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**Disease Control and Management of Fungicide
Resistance in Oomycetes Foliar Plant
Pathogens**

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Title
**Disease Control and Management of Fungicide Resistance in
Oomycetes Foliar Pathogens**

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c. Abstract

Disease control and management of resistance to phenylamide fungicides were studied with the *Phytophthora infestans* - potato pathosystem. In Israel, where resistant strains of the fungus prevail, research was directed at understanding the reasons for this prevalence, as well as to producing adequate tools for combating the disease they induce. We developed a new bioassay technique for estimation of the frequency of resistant sporangia in a sporangial population with a precession of 0.01%, measured non-competitive and competitive fitness parameters of MS and MR fungal stains, estimated the overseasoning of these strains in potato tubers, evaluated the efficacy of phenylamide fungicidal mixtures in controlling the disease, measured the selection pressure imposed by various fungicides and fungicidal cocktails on the pathogen and finally constructed a simulation model to predict the behavior of the fungus and the disease in potato crops treated and non-treated with fungicides. In the USA, where no resistance occurs, the behavior of metalaxyl in potato crops was followed in nature. Simulation models were constructed and the effects of initial inoculum size, mutation frequency, fitness of isolates and other host and pathogen parameters on selection pressure were computed. Fungicidal treatments were incorporated into the models and disease control and buildup of resistance computed.

The results obtained in this research are of a great and significant value not only for oomycetes foliar pathogens with respect to phenylamide fungicides but also to other site-specific systemic fungicides with respect to their target pathogens.

d. Objectives of the original research proposal

The main objective was to determine management strategies that would not only prevent and/or suppress the building up of fungicide resistance, but also would maintain effective control of diseases. Specifically, our goals were to:

1. develop mathematical models describing both disease development and buildup of resistance
2. generate theoretical predictions of disease development and resistance under various management practices
3. experimentally test the predictions generated from the models and revise the models according to biological data obtained from experimentation.

As a result of this work, we expected to make contributions to the basic understanding of pathogen population biology of resistance, and to make recommendations for rational resistance management.

e. Scientific Report

Part I: ISRAEL

A. Estimation of the frequency of metalaxyl resistance sporangia in fungal populations

An accurate bioassay technique was developed to estimate the frequency of R in a sporangial population (Kadish and Cohen, 1988a). Small potato tuber discs placed on metalaxyl are inoculated with about 6 sporangia each. Because a tuber disc may become infected (and support fungal sporulation), at a probability of 90%, by 0.625 sporangia, the detection > 5% R was accurately demonstrated. The bioassay was adapted to detect lower levels of R by increasing the number of sporangia inoculated to the discs. Thus, by applying 100, 400, and 800 sporangia/disc a R frequency of 1, 0.1 and 0.01% could be detected, respectively, with a high reproducibility. This is, so far, the most accurate technique available for determining % R in Oomycetes.

B. Non-competitive fitness of MS and MR isolates

Twenty MS and 20 MR field isolates of *P. infestans* were compared for their ability to germinate, infect, colonize and sporulate on potato foliage, tuber slices and whole tubers (Kadish and Cohen, 1988b, Bashan *et al.* 1989, Grinberger *et al.* 1990, Kadish *et al.* 1991).

MR isolates were found to germinate faster than MS isolates *in vitro*, to infect potato leaves within a shorter dew period compared to MS isolates (Bashan *et al.* 1989), to produce much larger lesions in potato leaves compared to MS isolates in cv. Alpha (Kadish and Cohen 1988b) and in other cultivars of potato (Grinberger *et al.* 1989) and to destroy potato crops (in single-isolate inoculation in walk-in plastic tunnels) faster compared to MS isolates (Kadish and Cohen 1988a). They, however, did not produce more sporangia per leaf lesion unit area compared to MS isolates (Kadish and Cohen 1988b), nor on potato tuber slices (Grinberger *et al.* 1990). MR isolates produced larger and deeper lesions in whole potato tubers of cv. Alpha (Kadish *et al.* 1991) at any age after sowing or after placed in storage.

MR isolates "suffer" from two disadvantages compared to MS isolates, both resulting from their relatively higher aggressiveness: first they had a shorter infectious period in potato leaves (Kadish and Cohen 1990) and second, they could over-season poorly in infected potato tubers (Kadish and Cohen 1991, submitted).

Indeed, MS isolates were predominant in initial foci of late blight in potato crops, although during the growing season MR isolates predominated the fungal population (Kadish and Cohen, 1991, submitted).

C. Competitive fitness of MR and MS isolates

Mixed (sensitive and resistant) isolates inoculations of potato leaves, potato tuber slices, potato plants, potato crops and potato tubers revealed that the competitive ability of isolates depended on the isolate, on the tissue inoculated, and under field conditions - on the total crop area infected (free space).

The following findings are based on a comparison of 20 isolates (10 MS and 10 MR) collected during 1983-1987 from blighted potato crops in various locations in the country:

1. Mixed-isolates inoculations of intact potato plants in growth chambers at 20°C resulted in an increase of the resistant isolate from 1 to 50% or from 10 to 100% within 10 sporulation cycles. Similar inoculations of detached leaves or tuber slices gave similar results only when the MR isolate had a higher sporulation capacity compared to its MS partner (Kadish and Cohen 1988c).
2. Mixed-isolates inoculation of potato crops (cv. Alpha) in walk-in plastic tunnels (90% sensitive + 10% resistant) caused a full devastation of the crops within 30 days. With all isolate-pairs competed, the frequency of the resistant isolate increased to 70-80% during the logarithmic growth of the epidemic (about 20 days). This frequency continues to grow to 100% for some resistant partners but decreased to about 30% for others (Kadish and Cohen, 1989).

The latter decrease in the frequency of some resistant isolates was attributed to their lower sporulation capacity and shorter infectious period compared to their sensitive partners (Kadish and Cohen, 1989). These dates suggest that the population dynamic of the fungus depended not only on fitness components of isolates, but also on the availability of uninfected host tissue.

D. Overseasoning of MS and MR isolates in potato tubers

Single-isolate inoculation of potato tubers (cv. Alpha) and fungal recovery tests made from such tubers after prolonged storage at various temperatures showed (Kadish and Cohen 1991, submitted) a significantly higher recovery of MS isolates after incubation at 20 or 25°C for 5 m and equal (or higher) recovery after such inoculations at 4 or 12°C. We attributed that difference to secondary contaminations of tubers associated with the highly aggressive MR isolates inoculations. Mixed MS + MR isolates inoculations of potato tubers and re-isolation tests made from such tubers showed (Kadish and Cohen, 1991, submitted) a

predominance of the MS isolates in the fungal populations recovered. We concluded that MS isolates may better overseason in potato tubers compared to MR isolates.

Indeed, late blight lesions collected from initial foci of the disease in potato fields in the country during 1987-1990 showed a predominance of MS isolates. However, in those same fields, MR isolates quickly and overwhelmingly predominated the fungal population during the growing season.

E. Chemical control of late blight in potato

The predominance of phenylamide resistant isolates of *P. infestans* in potato fields in Israel together with the poor efficacy of protectant fungicides such as mancozeb in controlling the blight forced the use of phenylamide fungicides in a way that their selection pressure will be minimal.

Bearing these principals in mind we found that:

1. Cymoxanil, a local-systemic fungicide, is equally effective in controlling both MS and MR isolates (Samoucha and Cohen, 1988c). Its efficacy in the field, however, lasted for about 5 days (Samoucha, Levy and Cohen 1988).
2. Propamocarb, another systemic fungicide, is equally effective in controlling MS and MR isolates (Samoucha and Cohen 1990). Its efficacy in the field lasted for about 7 days.
3. Phosetyl-Al and H_3PO_3 , acted as systemic fungicides against MS and MR isolates in potato, but their efficacy was dependent on potato cultivar, fungal isolate, and environmental conditions (Bashan, Levy and Cohen 1990).
4. Fungicidal mixtures containing cymoxanil, with either mancozeb (Mancur) , oxadixyl (Pulsan, Sandocur-M) or metalaxyl were strongly synergistic in controlling both MS and MR isolates in growth chambers (Samoucha and Cohen, 1988a), walk-in-plastic tunnels (Cohen and Samoucha 1989; Cohen and Samoucha 1990) and the open field

(Samoucha and Cohen 1989b; Samoucha, Ben-Hador and Cohen 1989; Samoucha, Levy and Cohen 1988).

5. Cymoxanil was an essential ingredient in mixtures. Mancozeb + oxadixyl or mancozeb + metalaxyl mixtures were not effective enough in delaying the buildup of resistance when mixed MS + MR isolates were used for inoculation (Cohen and Samoucha 1989).

Buildup of resistance was positively related to the initial frequency of resistant sporangia in the inoculum applied to the crop. Control efficacy of fungicides was inversely related to the selection pressure imposed by a fungicide on the pathogen (Cohen and Samoucha 1989).

6. Similar results were obtained for the control of downy mildew in cucumbers (Samoucha and Cohen, 1988b).
7. Synergistic action of fungicides in mixture was shown to be a broad phenomenon (Cohen and Levy 1990).

It was speculated that synergy involves energy production inhibition in fungal spores (Bashan, Levy and Cohen, 1990).

F. Simulation model

Based on the knowledge we acquired on fungus, fungicides, and fungus-fungicide interaction a model was constructed to predict the effect of fungicides on buildup of resistance and disease control in potato crops (Levy, Benderly and Levy 1991, in press). Validation studies showed that the model predicted accurately buildup of resistance and disease development in fungicide non-treated crops as well as in crops treated with fungicides (Levy, Benderly and Cohen, 1991, in press).

Part II. U.S.A.

Achievements concerned both methodology and evaluation of resistance management strategies. In methodology, we demonstrated that *in vitro* assessments of metalaxyl resistance provided very similar results as did *in vivo* (floating leaf disk) assessments. Isolates were evaluated for metalaxyl sensitivity (resistance) using both assays, and results between the two assays were consistent. There was some experimental error associated with each type of assay. A second methodological achievement was the improvement of the late blight simulation model. This model identifies the distribution and chronological dynamics of metalaxyl in a potato canopy. The improved model was tested against independently acquired field data, and was demonstrated to be very accurate with no consistent deviations (Doster *et al.*, 1990).

In terms of resistance management strategies, we demonstrated that both absolute population size and relative frequency of resistant forms are important criteria in evaluating resistance management strategies. Some previous models had employed relative frequency as the only criterion. Selection was identified as the most important force in the evolution of fungicide resistance in agriculture, and mutation contributed relatively little. The speed at which a population changes is related to population growth rate, thus the evolution of fungicide resistance is likely to be more rapid in an organism with many generations per season than in an organism with very few generations per season. An important result is that strategies which slow pathogen growth rates also slow the evolution of resistant forms. Unfortunately, slowed growth rates also slow evolution from resistance to sensitivity in the absence of selective forces for resistance. Many of these theoretical generalities are identified in Fry and Milgroom, (1990).

The influence of various factors on selection for fungicide resistance was quantified in a series of simulation experiments. An assumption of these simulation experiments was that the fungus occurred in discrete foci of single pathogen genotypes. We learned that 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. Sprays applied more frequently but at lower rates (same total amount of fungicide per season) resulted in less disease caused by either metalaxyl-sensitive or -resistant strains for both mixtures and alternations. The middle of the season (about eight weeks after emergence in a twelve week long season) resulted in the least final disease for the metalaxyl-sensitive strain. (Field experiments performed with a metalaxyl-sensitive strain indicated that an application of metalaxyl/chlorothalonil in the middle of the 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 was use of moderately resistant cultivars. Small differences in aggressiveness between resistant and sensitive 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 (Doster *et al.*, 1990).

The role of population size in the evolution of fungicide resistance was evaluated theoretically. A stochastic model was developed to relate pathogen population size, the probability of fungicide-resistant individuals (R) occurring, and the strength of selection for resistance (fungicide efficacy). The model was based on binomial probabilities that R individuals are present in the initial population size N_0 or occur during an epidemic after selective fungicide is applied. If an R mutant occurs early in the epidemic, the final frequency (q_1) and number of R (N_R) increased to higher levels than if the mutation occurs later. The probability of q_1 reaching some given value is always higher when N_0 , the pathogen growth rate (r), or mutation rate to resistance (p) is large. The effects of fungicide

efficacy ($1-\alpha$) depend on the other parameters. When N_0 is small, high values of $1-\alpha$ result in lower probabilities of R occurring. As N_0 increases, this trend is reversed. These results suggest that intensive use of fungicides may be a rational means of resistance management when population sizes are initially small (Milgroom 1990).

A preliminary assessment of the assumptions of various theoretical models was initiated. Competition was shown to be a factor when disease levels became quite high. Thus if models are used with large populations, competition effects must be considered.

We evaluated by computer simulation various strategies to time metalaxyl applications for improved control of potato late blight and reduced selection for metalaxyl resistance (Doster and Fry 1991). Timing spray applications according to some weather-based decision rules improved disease suppression relative to the standard schedule when the total amount of fungicide applied was reduced by amounts greater than 10%. Some programs in which spray dosages and intervals were adjusted according to time in the season resulted in much better disease suppression than did the standard treatment. Reduction of the dosage in each spray application resulted in better disease suppression than reduction in the number of sprays. The dosage of sprays applied late in the season can be reduced substantially (50%) without greatly affecting disease suppression. More frequent applications at a lower dosage resulted in better disease suppression than the standard treatment, even though less total fungicide was applied.

f. Description of Cooperation

Prof. Cohen and Dr. Levy visited Cornell University in 1988 in order to discuss the project. At that time ideas as per monitoring resistance at very low frequencies were raised. It was apparent that due to the fact that no resistant isolates of *P. infestans* occur in the U.S.A., all growth chambers and field experiments with resistant isolates would be conducted in Israel and data transferred to the U.S.A.

Prof. Fry's visit to Israel was postponed to the fall of 1990 but was then cancelled because of the Gulf War.

g. Evaluation of Research Achievements

This research focused on disease control and management of fungicide resistance in the late blight (*Phytophthora infestans*) - potato system. This major disease of potato in both Israel and USA is increasingly threatening crop production due to the actual appearance and spread of phenylamide resistant strains of *P. infestans* in Israel and the possible appearance of such strains in the USA. Intensive studies carried out in both countries with the financial support of BARD showed that appropriate management of resistance is required in order to achieve adequate control of the disease. In the USA, reduced-rate frequent applications of a phenylamide fungicide mixed with a protectant fungicide were predicted to result with the best results. In Israel, cymoxanil-oxadixyl based fungicides applied in early season followed by cymoxanil-, or propamocarb-based fungicides later in the season allowed for an acceptable control of the disease.

The results of this research were published in 26 scientific papers (22 by the Israeli group and 4 by the American group), one M.Sc. thesis and two Ph.D. theses (M. Grinberger, B. Bashan and D. Kadish).

h. List of Publications

1. Israel

1. Bashan, B., Kadish, D., Levy, Y. and Cohen, Y. 1989. Infectivity to potato, sporangial germination and respiration of isolates of *Phytophthora infestans* from metalaxyl-resistant populations. *Phytopathology* 79: 832-836.
2. Bashan, B., Levy, Y. and Cohen, Y. 1990a. Variation in sensitivity of *Phytophthora infestans* to fosetyl-Al. *Plant Pathology* 39: 134-140.

3. Bashan, B., Levy, Y. and Cohen, Y. 1990b. Synergistic interactions between phenylamide fungicides and respiration inhibitors in controlling phenylamide resistant and sensitive isolates of *Phytophthora infestans* on potato tuber disks. *Phytoparasitica* 19: 73-77.
4. Cohen, Y. and Samoucha, Y. 1989. Selection for metalaxyl resistance in potato crops infected with *Phytophthora infestans*: effects of fungicides and initial frequency of resistant sporangia. *Plant Pathology* 38: 382-390.
5. Cohen, Y. and Samoucha, Y. 1990. Competition between oxadixyl-sensitive and-resistant field isolates of *Phytophthora infestans* on fungicide-treated potato crops. *Crop Protection*. 9: 15-20.
6. Cohen, Y. and Levy, Y. 1990. Joint action of fungicide in mixtures: Theory and practice. *Phytoparasitica* 18: 159-169.
- 6a. Grinberger, M., Kadish, D. and Cohen, Y. 1989. Occurrence of the A₂ mating type and oospores of *Phytophthora infestans* in potato crops in Israel. *Phytoparasitica* 17: 197-204.
7. Grinberger, M., Kadish, D. and Cohen, Y. 1990. Infectivity of metalaxyl-sensitive and-resistant isolates of *Phytophthora infestans* to potato tubers. *Phytopathology* 81: (In press).
8. Kadish, D. and Cohen, Y. 1988a. Fitness of *Phytophthora infestans* isolates from metalaxyl-sensitive and resistant populations. *Phytopathology* 78: 912-915.
9. Kadish, D. and Cohen, Y.. 1988b. Estimation of metalaxyl-resistance in *Phytophthora infestans*. *Phytopathology* 78: 915-919.
10. Kadish, D., and Cohen, Y. 1988c. Competition between metalaxyl-sensitive and-resistant isolates of *Phytophthora infestans* in the absence of metalaxyl. *Plant Pathology* 37 558-564.

11. Kadish, D. and Cohen, Y. 1989. Population dynamics of metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* in untreated potato crops. *Plant Pathology* 38: 271-276.
12. Kadish, D. and Cohen, Y. 1991. Overseasoning of *Phytophthora infestans* from metalaxyl-sensitive and -resistant populations in potato tubers. *Phytopathology* 81: (Submitted).
13. Kadish, D., Grinberger, M. and Cohen, Y. 1990. Fitness of metalaxyl-sensitive and metalaxyl resistant isolates of *Phytophthora infestans* in susceptible and resistant potato cultivars. *Phytopathology* 80: 200-205.
14. Kadish, D., Grinberger, M. and Cohen, Y. 1991. Susceptibility of potato tubers to late blight infection after harvest or storage. *Phytophthora Newsletter*. 1991.
15. Levy, Y., Cohen, Y. and Benderly, M. 1989. Disease development and buildup of resistance to oxadixyl in potato crops inoculated with *Phytophthora infestans* as affected by oxadixyl and oxadixyl mixtures: experimental and simulation studies. *Journal of Phytopathology* (In press).
16. Samoucha, Y. and Cohen, Y. 1988a. Synergistic interactions of cymoxanil mixtures in the control of metalaxyl-resistant *Phytophthora infestans* on potato. *Phytopathology* 78: 636-640.
17. Samoucha, Y. and Cohen, Y. 1988b. Synergism in fungicide mixtures against *Pseudoperonospora cubensis*. *Phytoparasitica* 16: 337-342.
18. Samoucha, Y. and Cohen, Y. 1988c. Sensitivity of *Phytophthora infestans* to cymoxanil. *Phytoparasitica* 16: 39-45.
19. Samoucha, Y. and Cohen, Y. 1989. Field control of potato late blight by synergistic fungicidal mixtures. *Plant Disease* 73: 751-753.
20. Samoucha, Y. and Cohen, Y. 1990. Toxicity of Propamocarb to the late blight fungus on potato. *Phytoparasitica* 18: 27-40.

21. Samoucha, Y., Ben-Hador, G. and Cohen, Y. 1989. Chemical control of late blight epidemics incited by metalaxyl-resistant *Phytophthora infestans* in potato crops in Israel. *Phytoparasitica* 17: 185-195
22. Samoucha, Y., Levy, R.S. and Cohen, Y. 1988. Efficacy over time of cymoxanil mixtures in controlling late blight in potatoes incited by a phenylamide-resistant isolate of *Phytophthora infestans*. *Crop. Protection* 7: 210-215.

II. USA

1. Doster, M.A. and Fry, W.E. 1991. Evaluation by computer simulation of strategies to time metalaxyl applications for improved control of potato late blight. *Crop Protection* 10 April 1991 (in press).
2. Doster, M.A., Milgroom, M.G. and Fry, W.E. 1990. Quantification of factors influencing potato late blight suppression and selection for metalaxyl resistance in *Phytophthora infestans*: a simulation approach. *Phytopathology* 80: 1190-1198.
3. Fry, W.E. and Milgroom, M.G. 1990. Population biology and management of fungicide resistance. In "Managing Resistance to Agrochemicals: from Fundamental Research to Practical Strategies" (M.B. Green, W.K. Moberg, and H. LeBaron, eds.) American Chemical Society, pp. 275-285.
4. Milgroom, M.G. 1990. A stochastic model for the initial occurrence and development of fungicide resistance in plant pathogen populations. *Phytopathology* 80: 410-416.