Session 2

Phenylamides

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EARLY EXPERIENCES WITH PHENYLAMIDE RESISTANCE AND LESSONS FOR CONTINUED SUCCESSFUL USE

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ABSTRACT

The first and most dramatic cases of phenylamide resistance occurred in cucumber downy mildew in plastic houses in Israel and in potato late blight in Europe in 1980. They were associated with exclusive use of solo products under high disease pressure. This led to the withdrawal of the solo product in favor of prepack mixtures with residual compounds. Where phenylamides were used in mixtures from the start, as against grape downy mildew and in the UK against potato late blight, resistance was slower to emerge and spread. In addition, where mixtures were used, performance problems remained rare when resistant strains began to appear in the target pathogens. The successful use strategies throughout the 80s were based on the use of prepack mixtures, the avoidance of curative use and a limitation to 2-4 treatments early in the season. Where these strategies were implemented performance of phenylamides mixtures remained good even where resistance could readily be detected. Resistance tends to increase during seasons and recede again between seasons. This indicates a fitness deficit of resistant strains compared to the wild-type populations from which they emerged. Special studies confirmed that resistant strains are, as a rule, less fit for survival from season to season. In *P. infestans* a special situation may exist when resistant strains are imported with seed or plant material from areas with more virulent populations of late blight. Studies with DNA fingerprinting and other genetic markers show that for *P. infestans* migration with infected tubers may play a much bigger role in the initial establishment of phenylamide resistance in a region than previously thought. Early experiences showed that the phenylamides can be preserved as valuable tools for Oomycete control, if the anti-resistance strategies are implemented.

INTRODUCTION

Metalaxyl and furalaxyl were the first phenylamide fungicides to be introduced in 1977. Other companies presented four more representatives of this group over the next five years (Table 1) The six phenylamides differ considerably in their level of activity and in the versatility of their use (Schwinn and Staub, 1987). The high level of specific and systemic activity of the first phenylamides represented an exciting new technology for the control of most Oomycete pathogens; novel features were their high inherent level of activity, their rapid uptake and acropetal transport which led to extended spray intervals and to the protection of new growth. For systemic soil- and seedborne pathogens they offered effective chemical control for the first time. The biological properties of the phenylamides were so attractive that they were often used exclusively, especially in situations of high disease pressure which were difficult to handle with the old residual fungicides. It was also tempting for farmers to exploit their systemic and curative properties to the maximum, e.g. delay treatments until the epidemics were well established and hard to stop.

TABLE 1: Phenylamide introductions

Common name	Code	Year of presentation	Company
metalaxyl	CGA 48988	1977	Ciba-Geigy
furalaxyl	CGA 38140	1977	Ciba-Geigy
ofurace	RE 20615	1978	Chevron
benalaxyl	M 9834	1981	Montedison
cyprofuram	SN 78314	1982	Schering
oxadixyl	SAN 371F	1983	Sandoz

EARLY CASES OF PHENYLAMIDE RESISTANCE

The first cases of phenylamide resistance in practice occurred in the winter season 1980 in *Pseudoperonospora cubensis* on plastic-house grown cucumbers in Israel (Table 2). Up to that time metalaxyl had given excellent control under these conditions of heavy and continuous disease pressure both in trials and in the first seasons of use. Compared to the previously used residual fungicides, metalaxyl was so much better that it was used exclusively in many plastic houses. Therefore, the control failures in plastic houses where resistance appeared were complete and the solo product had to be withdrawn from this use.

TABLE 2: First major cases of phenylamide resistance

Year	Countries	metalaxyl use	disease control	special observations
Pseudoperonospora cubensis				
1980	ISL, CR	solo	lost	intensive use in plastic houses
Phytophthora infestans				
1980	CH, NL, IRL	solo	lost	heavy attacks, curative use
1981	UK, F , D	mixtures	good	few R samples in monitoring
Plasmopara viticola				
1981	SA, F	mixtures	good	few R samples in monitoring
Peronospora tabacina				
1981	Centr. Am.	solo	lost	problems restricted to shaded tobacco

The first occurrence of phenylamide resistance in a field crop was in *Phytophthora infestans* on potatoes during the 1980 season in Europe where metalaxyl had gained a high market share in the first two years of use (Table 2). Resistance appeared in an explosive fashion almost simultaneously in Switzerland, the Netherlands and in Ireland. In all three countries metalaxyl had been used season long as a solo product. The disease pressure was very high that summer and many farmers had problems spraying in time due to the bad weather. Therefore many applications were made in curative or eradicative way in potato fields with substantial levels of late blight attack. As soon as the surprisingly fast occurrence of phenylamide resistance was realized, the solo product was withdrawn from the market and later replaced by mixtures with residual fungicides. No performance problems occurred in the UK, where metalaxyl was available as a prepack mixture with mancozeb from the start. The rationale for the different use in the UK had been the improved late blight control by mixtures on older foliage late in the season where phenylamides alone were found to be less active than on younger, vigorously growing foliage.

The sudden appearance of phenylamide resistance so soon after the product introduction was most surprising since several risk analysis studies on the propensity of resistance development indicated a lower risk level than that known from experience for the benzimidazoles (see below). Equally surprising was the explosive nature of the first occurrences of resistance in the cases described above. In potato fields near Berne it took merely 10 days from the first observations of a possible problem until the foliage was completely destroyed in the foci where resistance appeared first (Table 3). This illustrates the futility of the "wait and see" attitude with such explosive diseases like late blight. A first lesson was that strategies have to be implemented early and that sensitivity methods have to be available for quick testing of samples from critical fields.

TABLE 3. Time course of events in 1980 related to the first occurrences of phenylamide resistance in *Phytophthora infestans* (Staub and Sozzi, 1983)

Day	1	- First indications of problems (active sporulation on treated plants)
Day	5	- 50% attack in actively sporulating foci (samples collected)
Day	10	- 100% attack in first foci
Day	20	- Resistance confirmed in leaf disc test in laboratory

In the following year phenylamide resistance appeared also in *Plasmopara viticola* on grapes in South Africa and southwestern France and in *Peronospora tabacina* on tobacco in Central America (Table 2). On tobacco, the phenylamides were so outstanding compared to the previously known residual fungicides, that the season-long exclusive use of the solo products led to resistance selection under the high disease pressure in that area. On grapes the first foci might have appeared in nurseries and spread from there to the vineyard, where mixtures with copper or folpet were used (Staub and Sozzi, 1981). The use of mixtures on grapes did not prevent the appearance and spread of resistant strains, but it did prevent wide spread performance problems.

WHERE HAD RESISTANCE-RISK STUDIES FAILED ?

The unexpected and dramatic occurrence of phenylamide resistance raised the question of the usefulness and the nature of the resistance risk studies done. Several studies done before the introduction of the first phenylamides showed that for *P. infestans* strains with decreased sensitivity could be selected in vitro, but such strains were either not pathogenic or they could not infect plants treated with phenylamides (Bruck et al, 1980; Staub et al, 1979). Furthermore, selection experiments on treated potato plants over 14 generations did not yield any resistant strains. Other labs obtained similar results with other pathogens (Bruin, 1980; Lukens et al, 1978). Mutagens were used only in some of these studies for fear of either creating a resistance problem or ending up with "unnatural" resistant mutants that would not occur in nature as is well known in the case of dicarboximides. Mutagens were included systematically in a detailed study on the possible development of phenylamide resistance in the soilborne pathogen *Phytophthora megasperma* (Davidse, 1981). In this study phenylamide resistant strains were produced that were fully pathogenic and that were not controlled on treated soybean plants. The lesson from these experiences is that resistance risk studies should include the use of mutagens, when other studies don't give sufficient information. However, as was the case with *P. megasperma*, such studies have to be done with pathogens that don't represent a risk for the farmers in the region. Resistance risk studies should assess the risk without increasing it.

RECOVERY THROUGH ADAPTED USE STRATEGIES

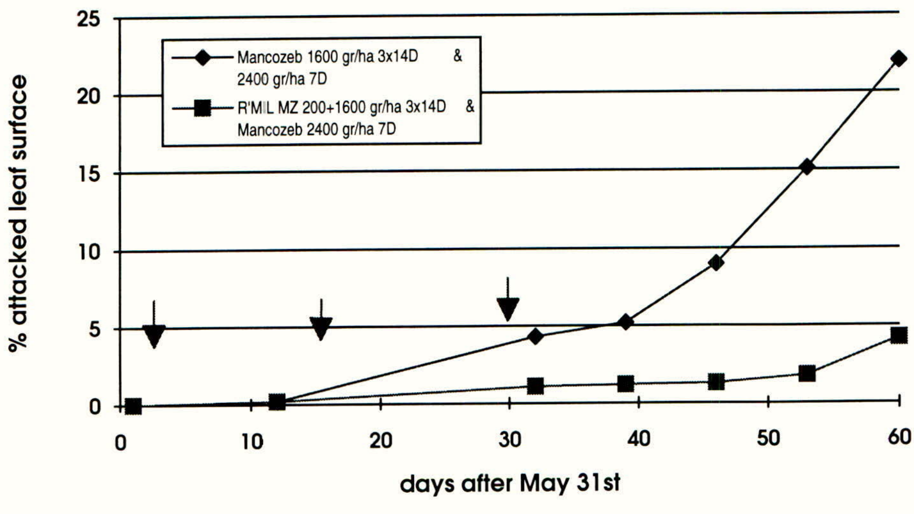
The dramatic developments in the second year after the first introduction of phenylamides led to a drastic revision of the use strategies against foliar pathogens. It was evident that all phenylamides belonged to the same cross-resistance group (Diriwächter et al, 1987), which meant that common anti-resistance strategies had to be defined. In 1981 the Fungicide Resistance Action Committee (FRAC) was established to coordinate the anti-resistance strategies of cross-resistant fungicides. This met the urgent need of the phenylamide producers, who established a working group to deal with the rather dramatic situation facing the further use of these novel fungicides. The working group established the following general guidelines for use of phenylamides against foliar pathogens (Urech and Staub, 1985):

- sell only prepack mixtures with residual partner fungicides
- include high rates of residual partner: ¾ to full rate
- intervals should not exceed 14 days
- limitation to 2-4 sprays early in the season
- no curative or eradicative applications
- no soil applications against foliar pathogens
- and no use on seed potatoes and nurseries

The strategies established in the early 80s proved to be very successful. A crucial factor for the success was the readiness of all members of the phenylamide working group to cooperate in designing reasonable ant-resistance strategies and to fight for their implementation within their companies and with officials and farmers through effective communication. Please refer to the next chapter for further details on the role of FRAC in dealing with phenylamide resistance.

Against late blight on potatoes the performance of the phenylamide mixtures remained stable in spite of the presence of resistant strains; this was also true for the Netherlands and Ireland, where phenylamides were reintroduced in mixture with residual fungicides in 1985 after the residual compounds failed to control the heavy epidemics of the previous year. L. Dowley describes the Irish experience with phenylamides against late blight since 1985 in more detail in a separate chapter in these proceedings. In the rest of Europe phenylamide mixtures continued to provide late blight control at a level clearly superior to that of residual compounds alone. Fig. 1 illustrates this for an area in Switzerland where phenylamide resistance in *P. infestans* could commonly be detected. In this trial area the resistance level was estimated at 30 % at the beginning of the epidemic. For this estimate, samples were taken from the first infections in untreated border rows and analyzed with a semi-quantitative sensitivity assay (Nuninger et al, 1992). The control in the RIDOMIL MZ plots was clearly superior to that in the mancozeb plots. In other similar trials comparable results were obtained as long as the first treatments with the metalaxyl mixture was applied protectively before the onset of the epidemic. Where curative or eradicative situations occurred because treatments could not be applied in time, neither the mixtures nor the residuals alone provided sufficient control under continuous high disease pressure. This example illustrates that phenylamides used strictly according to the FRAC guidelines continue to contribute significantly to disease control even when detectable levels of resistance are present.

FIGURE 1: Late blight control by Ridomil MZ and Mancozeb in large plot trials
(FR 125-90, 250m2/plot; 30 % resistance in initial inoculum on border rows)



It was frequently surprising how well phenylamide mixtures still worked, even where monitoring revealed high levels of resistance. This apparent discrepancy was often a consequence of the late monitoring and an overestimation of the resistance levels with the simple leaf disc test. Therefore, a semi-quantitative test was developed (Nuninger et al, 1992) and monitoring has been increasingly focused on the first infections of epidemics, before treatments are made.

In grape downy mildew phenylamide resistance appeared first in South Africa and south western France. The cases were associated with high disease pressure and it appeared that nurseries might have been the initial foci (Staub and Sozzi, 1981). In France, phenylamide resistance fluctuated somewhat from 1983-87 depending on the disease pressure and the extent of phenylamide use. Resistance was confined for several years to the western part, where downy mildew pressure is heavier, and has only more recently reached the eastern grape growing regions in France. Time of appearance of phenylamide resistance in the vine growing regions of France was clearly correlated with the general downy mildew pressure of an area. With moderate use of phenylamide mixtures, they continue to provide reliable downy mildew control. In grapes the best use of the special strength of the phenylamides is around flowering when downy mildew attacks on the young bunches can cause big yield losses.

For tobacco blue mold, special strategies were necessary for different regions because of extreme disease pressure and the relatively poor performance of the residual mixture partner available. In the critical regions of central America, it was recommended to use phenylamide mixtures with the full dose of the residual partner in alternation with applications of the residual fungicides alone. This strategy has been successful where both alternations and residual spray intervals were implemented.

WHY DID THE ANTI-RESISTANCE STRATEGIES WORK ? -- FITNESS OF R STRAINS

In addition to the proper design and the vigorous implementation of anti-resistance strategies for phenylamides, some basic biological properties of the resistant strains seem to have contributed to the success of these strategies; they are related to the fitness and the population dynamics of the resistant strains. The examples described above suggest that phenylamide resistance tends to decrease in the absence of selection pressure. It is this phenomenon that makes it possible to design use strategies that lead to relatively stable resistance situations and to continued good contributions by the phenylamide partners in mixtures in spite of detectable levels of resistance in the target pathogen populations. This is a more favorable situation than that of benzimidazole resistance which tends to persist at high levels and where interruption of selection pressure does not lead to a decrease in the resistance level in the population.

The basis for the reduction of phenylamide resistance in the absence of selection pressure is not well understood, but it may be due to decreased fitness of the resistant strains compared to the wild type populations from which they emerged through mutation and selection. Fitness parameters that were identified as playing a role in this context are increased sensitivity to high temperatures for *P. viticola* (Piganeau and Clerjeau, 1985) and decreased survival in potato tubers for *P. infestans* (Walker and Cooke, 1990). Our own studies on the survival of *P. infestans* in potato tubers at low temperatures gave similar indications (Table 4). Twenty-two populations from the 1989 monitoring program in Switzerland and a few populations from other countries with resistance levels between 0.04 and 78% were inoculated into potato tubers and stored at 6 C for 8 months. At the beginning, the middle and the end of the storage period, the populations were analyzed for the resistance levels with the semi-quantitative monitoring assay (Nuninger et al, 1992).

TABLE 4: Development of phenylamide resistance in field samples[1] of *Phytophthora infestans* collected in 1989 and stored for 8 months at 6ºC in potato tubers.

starting populations		development of resistance during storage				
% resistance	samples found	decrease		stable	increase	
		below DL[2]	2-5x	< 2x	2-5x	to 100%
0.04-0.39	4	4	.	.	.	.
0.4-3.9	3	3	.	.	.	.
4.0-39	11	6	.	1	3	1[3]
40-59	6	1	1	2	.	2[4]
>=60	3	3	.	.	.	.
Totals	27	17	1	3	3	3

[1] bulk samples collected 1989 in Switzerland (22), NL (2), IRL (2) and UK (1)
[2] DL = detection limit of semi-quantitative assay is 0.02%
[3,4] initial R frequencies were 35[3], 50 and 51%

At the end of the storage period 17 of the 27 populations had lost the resistant part of the population including several that had initial R frequencies of >40% (Table 3). In most of these cases resistance was no longer detectable after only 4 months of storage. This indicates a rapid decrease of the resistant portion in these populations by a factor of at least 2000x. In seven populations with relatively high initial R frequencies, the resistance level changed only slightly (less than 5x). In the three samples that reached 100% resistance at the end of the storage period, the initial R frequencies ranged from 35 to 51%. The cases where resistance was lost clearly dominate, so in most cases fitness for survival in potato tubers at 6 C seems to be reduced in resistant strains. The relatively small increases observed in some populations are most likely variation that occurred by chance or by the sampling procedures and they don't necessarily indicate fitness advantages of the resistant strains in these populations.

Reduced fitness for overwintering had also been described for strains of *P. infestans* collected in Israel (Kadish and Cohen, 1992) and Ireland (Walker and Cooke, 1990). The study from Israel showed a difference in the behavior of resistant strains in tubers and on the foliage. While survival of the resistant strains in tubers was clearly reduced they tended to be more aggressive than the sensitive ones on the foliage. This behavior was also confirmed by monitoring field populations between and during growing seasons.

THE ROLE OF MIGRATION VS INDEPENDENT MUTATION AND SELECTION EVENTS.

Already after the first cases of phenylamide resistance in 1980, the question was addressed whether resistance had appeared in many places independently or whether it appeared in one or a few places and spread from there throughout larger regions where it eventually led to the problems described above (Davidse et al, 1983). At the time race-typing with differential cultivars was used to look at some resistant Dutch *P. infestans* isolates collected in 1981. Phenylamide resistance was found in 10 of 23 physiological races detected in a survey. This indicated that several independent mutation and selection events had occurred in different places and that phenylamide resistance was genetically independent of any virulence traits in the different races. Only two of eight virulence genes detected in 1981 were not associated with phenylamide resistance at that time.

Improved methods of distinguishing between local developments and migrations were used to characterize the *P. infestans* populations on a world-wide basis (Fry et al, 1993). Interest in such studies was stimulated by the discovery of the A2 mating type in various parts of the world (Hohl and Iselin, 1984); previously, the A2 mating type was thought to be confined to regions of Mexico. The improved methods rely on allozymes patterns and on DNA fingerprinting to define clonal lineages. The main conclusion from the studies by Fry's group was that a large migration occurred during the 70s from Mexico into and through Europe and in the 80s from Europe to many other parts of the world (Fry et al, 1993). In the US and Canada a similar migration seems to have started in the last three years. The "new" population seems to be more aggressive than the "old" one. The long-distance spread is assumed to occur mainly via shipments of potato tubers and tomato seedlings infected by phenylamide resistant late blight strains.

For phenylamide resistance it is significant that it was mainly found in the "new" populations in Europe and that the "new" populations in the US seems to be largely resistant (Goodwin et al, 1994). Therefore, future anti-resistance strategies should include as a new element the prevention of the spread of resistant strains via seed and plant material. The presence of the A2 mating type is not linked genetically to phenylamide resistance, but it can be associated with it if clones with both A2 and phenylamide resistance happen to dominate an epidemic. The studies also showed that epidemics are often caused by only a few clones. Sexual recombination is still rare in Europe and the US in spite of the presence, side by side, of the two mating types. In Europe, the first indications have come from a study in Poland that sexual recombination may start contributing to the diversity in the populations of *P. infestans* (Sujkowsi et al, 1994). Increased sexual recombination will allow *P. infestans* to adapt more quickly to new sets of environmental factors. A new fungicide would be one such factor, fitness traits would be others that could be recombined through sexual recombination to the advantage of the pathogen and to the detriment of fungicidal efficacy.

CONCLUSIONS

From the early experiences with phenylamides, the following conclusion can be drawn that will help preserve the effectiveness of this valuable group of fungicides. Some of the lessons may be of a more general nature and apply also to other groups of fungicides with an inherent resistance risk.

<u>Phenylamide resistance is manageable</u>
Experience has shown that phenylamide resistance is manageable, if the proper anti-resistance strategies are employed. Depending on the pathogen, they emphasize the use of effective mixtures with residuals, a limitation of the application to the most crucial part of the epidemics, and the avoidance of curative use.

<u>Adherence to anti-resistance strategies is essential</u>
The strict implementation of the established anti-resistance strategies is absolutely essential. Non-compliance leads inevitably to performance problems and to a higher risk of a rapid build-up of resistant populations. This is especially crucial where detectable levels of resistance are already present.

<u>Fitness of phenylamide resistant strains is usually reduced</u>
Both circumstantial evidence and experimental results indicate that resistant strains are, as a rule, less fit than the wild type populations from which they emerged; resistant population diminish again when selection pressure is absent. The above strategies help prevent the selection of strains with both phenylamide resistance and normal fitness.

<u>For *P. infestans* migration is an important factor for the development of resistance</u>
This may lead to situations where the "new" imported resistant strains appear more fit and aggressive than the "old" sensitive ones. More care is required to minimize the spread of infected plant material that can carry with it resistant strains to areas previously free of resistance.

Phenylamides remain valuable part of arsenal against Oomycetes
Even where resistance is readily detectable, phenylamides used properly continue to contribute significantly to disease control over and above the one that can be achieved with residuals alone.

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DYNAMICS OF PATHOGEN RESISTANCE AND SELECTION THROUGH PHENYLAMIDE FUNGICIDES

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ABSTRACT

Phenylamide-based products continue to successfully control diseases caused by fungi of the Peronosporales. Pathogen resistance to phenylamides however, has developed since the early 1980`s in *Phytophthora* and *Plasmopara* species and may reduce efficacy if products are not used properly. Various antiresistance strategies contribute to lower the risk of resistant pathogen subpopulations becoming predominant. Mixtures of phenylamides with fungicides possessing a different mode of action delay resistance build-up significantly. The delaying effect is more pronounced if synergistic interactions between the components are at least as high for resistant as for sensitive strains. Sensitivity monitoring can assist resistance management and help explain problems of product performance. However, the sampling and sensitivity test methods used greatly influence the relevance of the data. Sensitivity monitoring by itself cannot be used to predict resistance development. It provides information on geographical and seasonal distribution, as well as on changes in pathogen resistance levels from year to year. Nevertheless, knowledge of the initial proportion of the resistant subpopulation at the beginning of the epidemics will help to better understand the subsequent process of selection. In order to interpret the sensitivity results, detailed studies should be made on the fitness and mating type distribution of field strains as well as their migration and appearance of novel pathogen genotypes.

INTRODUCTION

Phenylamide (PA-) fungicides, e.g. oxadixyl, metalaxyl, benalaxyl and ofurace, are single-site inhibitors with a high specific efficacy against fungi of the order Peronosporales. Their high specificity and consequent widespread use in agriculture selected phenylamide-resistant individuals within pathogen populations soon after introduction. Phenylamide resistance is a major-gene resistance and the selection process is disruptive. Phenylamide-resistant isolates may be as competitive as sensitive ones. Use strategies have been developed for phenylamide-based products to delay and reduce the development of resistance. As a result, they continue to contribute significantly to the control of diseases such as potato late blight and downy mildew of grape. Conditions under which such mixtures can be used successfully and their effect in delaying resistance build-up will be discussed in detail. Also, results on sensitivity monitoring from different countries generated according to the official PA-FRAC method descriptions (Gisi 1992) will be shown.

DISTRIBUTION OF PHENYLAMIDE-RESISTANT ISOLATES

The average amount of phenylamide resistant isolates of *P. infestans* collected in potato fields over the last years yielded the following approximate values: Switzerland 40%, The Netherlands 10-50%, England 10-30% and 50-80% in the early and late 80`s, respectively, Ireland 40-80% and France 50-90% (Table 1). The amount of resistant isolates significantly increased during the season (to 60-80%) according to the selection process, but started again at a low level (20-30%) at the beginning of the next season (Table 2). This observation was made in both treated and untreated fields in Switzerland. Samples of fields treated once, twice, three, and more than three times with PA-based products contained finally about 30%, 60%, 50%, and 80% resistant isolates, respectively (Majoros et al.,1993). Resistant isolates seem to be less fit than sensitive isolates in regard to overwintering capability (Walker and Cooke, 1990) but more fit in regard to colonization capability of leaves during epidemics (Cohen and Samoucha, 1990). According to O`Sullivan and Dowley (1993), resistant isolates became less frequent at the very end of the season (September) long after the last spray application of PA-based products.

In vineyards, phenylamide-resistant isolates of *P. viticola* are known to have occurred for at least 10 years. In vineyards not treated with PA-based products, sites containing resistant isolates were identified between 1983 and 1988 allover France, e.g. in the Bordeaux area

TABLE 1. Average amount (%) of phenylamide-resistant isolates of *P. infestans* from potato fields (annual means) in different european countries[a)]

year	% phenylamide resistant isolates					
	N.IRL[b)]	IRL[c)]	UK[d)]	NL[e)]	CH[f)]	F[g)]
1981	15	75	25	11	-	100
1982	25	21	15	5	-	90
1983	10	6	6	ld	-	97
1984	50	12	28	8	-	77
1985	15	8	20	12	-	46
1986	30	31	32	0	-	ld
1987	85	68	69	15	38	48
1988	90	84	81	43	45	67
1989	80	84	81	46	42	88
1990	67	57	78	-	41	71
1991	46	40	64	ld	ld	49
1992	65	38	46	-	73	93
1993	53	55	66	-	98	83

[a)] resistant isolates were also found in D, J, USA, CAN, Russia, South Africa, Israel
[b)]L. Cooke; [c)]L. Dowley; [d)]Ciba (80-85), ADAS (86-92), FRAC (93); [e)]L. Davidse; [f)]U. Gisi; [g)]S. Duvauchelle
- = no values available ld= low disease

17, 7, 6, 3, 30, and 60% of sites contained resistant isolates in the respective year, in the Bourgogne area 0, 20, 29, 17, 53, and 47% and in the Charentes area 93, 97, 74, 70, 48, and 67% of sites were found (Clerjeau, GRISP, 1988, pers. communication). In treated fields in France we have detected in 1988 up to 70% resistant isolates and in 1993, after several years of low downy mildew pressure, again a similar amount of isolates were resistant. Also in other countries (Italy, Switzerland, Spain, Portugal), resistant isolates were detected. Resistant isolates are known to occur also in *Pseudoperonospora cubensis, Bremia lactucae* and some other downy mildews (PA-FRAC information).

ESTIMATION OF RESISTANCE IN A POPULATION

Experience has shown that rather high numbers of resistant isolates can be found in the field but product performance remains good in most cases. An important question is then, what percentage of resistant sporangia in a field population may lead to performance problems.

TABLE 2. Seasonal variation of the amount (%) of phenylamide resistant isolates of *P. infestans* from potato fields treated with (PA+) or not treated with (PA-) phenylamide-based products [a)] over seven years in Switzerland

year		% phenylamide resistant isolates			No of
		PA-	PA+	m	isolates
	Jn	17	23		
1987	Jl	-	50	37	35
	Ag	-	(50)		
	Jn	(27)	33		
1988	Jl	-	48	41	27
	Ag	-	-		
	In	(40)	20		
1989	Jl	25	59	42	69
	Ag	69	61		
	Jn	15	15		
1990	Jl	33	61	41	106
	Ag	87	66		
	Jn	-	-		
1991	Jl	-	-	-	-
	Ag	-	-		
	Jn	-	-		
1992	Jl	(75)	(100)	-	21
	Ag	-	-		
	Jn	100	99		
1993	Jl	99	99	99	148
	Ag	100	100		

[a)]Phenylamide-based products (1-3 sprays per season) are used mainly during June and July
Jn = June, Jl = July, Ag = August, m = annual mean, - = no samples available
( ) = data base questionable, e.g. few isolates

So far, all routine sensitivity tests with *P. infestans* yield a resistant result for bulk samples if they contain at least 1-10% resistant sporangia. This figure was obtained by testing different mixtures of a sensitive and a resistant isolate of *P. infestans* against a range of fungicide concentrations (Table 3). The EC50 values increased more than 10 fold when amounts of r increased from 1 to 10%. For oxadixyl, a sharp increase of the RC50 values occurred between 10 and 100 mg/l (Table 3). Therefore, we should concentrate on the determination of amounts of resistant sporangia in a field isolate between about 1 and 10% rather than try to find levels down to 0.01%. More experimental work has to be done to determine the percentage of resistant sporangia present in field populations prior to the use of fungicide. In most cases, field isolates of *P. infestans* provide either a sensitive or resistant response, whereas in *P. viticola*, also intermediately resistant isolates can be found.

TABLE 3. Fungicidal activity of oxadixyl against a phenylamide-sensitive (0% r) and a phenylamide-resistant (100% r) isolate as well as against three different mixtures of both isolates (0.1, 1, 10%) of *P. infestans* in the potato leaf disc test

amount of r (%) in sporangium suspension	% inhibition of sporulation on leaf disc at .. mg/l oxadixyl					
	1000	100	10	1	0.1	EC50[a)]
0	100	100	60	20	0	3
0.1	100	95	40	10	0	9
1	95	80	30	5	0	26
10	60	50	10	0	0	270
100	10	0	0	0	0	>500
RC50[b)]	13	9	0.04	<0.01	<0.01	

[a)]EC50 = calculated concentration of oxadixyl resulting in 50% inhibition of sporulation
[b)]RC50 = calculated amount of r (%) allowing 50% inhibition of sporulation at a given fungicide concentation

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TABLE 4. Percent phenylamide resistant sporangia of *P. infestans* in a mixed population after repeated fungicide treatment [a)] with oxadixyl + mancozeb (o+ma = 1 + 7) and oxadixyl + mancozeb + cymoxanil (o+ma+c = 1 + 7 + 0.4) (Samoucha and Gisi, 1987)

sporangium generations	percent resistant sporangia collected from plants treated with oxadixyl + mancozeb					o+ma+c
0	0.01	0.1	1	10	50	50
1	<1	<1	8	12	63	45
2	1	6	12	20	72	46
3	8	12	22	40	90	40
4	10	32	45	60	100	45
5	22	70	90	100	100	43

[a)] each fungicide treatment (100+700±40 mg/l) was made on a new set of plants inoculated with sporangia of the previous generation (cycling every 7 days)

SELECTION PROCESS THROUGH FUNGICIDE APPLICATION

A sensitive and a phenylamide-resistant strain of *P. infestans* were mixed in proportions of 0.01, 0.1, 1, 10, 20, and 50% r in the sporangium suspension (Table 4). Plants were inoculated and produced symptoms with a new sporangium generation after one week in a growth chamber. The first sporangium generation was harvested and used for inoculation of the second plant set. The applications of a PA-based mixture was made for each inoculation, providing a repeated fungicide selection pressure. The resistant subpopulation did not increase to more than 12% after four, three, and two sporangium generations, when initial r populations were 0.01, 0.1, and 1%, respectively (Table 4). Therefore, when there is not more than 1% resistance in a population, two to four applications of fungicide mixtures per season are justifiable without getting into serious resistance problems. Thus, the FRAC recommendations (Urech and Staub, 1985) to restrict the number of applications are fully supported by experimental data. The population treated with the two-way mixture was fully resistant after four cycles, whereas no increase of the resistant subpopulation was detected with the three-way mixture, even after eight sporangium generations (Table 4). Results similar to those with *P. infestans* were also found with *P. viticola* on grapes (Samoucha and Gisi, 1987). Thus, the addition of cymoxanil in mixtures containing phenylamides and mancozeb strongly delay the build-up of resistance.

Field-grown potato plants in plastic houses were inoculated with a population containing 10% resistant sporangia (Fig. 1). The developing disease was treated four times with two different mixtures, either oxadixyl+mancozeb or oxadixyl+mancozeb+cymoxanil. Percentage of resistant sporangia in the population were recorded over a period of 60 days. As seen in the growth chamber results (Table 4), the three-way mixture imposed only a very low selection pressure, whereas treatments with the two-way mixture produced complete resistance after about 35 days (Fig. 1). The fungicide mixture oxadixyl+mancozeb still provided about 90% disease control after two applications, despite a proportion of about 60% resistance sporangia in the population (Cohen and Samoucha,1990).

SYNERGISTIC INTERACTIONS

Synergistic interactions occur when mixtures of a phenylamide fungicide and one or several other fungicides (contact or systemic) active against *Phytophthora* or *Plasmopara* are applied (Gisi *et al.*, 1985, Gisi 1991). If interactions between fungicides are to be investigated under field conditions, it is essential to apply the components alone and in the mixture at identical rates and intervals. As an example of many field trials, Table 5 illustrates interactions between oxadixyl, mancozeb and cymoxanil which gave synergy ratios between 1.7 and 5.3. Cymoxanil-containing mixtures produced higher synergism for resistant than for sensitive strains. Dosages yielding 90% disease control were reduced significantly in the mixtures. The three-way mixture o + ma + c was not much affected by different sensitivities of the strains, whereas the two-way mixture o + ma was clearly less effective against resistant than against sensitive strains. In another field experiment, the potato crop was inoculated with a *P. infestans* sporangium suspension containing initially 10% resistant sporangia. Half rates of phenylamide-based products applied weekly reduced resistance build-up more effectively than the full rates (n-rate) of the mixtures applied at biweekly intervals and it provided also much

Fig 1.: Percentage resistance frequency of a *P. infestans* population containing initially 10% resistant sporangia developing on field grown potatoes treated four times (Tr, arrows) with oxadixyl (o), mancozeb (ma), oxadixyl+mancozeb (o+ma) or oxadixyl+mancozeb+cymoxanil (o+ma+c). In = Inoculation; S_{un} = first symptoms on untreated plants: S_{tr} = first symptoms on treated plants (Cohen and Samoucha, 1990)

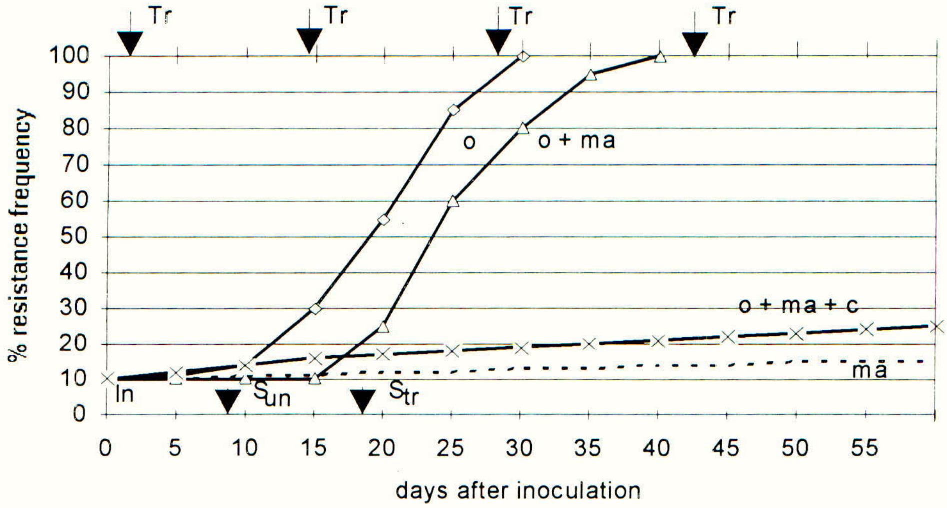

TABLE 5. Fungicidal activities (EC_{90}, g ha^{-1}) of oxadixyl (o), mancozeb (ma), cymoxanil (c) alone and in mixture and synergy ratio of mixtures against a phenylamide-sensitive and phenylamide-resistant isolate of *P. infestans* on potato under field conditions (Samoucha & Cohen, 1989)

fungicides	EC_{90} (g ha^{-1}) sensitive	resistant	Synergy ratio sensitive	resistant
o	60	>2000	-	-
ma	760	1130	-	-
c	350	310	-	-
o+ma = 1+7	100 (12+88)[a)]	550 (69+481)	3.2	2.3
ma+c = 7+2	220 (177+43)	260 (209+51)	2.8	2.9
o+ma+c = 1+7+2	110 (11+77+22)	150 (15+105+30)	2.8	5.3

[a)] Figures in parentheses are dosage of the individual components in the mixture. Recommended rates for *P. infestans* control in many countries are o+ma+c = 200+1400+80 g ha^{-1}

better disease control (Samoucha *et al.* , 1993). This observation shows that a reduced selection pressure (in this case lower dosage per treatment) delays the build-up of resistance.

CONCLUSIONS

Phenylamide-based products continue to control successfully diseases caused by fungi of the Peronosporales. Resistance to the phenylamide component can develop and cause problems if the products are not carefully used. Sensitivity monitoring can assist resistance management and help explain problems of product performance, but the sampling and sensitivity test methods used can greatly influence the relevance of the data. In the end, product performance is more important than the estimation of the amount of resistant subpopulations. Different antiresistance strategies can be used depending on the local conditions and the overall resistance risk. Mixtures of phenylamides and other fungicides represent an effective way to delay resistance build-up. Synergistic mixtures allow a decrease of the amount of active ingredients without reducing the overall activity. Decreased dosage of fungicides in synergistic mixtures lower the risk of selecting for resistant strains.

A devil's advocate may ask several questions after having read this paper: 1) Why should phenylamide-based products control the diseases in the field when sensitivity tests show that the populations are resistant against phenylamides and since contact fungicides may be removed by rain in many situations (and in case of a three-way mixture, cymoxanil may be degraded by the plant and the fungus within a few days)? 2) Why should we continue any sensitivity monitoring after having detected consistantly more than 50% of isolates to be resistant in several countries and crops? 3) What does the detection of phenylamide-resistant strains mean, since PA-based products are still showing good disease control in the field? 4) Why do we find an increasing amount of phenylamide-resistant isolates in fields never treated with phenylamide-based products?

Possible answers to the four questions may be given as follows: At the beginning of an epidemic, field populations are obviously not resistant to such a degree that products would give inadequate disease control. The overall product performance is a result of synergistic interactions also under situations of resistant subpopulations rather than a separate event of single ingredients. Mixtures extend the duration of activity of cymoxanil and delay rather than control resistance build-up. Today's routine sensitivity tests probably overestimate the amount of resistant sporangia in field populations. The frequency of resistant subpopulations fluctuates during the season and from year to year. During extensive monitoring programs one may estimate the actual amount of resistant isolates in a population, but more important, we also begin to understand many aspects of population biology like distribution and migration of the fungus in a region and over time, as well as fitness parameters (epidemiological and overwintering properties) and phenotypic and genotypic behaviour. Today`s monitoring methods may not be adequate to properly estimate the amount of resistant sporangia in a field population. Furthermore, we always neglect the majority of cases in which the products gave good control, because without detectable disease left in the field after application of products, we cannot collect samples for sensitivity tests. Moreover, sensitivity tests are normally done with a single active ingredient, not with products. Parallel to the appearance of resistant subpopulations, the migration of new races and the appearance of both mating types probably became more important for epidemics of *P. infestans*. More studies like those by Fry *et al.* will be needed to better understand the permanently changing scenery of late blight in potatoes and downy mildew in grapes.

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