation treatments (Fig. 3C). In general, as the number of sprays applied per season was increased (beyond four sprays), the ratio of MR to MS blight was higher, because the MS pathogen was very effectively suppressed.

Adjustment of component dosages in the mixture. Increased dosages of metalaxyl in the mixture reduced the final level of disease caused by the MS pathogen but made no difference in the final level of disease caused by the MR pathogen (the MR

TABLE 1. Influence of different timings of a single metalaxyl/chlorothalonil mixture spray on potato late blight in the 1987 field experiment

Spray day[u]	Disease (%)[v]	AUDPC[w]	Yield (× 1000 kg/ha)[x]	Tuber rot (%)[y]
40	98.1	15.9	19.4	7.7
54	97.7	17.2	16.4	6.0
68	99.1	20.9	11.8	3.0
none[z]	99.5	20.0	12.6	8.5
$LSD_{0.10}$	0.9	0.9	2.1	3.4
$LSD_{0.05}$	1.2	1.1	2.6	4.2

[u] Days after emergence that single mixture spray (0.15 and 1.21 kg a.i./ha of metalaxyl and chlorothalonil, respectively) was applied in an otherwise 7-day protectant spray scheme. Protectant fungicide applications (0.42 a.i./ha chlorothalonil) were resumed 14 days after the mixture spray.

[v] Percentage of foliage that had been diseased during the season.

[w] AUDPC = area under the disease progress curve. Data presented in units of porportion-days.

[x] Yield consisted of all rot-free tubers which had a diameter greater than 5.0 cm.

[y] Percentage of total number of tubers with diameters greater than 5.0 cm that had shown symptoms of rot. Besides tubers with visible late blight lesions, tubers with soft rot were included since soft rot often masks late blight symptoms.

[z] For this treatment, only the protectant fungicide was applied (on a 7-day schedule).

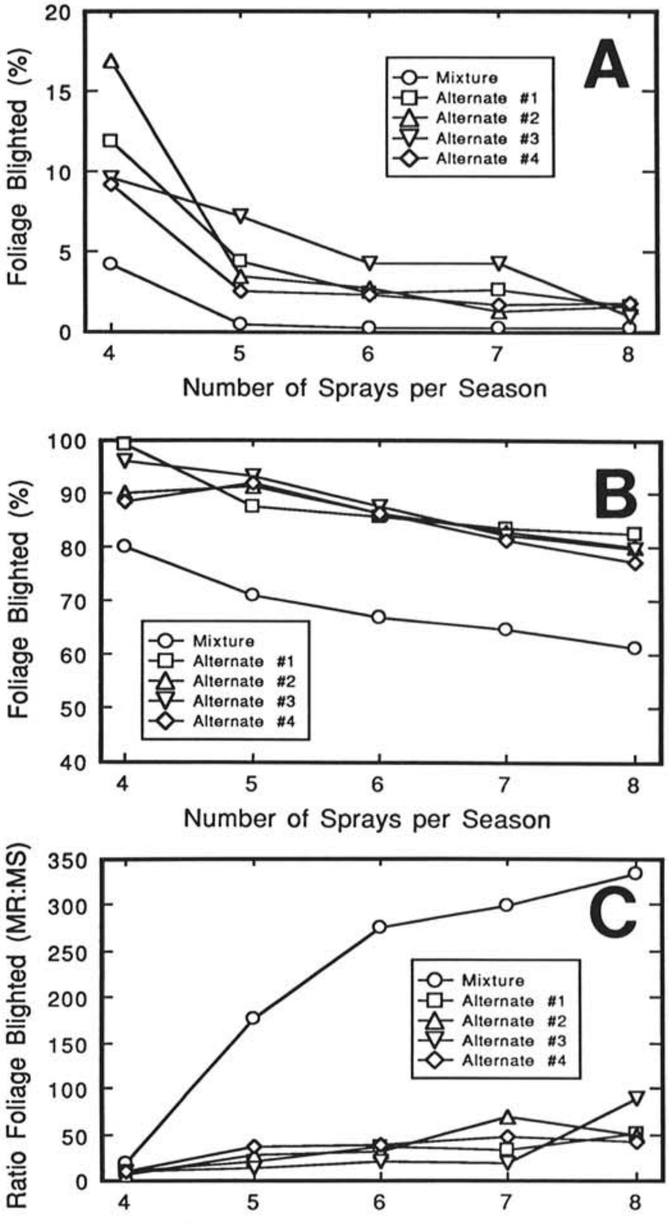

Fig. 3. Effect of metalaxyl/chlorothalonil mixtures and alternations of metalaxyl and chlorothalonil sprays on potato late blight development caused by metalaxyl-sensitive (MS) or -resistant (MR) strains of *Phytophthora infestans*. All treatments received the same amount of total fungicide (0.80 and 6.44 kg a.i./ha of metalaxyl and chlorothalonil, respectively) for the season. There were four alternation treatments: 1) first spray was metalaxyl and there were equal time intervals between sprays; 2) first spray was chlorothalonil and there were equal spray intervals; 3) first spray was metalaxyl and the time interval after metalaxyl spray was double that after chlorothalonil; and 4) first spray was chlorothalonil and the time interval after the metalaxyl spray was double that after the chlorothalonil. Results are means from simulations of 50 years. **A,** Percentage of foliage that had been diseased at the end of the season when the pathogen was metalaxyl sensitive. **B,** Percentage of foliage that had been diseased at the end of the season when the pathogen was metalaxyl resistant. **C,** Ratio of the disease caused by the metalaxyl-resistant strain to that caused by the sensitive strain.

strain was assumed to be completely insensitive to metalaxyl) (Fig. 4A). Adding more protectant to the mixture resulted in decreases in the amount of final disease caused by both the MR and MS strains (Fig. 4A). However, for all dosages of the protectant in the mixture, there were high final disease levels (>75%) for the MR pathogen. Adjusting the metalaxyl dosages in the mixture resulted in substantial differences in the ratio of disease caused by MR and MS strains (Fig. 4B). Increases in the amount of protectant produced a slightly higher ratio of disease caused by MR relative to MS strains (Fig. 4B).

Limited number of mixture sprays: Field study. The timing of the mixture spray affected disease suppression. Application of the mixture in the middle of the season (5–7 wk after emergence) resulted in the lowest AUDPC (Tables 1 and 2). In 1987, the treatment with the mixture applied on day 40 resulted in significantly ($P < 0.05$) higher tuber yields than the other treatments (Table 1). However, applying the mixture later in the season (day 68) resulted in significantly fewer rotten tubers (Table 1). When the mixture was applied late in the season (2 wk before vinekill), there was little or no reduction in AUDPC compared to the reference treatment of protectant alone (Tables 1 and 2).

Final disease was not necessarily correlated with AUDPC and yield loss. For example, in 1987 the lowest AUDPC and highest yield were associated with the earliest applied mixture spray (day 40), but the lowest final disease occurred in the treatment with a mixture spray applied later (day 54). (Three days before the end of the season, the treatment with the spray applied on day 54 had significantly [$P < 0.05$] less disease than any of the other treatments.) Applying the mixture spray late in the epidemic in 1988 resulted in the least number of sporulating lesions at the end of the season, although it did not have the least final disease (Table 2).

Limited number of mixture sprays: Simulation study. As expected, fewer numbers of mixture sprays applied in a spray schedule of weekly protectant sprays (relative to standard number of mixture sprays) achieved less suppression of disease caused by sensitive strains, but greater suppression of disease caused by resistant strains (Fig. 5). However, the time during the season in which the mixture spray(s) occurred had a noticeable impact on disease suppression both by the MR strain and by the MS strain. The range of effects associated with specific timings is indicated by the vertical bars in Figure 5. Thus, some spray schedules with a single mixture application were more effective at suppressing disease caused by the MS pathogen than were some schedules with two mixture applications. Typically, the most effective applications for suppressing disease caused by the MS

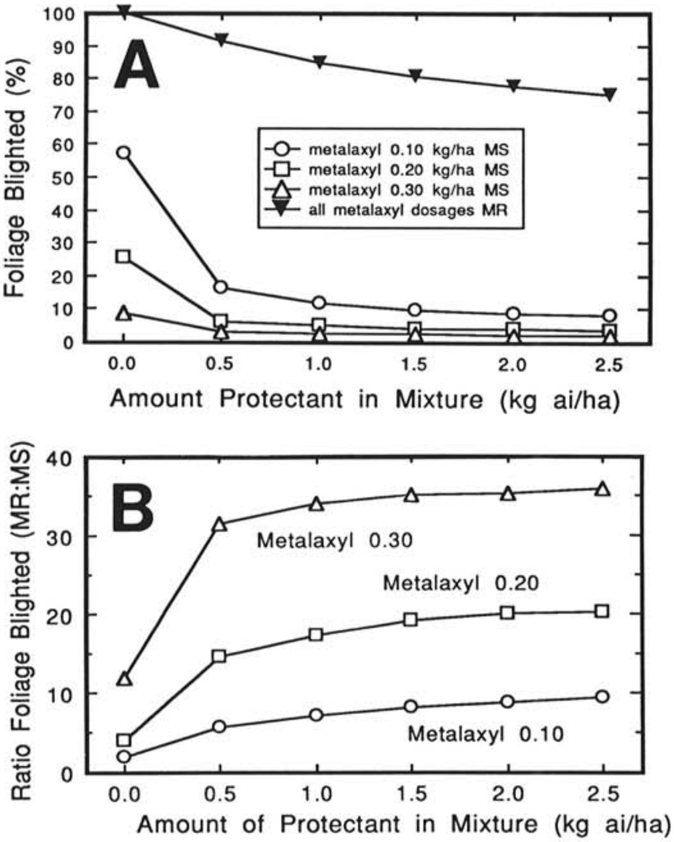

Fig. 4. The effectiveness of various dosages of metalaxyl and the protectant chlorothalonil in mixture on metalaxyl-sensitive (MS) and -resistant (MR) strains of *Phytophthora infestans*. Results are means from simulations of 50 years. All metalaxyl/chlorothalonil mixtures were applied on a 14-day schedule. Three amounts of metalaxyl (0.10, 0.20, and 0.30 kg a.i./ha) were used with the amounts of protectant identified on each graph. There were no differences due to different amounts of metalaxyl when treatments were applied to the metalaxyl-resistant pathogen. **A,** Increase in MS or MR phenotype as indicated by the percentage of foliage diseased. **B,** Relative resistance increase as indicated by ratio of disease caused by the metalaxyl-resistant strain to that caused by the sensitive strain produced under the experimental treatments.

TABLE 2. Influence of different timings of a single metalaxyl/chlorothalonil mixture spray on potato late blight in the 1988 field experiment

		Infected leaflets[t]		
Spray day[u]	Final disease (%)[v]	AUDPC[w]	No.	Log[x]
34	41	3.2	31	1.42
48	27	2.7	15	1.09
62	39	3.6	2	0.31
none[y]	52	4.1	73	1.83
$LSD_{0.10}$	13	0.8	...[z]	0.31
$LSD_{0.05}$	16	1.0	...[z]	0.38

[t] The number of leaflets per plant at the end of the season which had lesions with visible sporulation.

[u] Days after emergence that a single mixture spray (0.15 and 1.21 kg a.i./ha metalaxyl and chlorothalonil, respectively) was applied in an otherwise 7-day protectant spray scheme. Protectant fungicide applications (0.42 kg a.i./ha chlorothalonil) were resumed 14 days after the mixture spray.

[v] Percentage of foliage that had been diseased during the season.

[w] AUDPC = area under the disease progress curve. Data are presented in units of proportion-days.

[x] The common logarithm of the number of infected leaflets is presented for statistical comparisons.

[y] For this treatment, only the protectant was applied (on a 7-day schedule).

[z] Not applicable, since LSD is not appropriate without transformation because of unequal variances for treatments.

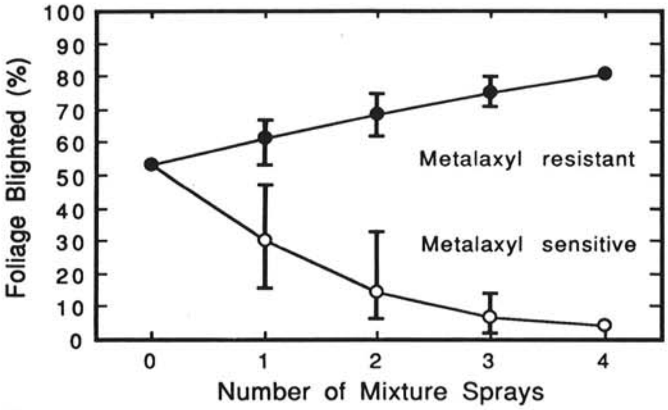

Fig. 5. Influence of the number of metalaxyl/chlorothalonil mixture applications and time in the season on suppression of late blight caused by metalaxyl-sensitive and -resistant strains of *Phytophthora infestans*. The vertical bars indicate the range in mean final disease severity achieved by different timings of mixture applications. The data points presented in the graph indicate the mean final disease achieved for all possible mixture timings. Each mixture application timing was incorporated into a weekly application schedule of protectant fungicide. There were seven distinct spray schedules for applying a single mixture spray per season, fifteen spray schedules for applying two mixture sprays, seven spray schedules for applying three mixture sprays, and one spray schedule for applying four mixture sprays.

strain occurred in the middle portion of the season, whereas the least effective applications occurred at the beginning and at the very end.

There were large differences in effectiveness of disease control for the various timings of a single mixture spray. Depending upon the date of application, the mean AUDPC ranged from 1.9 to 5.9 proportion-days for the MS strain and from 6.7 to 9.5 proportion-days for the MR strain. The final disease severity ranged from 15.7 to 48.7% foliage diseased for the MS strain and from 53.1 to 66.4% for the MR strain (Fig. 6A). Of the 50 yr simulated, application of the mixture in the eighth week for 66% of the years and in the seventh week for 34% of the years resulted in the least final disease. There tended to be slightly less final disease caused by the MR pathogen when the mixture was sprayed later in the epidemic (Fig. 6A). There was the highest ratio of disease caused by MR to disease caused by MS when the single mixture spray was applied in the middle of the season (week 8) (Fig. 6B).

There was a wide range in the effectiveness (as measured by AUDPC or final disease) of the various timings for two mixture sprays applied during the season. In general, application of the two mixture sprays in the middle of the epidemic was the most effective (smallest AUDPC and least final disease) against the MS strains. A treatment which effectively controlled the MS strain was usually (but not always) relatively ineffective at suppressing the MR strain. Applications of the mixture 5 and 8 wk after emergence resulted in the least final disease (6.1%) when the MS strain was present. Applications of the mixture 4 and 10 wk after emergence resulted in the least disease (62.8%) when the MR strain was present.

Host resistance. Use of a moderately resistant cultivar in combination with reduced dosages of both metalaxyl and chlorothalonil resulted in better suppression of disease and of metalaxyl resistance than did higher doses on susceptible cultivars. Application of half dosage of the standard mixture on a moderately resistant cultivar resulted in less disease caused by MR and MS strains (Fig. 7A) than did the full standard mixture rate on a susceptible cultivar. The final level of disease caused by MR strains was substantially less on the resistant cultivar than on the susceptible cultivar (Fig. 7A).

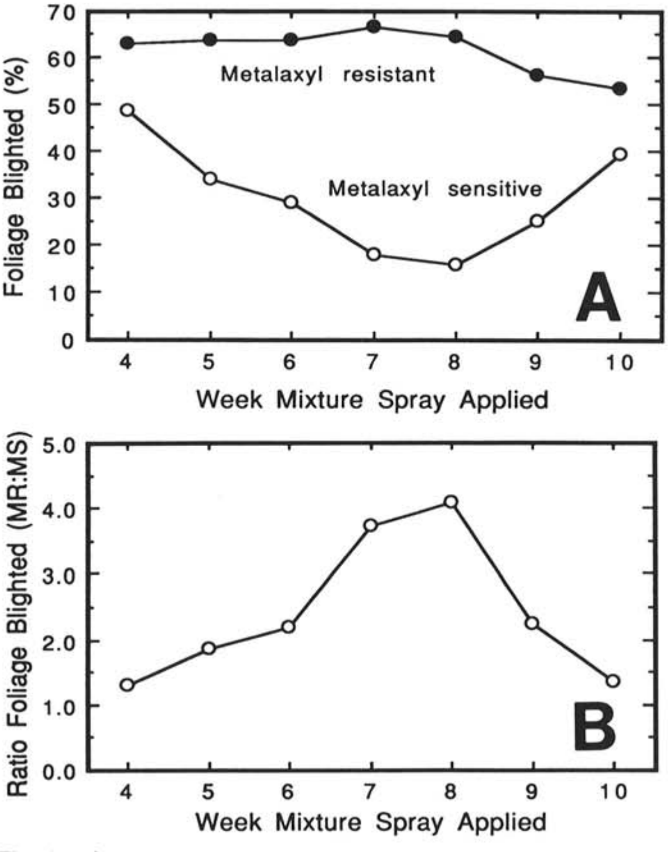

Fig. 6. Influence on potato late blight suppression of the timing of a single metalaxyl/protectant mixture spray applied at different times during the epidemic caused by metalaxyl-sensitive (MS) or -resistant (MR) strains of *Phytophthora infestans.* **A,** Percentage of foliage diseased by metalaxyl-sensitive or -resistant phenotypes. **B,** Ratio of disease caused by the metalaxyl-resistant strain to that caused by the sensitive strain produced under the experimental treatments.

The ratios of disease caused by MR relative to MS strains were much higher on moderately resistant cultivars than on susceptible cultivars (Fig. 7B). And as the dosage of the mixture was increased, the ratio of MR/MS increased. Both of these results derive from the especially small amount of disease caused by MS strains.

Influence of fitness of metalaxyl-resistant strains. Only large decreases in aggressiveness (relative lesion expansion rate, relative sporulation rate, or relative infection efficiency) of the MR strains substantially reduced the final level of disease caused by MR strains in the standard protectant and mixture treatments (Fig. 8A). When the MR strain had a relative lesion expansion rate half that of the MS strain, then the mixture provided excellent control of the MR strain (Fig. 8A). Decreased aggressiveness reduced the ratio of disease caused by MR to MS strains substantially for the standard mixture treatment (Fig. 8B). For example, when the MR strain had a relative lesion expansion rate half that of the MS strain, then use of the mixture resulted in a ratio of MR/MS sporangia of 2.3 (which was one-twelfth the ratio when the strains had equal lesion expansion rates) (Fig. 8B). Increased aggressiveness (relative colony expansion rate >

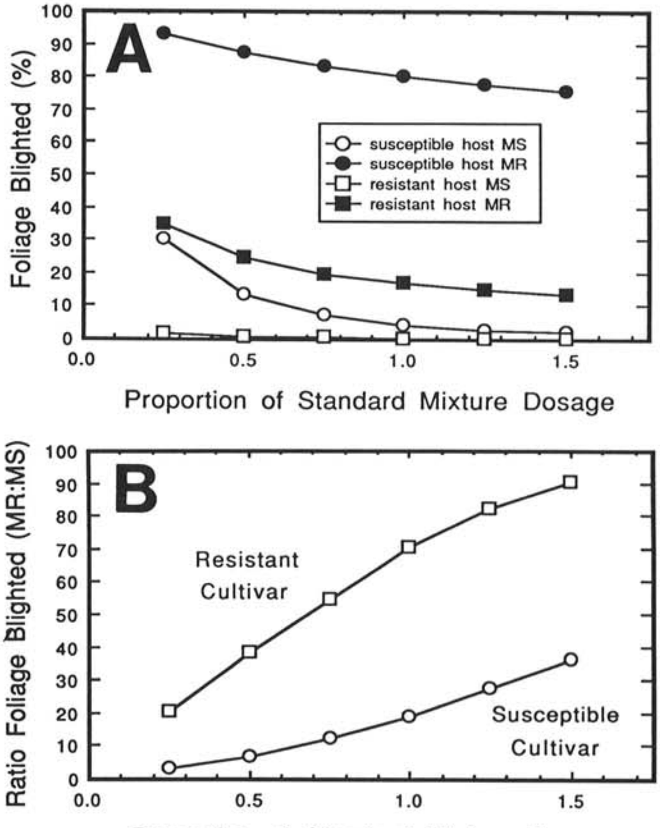

Fig. 7. Influences of different dosages of the standard metalaxyl/chlorothalonil mixture and of host susceptibility on potato late blight caused by metalaxyl-sensitive (MS) and -resistant (MR) strains of *Phytophthora infestans.* The results are means from simulations on 50 years. The standard mixture dosage (0.20 and 1.61 kg a.i./ha metalaxyl and chlorothalonil, respectively) was applied every 14 days. **A,** Percentage of foliage diseased by metalaxyl-sensitive or -resistant phenotypes. **B,** Relative resistance increase as indicated by ratio of disease caused by the metalaxyl-resistant strain to that caused by the sensitive strain produced under the experimental treatments.

1.0) resulted in a larger ratio of disease caused by MR to MS strains (Fig. 8B). Decreasing the relative sporulation rate or relative infection efficiency of the MR strain gave results similar to those observed with decreased relative lesion expansion.

DISCUSSION

Predictions by the simulation model compared favorably with the observed disease progressions by visual comparison (Fig. 1) and by simple statistical tests. The late blight simulation model performed reasonably well in indicating the relationships between the treatments in the 1987 experiment (Fig. 2). We did not expect the model to perfectly predict disease progressions in the field because the model is a simplification of reality and there are numerous factors and interactions involved in field experimentation that are not included in the model. We concluded from these tests that the model represents reality sufficiently well to provide credible evaluation of management strategies. Although many models have been used to evaluate strategies for managing fungicide resistance (8,19,20,22,29), these models have been much simpler than the model used in our study. A complex model provides more realism in the sense that there is consideration of more factors involved in the complex fungicide-pathogen-host interactions. The realistic simulation model for potato late blight provides a tool for quantitatively evaluating management strategies for the suppression of disease and metalaxyl resistance. Although our complex model is specific for a particular host-pathogen system, the conclusions still provide guidance for other systems.

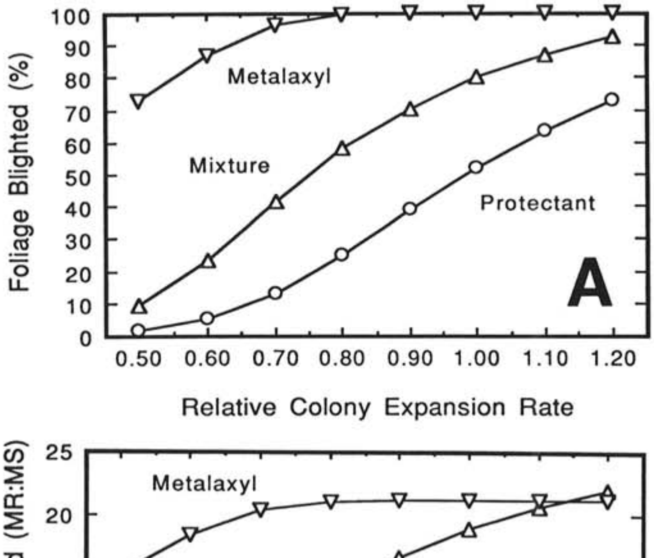

Fig. 8. The effect of aggressiveness of the metalaxyl-resistant (MR) strain of *Phytophthora infestans* on the effectiveness of the standard protectant (chlorothalonil), standard mixture, and metalaxyl-alone spray treatments. Results presented are means from simulations on 50 years. Aggressiveness was adjusted via relative colony expansion rate. **A,** Increase in metalaxyl resistance as indicated by the percentage foliage blighted by the metalaxyl-resistant strain. **B,** Relative resistance increase as indicated by ratio of disease caused by the metalaxyl-resistant strain to disease caused by the sensitive (MS) strain as influenced by use of metalaxyl alone, the protectant alone, or the mixture.

A fungicide-use strategy should limit selection for fungicide resistance. However, the evaluation of strategies for suppression of fungicide resistance requires knowledge of whether it is better to minimize the total number or the frequency (or ratio) of fungicide-resistant individuals. It can be seen that this is an important distinction by observing that the mixture treatments in our study resulted in the least disease caused by the metalaxyl-resistant pathogen (Fig. 3B), but also the highest ratio of metalaxyl-resistant to sensitive disease severity (Fig. 3C). The proportion of metalaxyl-resistant individuals in the population is influenced by the number of sensitive individuals. A high frequency (or proportion) of metalaxyl resistance may result from an especially small number of sensitive individuals, and, therefore, is not always an accurate measure of suppression of metalaxyl-resistant strains. Some models of selection for fungicide resistance consider only the frequency or proportion of fungicide resistance (8,20,29), although other models predict resistance buildup in terms of number of resistant individuals (12,19). The two approaches may consider different problems. The ratio of resistant to sensitive indicates the severity of the fungicide resistance problem. The amount of disease caused by the resistant strain indicates the severity of the disease suppression problem. We consider the increase in resistant individuals to be useful as well as the ratio of resistant to sensitive individuals for evaluating management strategies for the late blight system.

Unexpectedly, our study showed that increasing the amount of protectant in the mixture can actually result in more selection for metalaxyl resistance (larger ratios of disease caused by the metalaxyl-resistant strain to disease caused by the sensitive strain in Fig. 4B). These results are different from those we and others have obtained previously (24,28) which showed that increasing the efficacy of the protectant fungicide in the mixture would improve suppression of fungicide resistance. Another way in which results from our study differ from those of a previous study (24) is that use of a resistant cultivar resulted in a higher ratio of disease produced by the metalaxyl-resistant strain to disease caused by the sensitive strain (Fig. 7B). The explanation of these differences in results is the difference in assumptions concerning the extent that pathogen strains compete for healthy foliage. For our simulation studies, we assumed that little or no competition between strains of *P. infestans* occurs in the small area being simulated, and so simulations were run separately for the different strains. The other studies (24,28) assumed strong competition between strains. When we ran additional simulations with both strains together (strong competition), the ratio of disease caused by the resistant strain to disease caused by the sensitive strain decreased (unlike our results presented in Figure 4B) with increased protectant in the mixture (unpublished). Although the extent of competition between strains seems to be an important factor, it is difficult to accurately quantify competition and to adequately incorporate competition into a simulation model.

Several of the simple models predicted that use of a mixture would suppress fungicide resistance better than alternations of the two fungicides (19,20,22). Our simulation results confirmed this, since mixtures (relative to alternations) resulted in less disease caused by metalaxyl-resistant strains (Fig. 3B). However, use of mixtures resulted in a higher ratio of disease caused by resistant to disease caused by sensitive strains (Fig. 3C). Use of mixtures did result in smaller AUDPCs than use of alternations, indicating that mixtures are superior for general disease management. Prepacked metalaxyl/protectant mixtures have been used commercially instead of alternations, since this strategy is enforceable (31).

Although it has been recommended that a high rate of protectant be used in a mixture (31), we found that increasing the amount of the protectant chlorothalonil in the mixture (for example, from 1.0 to 1.5 kg a.i./ha) had only a small effect in reducing the final level of disease resulting from the metalaxyl-resistant strain (Fig. 4A), and even slightly increased the ratio of resistant to sensitive (Fig. 4B). A more effective use of fungicide resulted from changing from a 14-day (four sprays per season) to a 7-day (eight sprays per season but with the same total amount of each fungicide applied during the season) spray schedule (thereby substantially reducing the level of disease caused by the metalaxyl resistant strain [Fig. 3B]). Because of the high rate of weathering and decay (7), the protectant fungicide, chlorothalonil, which is responsible for control of metalaxyl-resistant strains, is not as effective in late blight control when applied at 14-day intervals.

Limiting the number of applications of the fungicide with the potential pathogen resistance problem should decrease selection for fungicide resistance (12). However, a trade-off is poorer control of the fungicide-sensitive pathogen strains. The importance of the specific timing of the metalaxyl/protectant mixture sprays is illustrated by the fact that a single, well-timed application of the mixture can be more effective in suppressing disease caused by the sensitive strain than two applications that are less well timed (Fig. 5). Conversely, two well-timed applications of the mixture (late in the season) can be less conducive to disease caused by a resistant strain than a single poorly timed application. Thus, for some comparisons the time during the season in which a metalaxyl application is made can be as influential on disease suppression (and fungicide resistance selection) as adjusting the number of mixture applications. By carefully selecting the timing (for example, applying in the middle of the season) of a decreased number of mixture sprays, a grower may obtain a substantial reduction in selection for metalaxyl resistance and excellent control of metalaxyl-sensitive strains.

Foliar growth of the host may influence which spray timing for the mixture is most effective in disease suppression. Application of a mixture spray was most effective against metalaxyl-sensitive strains during the middle of the season (Table 1 and Fig. 6A) corresponding to the period of most rapid foliage growth. Foliage area increases relatively slowly at first, increases very rapidly during the middle of the season, and then slows as foliage area approaches a maximum (1,18). Rapid foliar growth may result in more host tissue which needs more effective fungicidal protection.

Using different measurements (final disease, AUDPC, number of actively sporulating lesions, yield, or tuber rot) for evaluating disease control can result in slightly different conclusions regarding the most effective timing of metalaxyl sprays. For example, simulations and field results showed that mixture sprays applied early in the epidemic of the metalaxyl-sensitive strain tended to be more effective in lowering the AUDPC than in decreasing final disease. In the 1987 field experiment, applying the mixture on day 40 resulted in the lowest AUDPC and highest yield (Table 1). However, application of the mixture on day 54 resulted in the least final disease, and application on day 68 had the least tuber rot. Although in general, growers might be most interested in minimizing yield loss, limiting pathogen population growth would be of benefit in situations where there is concern over tuber blight, buildup of initial inoculum for next year's epidemic, or increase in a specific pathogen phenotype (i.e., metalaxyl resistance).

Moderately resistant cultivars would be especially useful in cases where fungicide-resistant strains may be present. Such cultivars reduce the risk of yield loss and limit the development of metalaxyl-resistant individuals in comparison to susceptible cultivars (Fig. 7A). The dosage of the standard mixture can be reduced in half on moderately resistant cultivars and still achieve effective disease suppression of the sensitive pathogen while causing little difference in the final level of disease caused by the metalaxyl-resistant strain (Fig. 7A). In fact, because the increase in metalaxyl resistance relative to sensitivity is so large on resistant cultivars with standard dosages (Fig. 7B), it might be even more desirable to use lower rates of fungicide on resistant cultivars.

Increased or decreased fitness of a metalaxyl-resistant strain changes the selection for metalaxyl resistance (Fig. 8A and B). Components of aggressiveness such as colony expansion rate, infection efficiency, or sporulation rate of the pathogen can vary greatly from one isolate of *P. infestans* to another (21,30). Unfortunately, decreased fitness is not a consistent characteristic of metalaxyl-resistant strains. Some metalaxyl-resistant isolates from Israel were more aggressive (larger resultant lesions) (21), but others from Ireland were less aggressive (less sporulation) than metalaxyl-sensitive isolates (13). Also, typical differences in aggressiveness among isolates are not large enough to impact greatly management of disease and fungicide resistance.

Metalaxyl is so effective against sensitive individuals that it is difficult to find a management strategy using metalaxyl which does not have a strong selection for metalaxyl resistance (relative to sensitivity). Because the effects of selection for metalaxyl resistance can be multiplicatively compounded from year to year, a change in the management strategy from the standard protectant to one with slightly increased selection for metalaxyl resistance may cause a substantial increase in metalaxyl resistance in the pathogen population within just a few years. In our simulation study, metalaxyl treatments resulted in very high ratios (for example, greater than 5) of disease caused by the resistant pathogen to disease caused by the sensitive pathogen (Fig. 3C, 4B, and 7B). Therefore, use of metalaxyl/protectant mixtures may not reduce the selection for metalaxyl resistance sufficiently to enable long-term use. In the Netherlands, control problems have occurred in fields treated with metalaxyl/protectant mixtures because of metalaxyl resistance in *P. infestans* (11). In Israel, the use of mixtures did not prevent the development of metalaxyl resistance (9).

A single strategy involving metalaxyl will probably not sufficiently suppress metalaxyl resistance. Therefore, management strategies are needed in addition to the standard practice of using metalaxyl/protectant mixtures. Since the fungus overwinters in seed tubers, volunteer tubers, or in cull piles (2,17), it is imperative that care be taken to avoid planting seed potatoes infected with metalaxyl-resistant strains and to avoid metalaxyl-resistant inoculum from cull piles. For the seed crop, only metalaxyl-use strategies that greatly reduce selection for metalaxyl resistance should be used, but for the commercial crop, selection for metalaxyl resistance may not be so important in choosing a control strategy if the overwintering of *P. infestans* is controlled. Adjustments in fungicide dosages or timings may not be as valuable in limiting fungicide resistance as changing (if possible) other factors such as the susceptibility of the potato cultivar.

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