Annu. Rev. Phytopathol. 1996. 34:549–72

RESISTANCE TO PHENYLAMIDE FUNGICIDES: A Case Study with *Phytophthora infestans* Involving Mating Type and Race Structure

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KEY WORDS: metalaxyl, oxadixyl, fitness, virulence, resistance management, late blight, potato, tomato

ABSTRACT

Phenylamide-resistant isolates of *Phytophthora infestans* have gradually become an important part of populations in many countries. However, fungicide mixtures containing a phenylamide component are still an effective strategy for the control of late blight in potato and tomato. The proportion of phenylamide-resistant isolates fluctuates from year to year and within the season. Almost concurrent with the appearance of resistant isolates was the discovery of the A2 mating type of *P. infestans* in many European countries and in other parts of the world. However, no genetic correlation exists between resistance and mating type, and the proportion of A2 isolates in European populations remains small. Resistance to phenylamides became established in A1 populations before the appearance of A2 type. Resistant isolates express equal or greater fitness than sensitive isolates, but no correlation was detected between resistance and race structure. The continuous changes in *P. infestans* populations require careful adaptation of successful disease control programs.

0066-4286/96/0901-0549$8.00

INTRODUCTION

Phenylamide fungicides (PAFs) are a class of systemic compounds including metalaxyl (Ridomil®), oxadixyl (Sandofan®), benalaxyl (Galben®) and ofurace (Patafol®) that show excellent protective, curative, and eradicative antifungal activity and exclusively control diseases caused by Peronosporales (76). Although different in their intrinsic activity, systemic behavior, and persistence of activity, all four molecules are specific inhibitors of ribosomal RNA polymerases in the target fungi (14). The incorporation of uridine into rRNA is reduced by PAFs (20–60% of the untreated control, depending on the fungal species). However, even at concentrations that fully suppress fungal growth, inhibition of uridine incorporation is incomplete, suggesting that only a part of the cellular RNA synthesis is sensitive to PAFs. In phenylamide-resistant isolates, this process remains completely unaffected by PAFs (14). Endogenous RNA polymerase activity of isolated nuclei from phenylamide-sensitive isolates of *Phytophthora megasperma* and *P. infestans* is highly sensitive to PAFs but not that of phenylamide-resistant isolates, suggesting that a target site change is responsible for resistance. This hypothesis was further supported by binding studies: (^{3}H) metalaxyl binds to cell-free mycelial extracts of sensitive but not of resistant isolates (14). The binding site in isolates of *P. megasperma* showed a lower affinity for oxadixyl than for metalaxyl or benalaxyl. Although the four phenylamides show different intensities of inhibition of rRNA polymerases or the various developmental stages of the target fungi, positive cross-resistance behavior was observed for all molecules. The level of resistance (resistant factor), however, is specific for the compound and the isolate (21, 30). Detailed review articles are available on the mode of action and systemic behavior of PAFs as compared to other fungicides controlling diseases caused by oomycetes (6, 76). This review concentrates on population biology and dynamics of *P. infestans* in regard to sensitivity and resistance to PAFs, and on resistance management using synergistic fungicide mixtures. Special emphasis is given to the changes in population genetics, mating type, and race structure, and their possible effects on resistance.

Since their discovery in 1977 (93), PAFs have significantly contributed to effective control of diseases caused by Peronosporales in general and of *P. infestans* in particular. In addition to the conventional fungicides like dithiocarbamates, triphenyltins, chlorothalonil, cymoxanil, and more recent antifungal compounds (propamocarb, fluazinam, dimethomorph), the PAFs play an important role in modern disease control programs. As early as 1979, two years after metalaxyl was introduced in some countries as a straight product (without mixing with a second fungicide), the first phenylamide-resistant isolates of *Pseudoperonospora cubensis* were isolated from cucumbers grown in

plastic houses in Israel (68). In 1980, PA-resistant isolates of *P. infestans* were detected on field-grown potatoes in Ireland (IRL), The Netherlands (NL), and Switzerland (CH) (Figure 1). Associated with the detection of resistant isolates was a decline in disease control. When used in mixture with other fungicides (e.g. mancozeb), PAFs performed well and resistant subpopulations evolved much slower (87, 88). Resistant isolates were also detected in other pathogens, e.g. *Plasmopara viticola* on grapes in France in 1983 (61), *Bremia lactucae* on lettuce (12, 62), *Pythium spp.* in turf (70), and *Peronospora tabacina* in tobacco (3).

ORIGIN AND DEVELOPMENT OF RESISTANCE

Resistance to PAFs originated from naturally occurring resistant (insensitive) isolates existing at a very low proportion in the population even before any exposure to the fungicide. Daggett et al (13) recently reported that PA-resistant isolates existed in 1977 north of Berlin, prior to any application of metalaxyl in Germany. This is most probably not the only case of pre-existing PA-resistant isolates worldwide. Whether PA-resistance in Europe resulted from immigration of resistant genotypes or from the simultaneous occurrence of mutations at different sites is unknown. Such pre-existing resistant individual isolates are produced by random mutation in the absence of PAFs; the mutation frequency is not altered by applying PAFs. However, the use of PAFs resulted in selection and increase in the frequency of resistant individuals until a distinct subpopulation became an important factor in disease epidemics. Phenylamide resistance is a monogenic trait (29, 79), originating from one or at most a few mutations. In crossing experiments (79), the majority of the F_1 progeny from metalaxyl-resistant and metalaxyl-sensitive parental isolates of *P. infestans* were intermediately sensitive to metalaxyl. Crosses between two intermediately sensitive F_1 isolates yielded a 1 : 2 : 1 ratio of sensitive : intermediate : resistant progeny in the F_2 generation. This Mendelian segregation patern reflects a single-gene controlled (monogenic) resistance. The selection process imposed on the fungal population by a PAF-treatment is disruptive and usually results in two distinct subpopulations with either sensitive or resistant individuals. The proportion and persistence of the resistant isolates depend on the selection pressure (e.g. concentration of fungicide and number of treatments), as well as the relative fitness of the resistant individuals in the population. The PA-resistant isolates of *P. infestans* are generally at least as fit as the sensitive isolates even in the absence of any fungicide selection (43, 46). PA-resistant isolates are much less sensitive to PAFs compared to a sensitive reference isolate with resistance factors usually higher than 100.

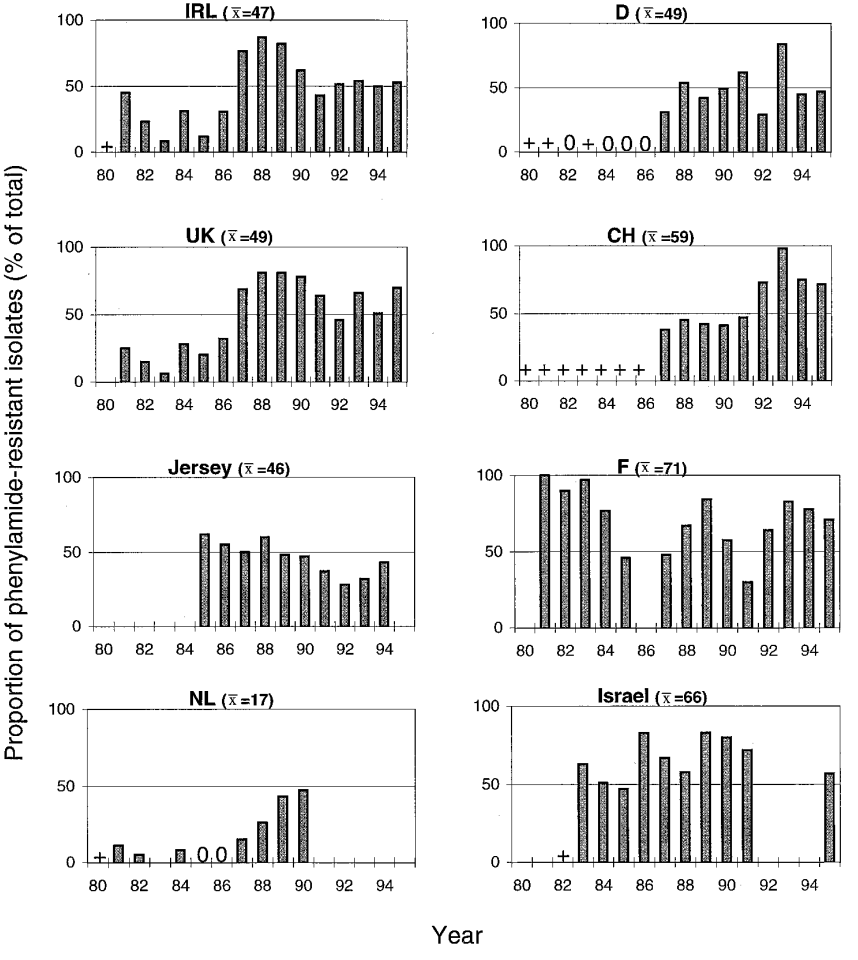

Figure 1 Annual proportion of phenylamide-resistant isolates (% of total) in *P. infestans* collected from potato fields in different European countries. Resistant isolates were also found in Finland (50), Poland (92), Spain (59), Japan (63, 64), United States/Canada (17, 20), Russia (FRAC), and South Africa (FRAC). IRL : L Cooke/L Dowley (11, 23, 34); UK: Ciba (1981–1985), ADAS (1986–1992), FRAC (1993–1995) (34); Jersey: R Collier (10); NL: L Davidse (15, 27); D: B Schöber (personal communication; 72)/S Dagget (13)/E Götz (first record of PA-resistance in Europe 1977); CH: U Gisi (34 and FRAC); F : S Duvauchelle (personal communication; 34)/D Andrivon (2); Israel: Y Cohen (48). + = few resistant isolates detected; 0 = no resistant isolates detected; blank = no values available.

ANTIRESISTANCE STRATEGIES

Since PAFs are considered to bear a high inherent risk of resistance (35), it was essential for the durability of PAF-products to formulate and enforce antiresistance strategies. Therefore, starting in 1982, PA-FRAC-Working Groups (**p**henyl**a**mide **f**ungicide **r**esistance **a**ction **c**ommittee) have been established on an international as well as local basis for some countries, with members of all PA-producing companies required to formulate recommendations for the proper use of PAFs. These strategies (94) include **preventive** use of prepacked **mixtures** of PAFs with well-defined rates of nonphenylamide fungicides, **limitation** on the number of applications per crop and per season (a maximum of 2–4 consecutive treatments with 14-day intervals), and **no soil use** for the control of airborne pathogens.

Based on the rapid appearance of resistant isolates of *P. infestans*, the use of metalaxyl alone was suspended from 1981–84 in Ireland and The Netherlands (86). Several two- and three-way mixtures were introduced to the market in other countries, and in Ireland and The Netherlands after 1985. Mathematical models and experimental studies support the usefulness of PA-based mixtures as a valid antiresistance strategy. A model proposed by Levy & Strizyk (57) suggests that the selection for resistant subpopulations is slow (about 3% increase over six seasons) when a PA-mixture with as high or even higher synergistic interaction is used to control resistant compared to sensitive subpopulations. In many greenhouse and field tests, oxadixyl-mancozeb mixtures (and any other PA with contact fungicides) provided strong synergistic interactions (30–32) and significantly delayed the effects of resistance build-up in population dynamics experiments (69, 87). Potato crops in plastic houses, inoculated with a mixed population of *P. infestans* that initially contained 10% resistant sporangia, were treated four times with either oxadixyl alone, a two-way oxadixyl-mancozeb, or a three-way oxadixyl-mancozeb-cymoxanil mixture, and the proportion of resistant sporangia was recorded over a period of 60 days. After three treatments, oxadixyl alone selected for a 100% resistant subpopulation, whereas the two- and three-way mixtures allowed an increase to about 70% and 20% resistance, respectively (9). The addition of cymoxanil to the two-way mixture is an enforced antiresistance strategy, especially under heavy disease conditions, compared to oxadixyl-mancozeb; the latter mixture is still a valid strategy, provided initial populations contain no more than about 1% resistant sporangia. In discontinuous epidemics of *P. infestans* containing initially 0.01, 0.1, and 1% resistant sporangia, the resistant subpopulation did not increase to more than 12% after four, three, or two sporangium generations, respectively, when the tomato plants were treated once with the two-way mixture (69). Thus, the FRAC recommendations to restrict the number of treatments to two to four per season are fully supported by experimental results. Synergistic interac-

tions between fungicides in a mixture reduce the risk of selecting for resistant subpopulations and enforce the level and duration of activity of such mixtures (31). Attempts to explore the physiological and biochemical background of the synergistic interactions between PAFs and cymoxanil and/or contact fungicides showed that these mechanisms remain speculative (7).

ESTIMATION OF RESISTANCE AND SENSITIVITY MONITORING

Three approaches can be used to estimate resistance; they are not always clearly distinguished: (*a*) the proportion of sites (fields, farms) containing resistant isolates; (*b*) the proportion of isolates resistant to PAFs; and (*c*) the proportion of resistant sporangia in a bulk sample (isolate). The first approach may give an adequate distribution pattern over large areas (e.g. a country), but it overestimates the frequency of resistant individuals in populations. The second approach is more accurate in that it also estimates the proportion of resistant individuals within small populations, but it needs a minimum of 20–50 isolates per agronomic "unit" (e.g. north of France) and a definition of isolates (bulk samples, single-spore isolates). The third approach estimates the amount of the resistant subpopulation in an epidemic rather accurately (e.g. in population dynamics studies), but it is too laborious for studies addressing the distribution pattern of resistant isolates in a given area.

The method most widely used to determine the sensitivity of *P. infestans* to PAFs is based on the sporulation intensity of the pathogen on leaf discs that either have been pretreated (oxadixyl-method) or are floating on fungicide suspension (metalaxyl-method). This method is recommended by FRAC (85) and is adapted to large tests of bulk samples (sporangium suspension of original field samples) without the need to transfer or purify the fungus prior to testing. This "qualitative or semi-quantitative" test method results in a resistant response when as few as 1–5% resistant sporangia are present in the test suspension. If more accurate analyses are needed, the tuber disc method (8) is recommended. Some researchers (e.g. 17) have used linear mycelial growth on fungicide-amended agar to determine the response to PAFs. However, the growth characteristics or sporulation capacity of some isolates of *P. infestans* are different in vitro than on planta (discs or plants). Therefore, in vitro sensitivity results must be double-checked with in vivo tests before conclusions can be drawn. The time of sampling (early vs late in the season) may result in detection of different levels of resistance. Invariably, the proportion of resistant isolates increases during the season, more rapidly in fields treated with PAFs than in fields not treated with PAFs (Figure 2) (23, 97), and resistance levels at

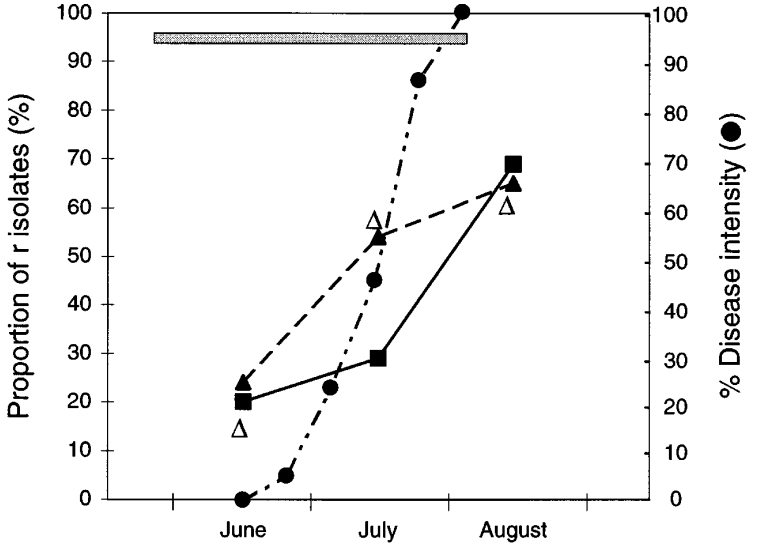

Figure 2 Disease progress curve (●) and increase of phenylamide-resistant isolates during epidemics of *P. infestans* in potato fields in Switzerland treated (▲) or not treated (■) with phenylamide-based products (mean of the years 1987–1990, 34) as compared to epidemics in Ireland (△), 1981–1985, 23). (▬) period of PAF applications.

the start of the next season are lower than those detected at the end of the previous year. Therefore, selection pressure by PAFs cannot be the only reason for the increase of resistance. There must also be some advantages/disadvantages in fitness in resistant isolates over sensitive isolates during the epidemic and overwintering phase of the fungus' life cycle (see below).

DISTRIBUTION OF RESISTANCE

A decrease in resistance was observed between 1981 and 1984, the period during which PAFs were suspended in Ireland and The Netherlands (Figure 1). Over the past 15 years since PAFs have been used in Europe, the average proportion of PA-resistant isolates detected is close to 50% for Ireland (Northern and Republic), UK, Jersey, Germany, and Poland (92), whereas it is lower in The Netherlands (20%) and higher in Switzerland, France, and Israel (ca 60, 70, 70%, respectively) (Figure 1). In Spain (59) and Finland (50), average resistance levels of 80 and 75%, respectively, have been reported in the past few years. In single years the values varied significantly (between 0–100%), with maximum levels at 1987–90 in most countries. In the past five years, resistance

levels remained more or less stable in all European countries. In Switzerland, however, the s : r ratio was about 1 : 1 until 1991, with a very low proportion of intermediates; in 1992, the majority of sensitive isolates disappeared, whereas the intermediates doubled and further increased to about 30% until 1995 (33; Figure 3). This interesting development might involve sexual recombination, although the A2 mating type is extremely rare in Switzerland (Figure 4).

In the United States and Canada, the appearance of PA-resistant isolates is recent: they were first detected in 1991 in the Pacific Northwest [Washington, British Columbia (20; Table 1)]. During 1992–95, the proportion of PA-resistant isolates steadily increased in the United States: from 46% in 1992 to 71% (1993), 87% (1994) and 82% (1995) (16). High proportions (60–100%) were found only in the south and west (Florida, Texas, California, Washington, British Columbia), whereas low values (< 20%) were observed in the north and east (Alberta, North Dakota, Minnesota, Maine, Prince Edward Island) (17). In Canadian provinces other than British Columbia, the average frequency of resistance in 1994 was reported to be around 55% (67; Table 1). Although complete studies over several years in the described regions have not yet been done, the migration and development of PA-resistance was obviously much faster in North America than in Europe.

In Japan, PA-resistant isolates were first detected in 1988 (53, 63, 64) at a low proportion (< 20%) and steadily increased to about 50%, mainly in the Hokkaido area.

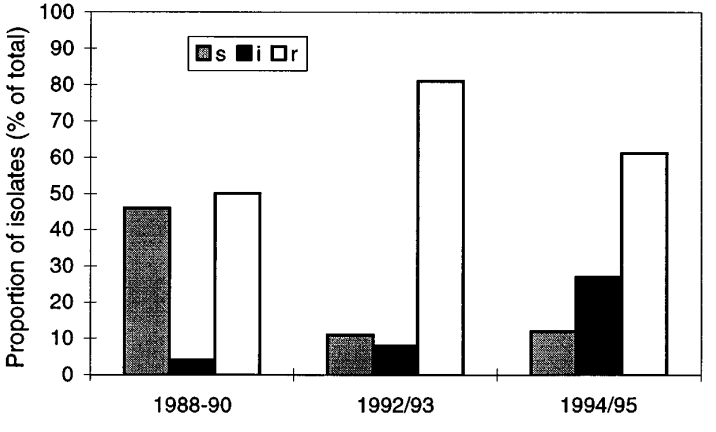

Figure 3 Proportion of *P. infestans* isolates (% of total) collected in potato fields in Switzerland between 1988-95 showing a sensitive (s), intermediate (i) and resistant (r) response to phenylamide fungicides (33).

Table 1 Annual proportion of phenylamide-resistant isolates (r, % of total) and A1/A2 mating type isolates (% of total, and % sensitive and resistant) of *P. infestans* collected from potato fields in Mexico, USA, and Canada between 1991 and 1994

Location	%r	A1/A2	A1s/A1r	A2s/A2r	Reference
California (1993)	100	100/0[a]	0/100	–	17
Washington (1991)	100	100/0	0/100	–	17
North Carolina (1994)[b]	96	0/100	–	4/96	25
British Columbia (1991)	87	93/7	?	?	17
All USA (1994)	87	?	?	?	16
Mexico (1992)	85	17/83	10/90	16/84	17
Texas (1993)	79	22/78	93/7	0/100	17
British Columbia (1993)	73[c]	16/84[d]	16/84	30/70	5
Florida (1993)	61	39/61	100/0	0/100	17
All Canada (1994)	55	38/62	?	?	67
Minnesota (1994)	20	100/0	80/20	–	77
Alberta (1993)	16	100/0[e]	84/16	–	17
North Dakota (1994)	10	100/0	90/10	–	77
Prince Edward Island (1992)	7	100/0	93/7	–	17
North Dakota (1992)	0	100/0	100/0	–	17
Maine (1992)	0	100/0	100/0	–	17

[a] Also A2 and r isolates present (M Coffey, personal communication).
[b] Mostly tomato isolates.
[c] r isolates present only in British Columbia of all Canadian provinces.
[d] 1993: A2 only in British Columbia; 1994: A2 also in New Brunswick.
[e] Also true for 1994 (67).

APPEARANCE OF A2 MATING TYPE OUTSIDE MEXICO

Almost concurrent with the initial appearance and increase of PA-resistant isolates in Europe was the discovery of A2 mating type outside Mexico: 1980 in the former East Germany (13), 1981 in Switzerland (42), United Kingdom (90), and The Netherlands (26). The A2 mating type has subsequently been isolated repeatedly in almost all countries in Europe, except France (2, 56) and Spain (59), although usually at very low proportions ($\bar{x} < 20\%$; Figure 4). Despite experimental evidence that oospores can infect potato and tomato plants (24, 73), it is debatable, whether the A2 mating type, given its low frequency (Figure 4), can or will play an important role in the epidemiology of late blight in Europe. More information is required on the temporal and spatial distribution of A2 isolates in mixed A1+A2 populations and their effects on oospore production in planta. Figure 4 shows that the proportion of the A2 mating type increased in Europe from undetectable levels to about 20–30% for a few years and declined thereafter. This pattern might involve some inferior epidemiological properties of the A2 type that render it less competitive than the A1 type. Otherwise,

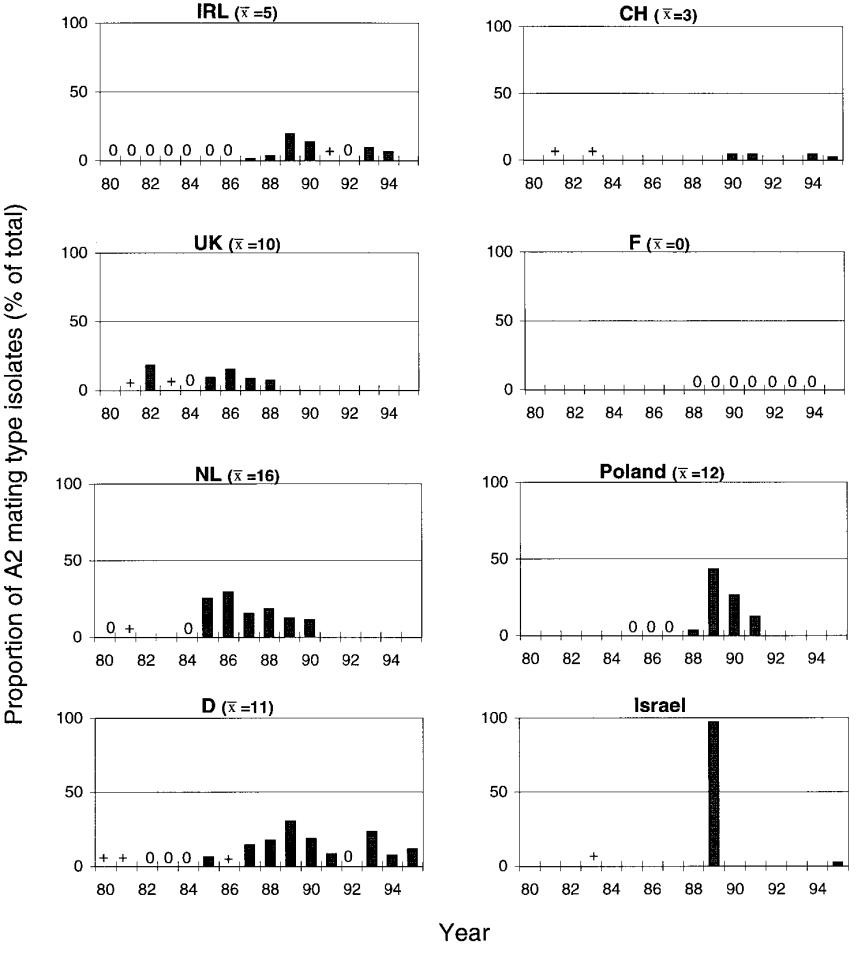

Figure 4 Annual proportion of A2 mating type isolates (% of total) in *P. infestans* collected from potato fields in different European countries. A2 isolates were also found in Egypt 1984 (83), Sweden 1985 (44), Japan 1985 (63), United States 1987 (18), Canada 1989 (18), Norway (41)/Finland 1994 (50), and in some Asian and South American countries. IRL: E O'Sullivan/L Dowley/L Cooke (66); UK: P Tantius (90)/R Shattock (82); NL: H Frinking (26)/A Drenth (24); D: B Schöber (74)/S Daggett (13)/E Götz (first record of A2 in Europe 1980 (36); CH: HR Hohl (42) /U Gisi (33, 58); F: D. Andrivon (2, 56)/S. Duvauchelle (personal communication); Poland: C Therrien/L Sujkowski (89, 92); Israel: M Grinberger/Y Cohen (39; unpublished data). + = few isolates detected; 0 = no A2 isolates detected; blank = no values available.

the low proportion of A2 type isolates in Switzerland and Israel (Figure 4) is hard to explain, especially for isolates that are intermediately sensitive to PAFs. Intermediates are believed to be the product progeny of sensitive A1 × resistant A2 (or vice versa) and should produce A1 and A2 at a ratio of 1 : 1. In Switzerland, the frequency of the A2 type never surpassed 4% of the isolates tested, and in Israel, the population that was once predominately A2 (1989) changed drastically, and today (1995) the A2 type has almost disappeared (Figure 4).

Interestingly, in France, Switzerland, and Spain with high levels of PA-resistant isolates, very few or no A2 isolates were detected (Figures 1, 4). Elsewhere A2 isolates tended to be less frequent during years with a high level of PA-resistance. In our analysis, however, no correlation exists between resistance levels and the proportion of either mating type (Figure 4). In Poland (92), Germany (13; B. Schöber, personal communication) and The Netherlands (27), the A1/A2 frequency is about 80%/20%. When the PA-sensitivity distribution of the two mating types was analyzed separately, an average frequency of 50%/50% and 80%/20% for A1s/A1r and A2s/A2r, respectively, was observed for the three countries. Obviously, the A2 populations in European countries contain primarily PA-sensitive individuals; thus, the almost simultaneous appearance of PA-resistant and A2 type isolates is not linked. Genetic studies

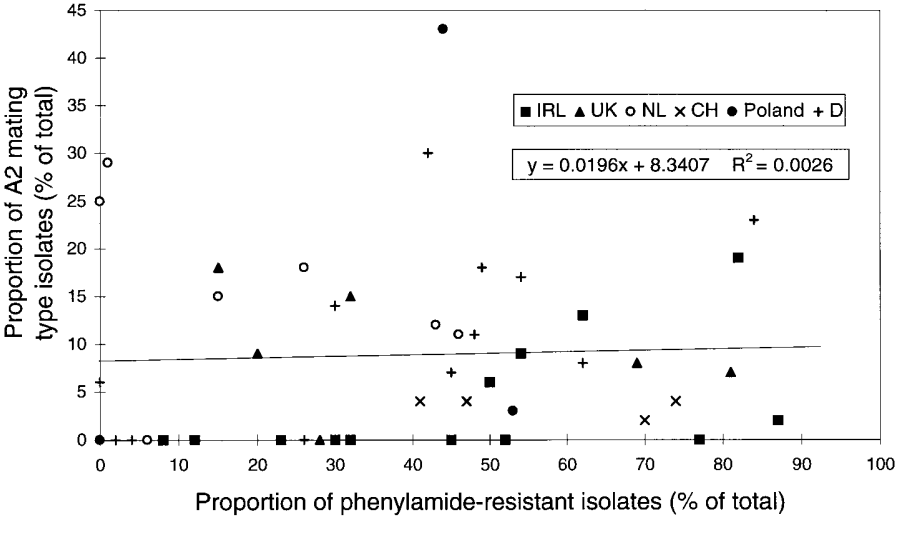

Figure 5 Proportion of phenylamide-resistant isolates vs A2 mating type isolates (% of total) in *P. infestans* collected from potato fields in different European countries between 1981–95 (data compiled from Figures 1 and 4).

(see below) support the observation that mating type and resistance to PAFs are independent traits.

In the United States and Canada, the A2 mating type was very rare until 1991; single A2 isolates had been recovered in 1985 in Pennsylvania and in 1989 in British Columbia (18). In 1992, A2 became more frequent, but there are still areas with predominating A1 populations (California, North Dakota, Alberta, 1993; Table 1). A1 populations in North Dakota and Alberta are mostly sensitive to PAFs, as in Europe, but in California are mainly resistant (17). A few, mostly sensitive A1 and predominately resistant A2 isolates were found in other states (Florida, Texas), whereas most isolates in British Columbia and Mexico are of the A2 type and are PA-resistant (1993, Table 1).

CHARACTERISTICS OF A2 MATING TYPE

Shortly before or concurrent with the discovery of PA-resistant isolates and the appearance of the A2 mating type in Europe, the dominant ("old") populations of *P. infestans* were claimed to be displaced by a "new" population, probably introduced in 1976 by a potato shipment from central Mexico to Europe (28, 65). The new populations belonged to the A2 mating type, and had a distinct DNA fingerprint pattern and allozyme profiles; they were claimed to be more pathogenic and hence fitter than the old ones. The old genotypes were 86/100 for glucose phosphate isomerase (Gpi) and represented a single clonal lineage. The new genotypes detected in The Netherlands and Germany between 1978 and 1982 were described as 90/100 or 100/100 for Gpi and genetically diverse (28). However, the proportion of A2 isolates rose higher than 10% only in 1985 (The Netherlands) and 1987 (Germany) (Figure 4). Despite evidence that the old European population was displaced by a new one from central Mexico, two other hypotheses have been advanced to explain the appearance of A2 in Europe: (*a*) A2 evolved from A1 either by hormonal induction (51) and/or through mutation or mitotic recombination (82); or (*b*) A2 was always present in Europe in a very low and undetectable proportion. Its relative fitness might have changed and its frequency increased through either mutation or a change in environment. Whatever the source of the "new" genotypes, there was a lag of several years before they became a distinct part of the population. Probably, the new traits from A2 were **incorporated** into the old A1 genotypes by mating rather than by displacement. The A2 trait seemed to decline a few years later for reasons not yet understood (see above).

Castro (65) showed that homothallic *P. infestans* may asexually segregate into homothallic A1A2 (which give rise to A1 but not A2) and A2 cultures. This finding confirms the well-known phenomenon in *Phytophthora* that variation among zoospore progenies from a single isolate might also be a result of

parasexual recombination (fusion) in the vegetative phase (54). In contrast, Ko (52) presented evidence that A2 may evolve from A1 by selfing. When single zoospore A1-cultures were stimulated to self by hormonal induction, 2–13% of the offspring of each selfed progeny were A2. Similarly, when A2 was allowed to self, some A1-offspring were formed in addition to the A2 parental type. Ko (52) disagreed with the hypothesis of Sansome (71) and Goodwin & Fry (37) that A1 is homozygous recessive *aa* and A2 is heterozygous *Aa*. He suggested that the appearance of A2 *P. infestans* outside Mexico in the early 1980s may have originated from selfed oospores produced by the A1 mating type. On the other hand, Kadish & Cohen (D Kadish & Y Cohen, unpublished), using Israeli isolates, showed that, upon selfing, A1 produced A1 progeny only, whereas A2 produced both A1 and A2. The F_1 progeny from the cross A1 $\times$ A2 segregated to A1 and A2; the F_2 of A1 gave rise only to A1, whereas the F_2 of A2 gave rise to both A1 and A2 mating types. The backcrosses of F_1 to either parent also produced both mating types, supporting the hypothesis of the recessive homozygosity of A1 and heterozygosity of A2.

INHERITANCE OF PHENYLAMIDE RESISTANCE

Analysis of inheritance of resistance to metalaxyl suggested (80) that a single, incompletely dominant gene was involved, even though backcross progeny showed unexpected frequencies of phenotypes, isozymes, and sensitivity to metalaxyl (84). The results of a study by Kadish & Cohen (D Kadish & Y Cohen, unpublished) are shown in Table 2. Israeli field isolates ($2c = 2n$) were used for mating; analysis of resistance to metalaxyl was conducted with sporangia inoculated onto potato tuber discs placed on metalaxyl (45). The sensitive parent failed to sporulate on tuber discs treated with 1 μg/ml metalaxyl and the resistant parents sporulated on all discs treated with 100 μg/ml. The F_1 progeny sporulated on all discs treated with 1 μg/ml and on some discs treated with 10 μg/ml but not on discs treated with 100 μg/ml. The data (Table 2) suggest that resistance to metalaxyl is controlled by a single codominant nuclear locus. The single deviation in the F_1 progeny probably resulted from selfing.

The possible linkage between the locus for metalaxyl resistance and other loci, e.g. A2 mating type, enhanced fitness, polyploidy, and complex virulence, has frequently been raised (4, 24, 52, 75). Majoros et al (58) found no association between mating type and resistance to oxadixyl among 139 isolates from Switzerland. Therrien et al (91) analyzed isolates collected in The Netherlands in 1987; the frequency of resistance to metalaxyl in both mating types was about equal. They proposed that the initial mutation for metalaxyl resistance in The Netherlands occurred in A1 and only later in A2, either spontaneously or via recombination with A1. Therrien et al (92) did not detect any relationship

Table 2 Inheritance of resistance to metalaxyl in field isolates of *P. infestans* (D Kadish & Y Cohen, unpublished)

Parental isolate(s)[a]	Generation	No. of single oospore cultures			Expected ratio	X^2		
		Sensitive	Intermediate	Resistant		Value	df	P
ms3 (A1)	P_1	21						
mr1 (A2)	P_2			52				
mr2 (A2)	P_3			75				
$P_1 \times P_2$	F_1	1[c]	58	1[c]				
F_1, self[b]	F_2	22	46	19	1:2:1	0.22	2	0.90
$F_1 \times P_1$	BC	14	15		1:1	0.034	1	0.85
$F_1 \times P_2$	BC		15	14	1:1	0.034	1	0.85
$P_1 \times P_3$	F_1		47	1[c]				
F_1, self[b]	F_2	14	32	14	1:2:1	0.27	2	0.87
$F_1 \times P_1$	BC	10	8		1:1	0.22	1	0.64
$F_1 \times P_3$	BC		7	9	1:1	0.25	1	0.62

[a] All three isolates had a ploidy of 2c=2n and were sensitive (ms) or resistant (mr) to metalaxyl. Parental isolates (P_1, P_2, P_3) were stable through 5 generations of selfing and many zoosporic "generations". BC = backcross.
[b] Selfing was conducted by hormonal induction.
[c] Possible "self".

between ploidy level and either mating type or sensitivity to metalaxyl. They proposed that resistance became established in the A1 population long before the appearance of A2 (1988) in Poland.

RACE STRUCTURE

For a new population to displace an old one, an improved relative fitness of the former is required. Virulence gene complexity was argued to serve as a major driving force for changes in populations. Schöber (74, 75) described an increase in the diversity of the virulence gene pool and in the number of virulence genes per isolate in *P. infestans* populations in Germany between 1950 and 1990. Highly complex races of *P. infestans* were already present in the UK (81) by the beginning of the 1970s, long before the appearance of the new races in Europe. Andrivon et al (2) discovered a diverse virulence pattern for isolates of *P. infestans* in France. Compared to their results, a lower diversity of pathotype distribution was described in *P. infestans* populations in Switzerland (33). Generally, few races (phenotypes, pathotypes) dominate *P. infestans* populations in Europe. Most frequent in many countries are races [1.3.4.7.10.11] and [1.3.4.7.8.10.11] but [1.3.4.7.11] and [1.3.4.6.7.8.10.11] are also important, the latter so especially in Switzerland and Israel in 1994/95 (Table 3). Most of the isolates tested in Switzerland in 1994 (33) originated

from Bintje, a cultivar without any R-resistance genes and therefore imposing little selection pressure on the pathogen population. Unnecessary virulence factors are frequent in most populations, and rare virulence factors are found in highly complex races (1). The race structure in the United States seems to be different: in 1992–94 about half of the isolates tested had 0–4 virulence factors, whereas the other half carried 6–9 factors (38). The simple races were mostly sensitive to PAFs.

In the population of *P. infestans* in Switzerland in 1994, the proportion of PA-resistant isolates was lower in highly complex races (7–8 virulence factors) than in less complex races (5–6 virulence factors) (33; Table 3). In Israel, the same proportion of resistant isolates (about 50%) was found in all races (Table 3). Further analyses are needed to characterize the virulence gene complexity and sensitivity to PAFs in populations of *P. infestans* for different countries. So far, no linkage has been found between the number or composition of virulence genes and the sensitivity profile. The population of isolates of *P. infestans* in

Table 3 Race structure and proportion (% of total) of *P. infestans* isolates collected from potato fields in different European countries

Number of virulence factors	Race structure	Proportion of isolates %					
		NL	Switzerland		France		Israel
		1988[a]	1991[b]	1994[c]	1991[d]	1994[e]	1995[f]
		N = 77	N = 51	N = 53	N = 23	N = ?	N = 54
≤ 3		26	4	0	8	0	0
4	1.3.4.7 and others	14	10	0	4	12	0
5	1.3.4.7.11	7	33	3	13	16	2
5	Others (3 types)	5	6	0	4	?	0
6	1.3.4.7.10.11	44	4	53[g]	27	20	30[g]
6	1.3.4.7.8.11	0	11	0	9	?	0
7	1.3.4.6.7.10.11	1	0	19[h]	0	?	0
7	1.3.4.7.8.10.11	1	18	12	22	40	55[h]
7	1.2.3.4.7.10.11	0	3	0	9	?	2
8	1.2.3.4.6.7.10.11	0	1	2	0	?	0
8	1.3.4.6.7.8.10.11	0	0	11[i]	4	?	11[i]
9	1.2.3.4.6.7.8.10.11	0	4	0	0	?	0
	Others	2	6	0	0	12	0

[a]Schöber & Turkensteen (75).
[b]Gujer & Schöber (pers. communication).
[c]Gisi et al (33).
[d]Andrivon et al (2).
[e]Lebreton & Andrivon (56).
[f]Cohen (unpubl.).
[g]Switzerland: 92% r + i; Israel: 50% r + i.
[h]Switzerland: 61% r + i; Israel: 50% r + i.
[i]Switzerland: 33% r + i; Israel: 50% r + i.

Switzerland in 1994 (all A1 mating type) has been further analyzed by RAPD-PCR. At least five genetically different classes were detected, although no clear distinction was possible between PA-sensitive, intermediate, and resistant isolates; between isolates of different origins (potato cultivar, geographical site); or between different races (T Bruyère & U Gisi, unpublished). Population changes might be driven by factors like competitiveness and fitness of isolates rather than by virulence gene composition.

COMPETITIVENESS AND FITNESS OF METALAXYL-RESISTANT ISOLATES

Several research groups reported a higher competitiveness of metalaxyl-resistant isolates of *P. infestans* as compared to sensitive ones. Kadish & Cohen (45) measured the progress of epidemics artificially produced on untreated potato crops in walk-in plastic tunnels. They observed faster progress of the disease epidemics imposed by the PA-resistant than by sensitive isolates, i.e. 50% of the foliage was blighted after 11–13 days compared to 18–23 days, respectively. Three fitness parameters in each of ten sensitive and resistant field isolates of *P. infestans* collected in Israel were measured on potato plants (45). PA-resistant isolates produced 4.7 times larger lesions on leaves as compared to sensitive isolates. The isolates did not differ in mean sporulation capacity or in infection efficiency. A similar study with 14 potato cultivars (49) confirmed that resistant isolates were fitter than sensitive ones. The competitive ability of PA-resistant and sensitive isolates in mixture was studied on potato plants in the absence of metalaxyl (46). The proportion of the resistant subpopulation increased from an initial 10% to 100% after 8–10 sporulation cycles.

In a similar study by Kadish & Cohen (47), the disease progress and the population dynamics of mixed PA-resistant and sensitive isolates were followed in walk-in plastic tunnels on fungicide-untreated potato crops. Six pairs of isolates were inoculated as $r + s = 1 + 9$ mixture onto the plants, and infected leaf samples were taken for sensitivity monitoring. In all tunnels, late blight killed the crops in 26–31 days, and resistance frequency increased during the logarithmic phase of the epidemic to 70–85% of the population. Later, the resistance frequency decreased to 7–38% in six populations but continued to increase to 94–100% in the other six. Although all resistant isolates had a higher cumulative sporangium production during the first three nights of the infectious period, the resistant isolates that predominated in the population at the end of the epidemic had a cumulative sporangium production 1.1–1.7 times higher compared to the sensitive partners. In contrast, those that declined toward the end of the epidemic had a lower (0.6–0.7 times) cumulative sporangium production. Obviously, when host leaf area is severely limited at the end of the

epidemic, the slower lesion expansion of the sensitive subpopulation together with its longer infectious period works in favor of the sensitive subpopulation.

The studies in growth chambers and walk-in plastic tunnels are supported by open field experiments. Blight development and the frequency of resistant sporangia were measured as a function of time and of distance from the infectious focus (D Kadish & Y Cohen, unpublished; Figure 6). Potato crops were totally devastated by the blight in 50–60 days. The proportion of resistant sporangia (initially 10%) sharply increased during the logarithmic phase throughout the field in all four epidemics, but toward the end of the season declined markedly in two epidemics, while reaching 100% in the other two (Figure 6), as was observed for the same isolates in tunnel experiments. In similar experiments, Matuszak et al (60) measured the frequency of metalaxyl-resistant isolates over the course of an epidemic at three locations in the Toluca Valley in Mexico. They found no significant changes from the initial value (35–47% resistance) over a sampling period of 40 days and concluded that resistance to PAFs in *P. infestans* is not associated with decreased fitness. They assume that sexual reproduction in central Mexico has overcome any association between resistance and reduced fitness. Cooke (11) hypothesized that after many years of selection by PAFs, resistance and fitness have probably been combined to produce strains as fit as the wild type.

Information on fitness and competitiveness of resistant and sensitive populations in potato tubers is particularly important because of the central role of

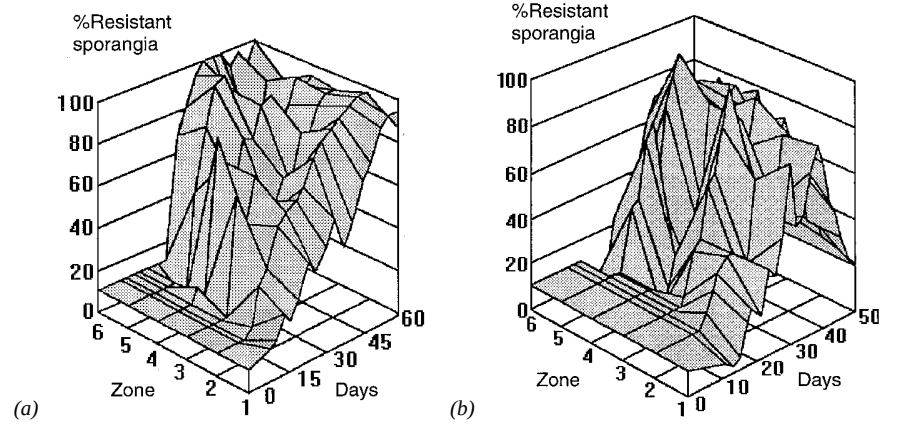

Figure 6 Increase of resistant sporangia in two epidemics (a, b) produced by r + s = 1 + 9 mixtures of isolates of *P. infestans* in untreated potato fields. A single plant in one corner of each plot (size 600 m^2) was inoculated with the mixed sporangium suspension, and % resistance monitored at different zones (1–6, 25 m apart, original focus at 1) (D Kadish & Y Cohen, unpublished)

tubers in overwintering and long-distance dispersal of *P. infestans* (78). Dowley (22) and Grinberger et al (40) showed that PA-resistant isolates of *P. infestans* did not differ significantly from sensitive isolates in the percent tubers they can infect. Grinberger et al (40) showed that resistant isolates produced larger and deeper lesions compared to sensitive isolates and concluded that the former isolates cause more severe tuber blight. Walker & Cooke (95, 96) observed that resistant isolates caused more tuber rot than sensitive isolates, thus enabling sensitive isolates to better survive and overwinter. Kadish & Cohen (48) examined the recovery of *P. infestans* from tubers inoculated artificially with either sensitive or resistant isolates after 20 weeks of storage. Recovery of sensitive isolates was significantly greater than of resistant isolates because rot was significantly more frequent in tubers inoculated with resistant than sensitive isolates. Thus, sensitive isolates survive and overseason in tubers better than resistant isolates. Similar results were found in competition experiments at 1:1 ratios between sensitive and resistant isolates in mixed-inoculated potato tubers (48, 86). These results help explain the higher proportion of sensitive isolates often observed in the initial foci of the disease (Figure 2).

OUTLOOK

Due to their enhanced competitiveness, resistant isolates survive poorly in infected tubers, and therefore their chance of appearing in first foci in the field is lower than that of sensitive isolates, provided that seed potatoes are the only source of overwintered inoculum. First foci of blight lesions are generally composed mainly of sensitive isolates (see above). However, despite their small proportion in the initial inoculum (as low as 0.1%), resistant isolates compete favorably with the much larger population of sensitive isolates and predominate the population later in the season, also in the absence of PAF applications. Some populations are composed almost 100% of resistant isolates, whereas in others resistant isolates decline at the end of the epidemic, allowing the sensitive subpopulation to take over. Those that decline have a shorter infectious period and a reduced sporulation capacity relative to their competitors. The tubers in soil become infected by sporangia washed down from the foliage, and these sporangia will mainly be composed of resistant isolates. This scenario may also be true for crops grown without selection pressure of PAFs. Application of PAFs may further decrease the frequency of sensitive isolates and thus leave resistant isolates to dominate the population more rapidly.

Fitness is a multicharacter trait comprising all capacities of a fungal isolate to successfully complete the many steps in the disease cycle relative to other isolates. The "new" genotypes of *P. infestans* that became dominant in Europe over the past two decades are obviously more fit than the "old" genotypes. Also, PA-resistant isolates have equal or greater fitness than have sensitive isolates. The mode of inheritance of a major fitness component, e.g. lesion size, was

Table 4 Inheritance of one fitness component, lesion size, in field isolates of *P. infestans* resistant to metalaxyl (D Kadish & Y Cohen, unpublished)

Parent or cross	Generation	No. of single oospore cultures							
		Sensitivity to metalaxyl			Mating type		Lesion size, cm^2		
		s	i	r	A_1	A_2	$> 1 < 2.5$	2.5–4.5	> 4.5
mr1[a]	P_1 self			52	21	31	11	19	22
ms3[b]	P_2 self	21			21		9	7	5
$P_1 \times P_2$	F_1	1	58	1	34	26	13	27	20
F_1 large[c]	F_2	12	20	9	15	26	8	18	15
F_1 small[d]	F_2	10	26	10	18	26	14	19	13
F_1 large $\times$ P_1	BC		15	14	17	12	7	12	10
F_1 large $\times$ P_2	BC	14	15		15	14	9	11	9

[a]Single zoospore cultures produced large lesions (> 4.5 cm^2) for many asexual generations.
[b]Single zoospore cultures produced small lesions ($> 1 < 2.5$ cm^2) for many asexual generations.
[c]F_1 single oospore cultures that produced large lesions.
[d]F_1 single oospore cultures that produced small lesions.

studied recently (Table 4). A resistant A2 isolate producing lesions of about 5 cm^2 was crossed with a sensitive A1 isolate that produced lesions of about 1 cm^2. Selfing of either parent by hormonal induction resulted in a progeny that segregated for fitness (lesion size) but not for sensitivity to metalaxyl (selfing of the A2 parent segregated also for mating type). The F_1 progeny was intermediate for metalaxyl-sensitivity, segregated 1:1 into A1 and A2, and also segregated for lesion size. F_2 and backcrosses also segregated for lesion size (Table 4). It thus became apparent that greater fitness and metalaxyl-resistance are nonlinked traits, as are greater fitness and mating type. This implies that, in the absence of selection pressure by phenylamide fungicides, random mating of *P. infestans* in nature may lead to the reappearance of sensitive isolates with greater fitness that can favorably compete with the rest of the population.

For a better understanding of the population dynamics of *P. infestans*, other important aspects must be considered. Potato tubers play a major role in the worldwide dissemination of the disease, together with mating type, fungicide resistance, race structure, and any other property of the pathogen. Equally important is the tuber as a source of the inoculum for the next epidemic. However, tubers do not necessarily represent the nature and frequency of the isolates harbored by the foliage. Studies in The Netherlands (27), Norway (41), and United States (77) indicate that the A2 mating type was less frequent in tubers than A1. On the other hand, Cara, the principle cultivar in Ireland, hosted primarily the A2 mating type (75% of the isolates) and exclusively PA-sensitive isolates on the foliage (66). If tomatoes are planted close to or sequentially after potatoes, cross contamination may cause an additional selection of certain races of *P. infestans*. In 1989, the A1/A2 proportion in fields in The Netherlands was 86%/14% on potato foliage but 30%/70% on tomato plants, with 43% and 0%

of PA-resistant isolates on the respective crops (27). On the other hand, in North Carolina, the 1993/94 isolates of *P. infestans*, primarily of tomato origin, were all A2 mating type and mainly PA-resistant (25). The cross contamination between tomato and potato may be of special importance in states like California and Florida with large acreage of tomato.

Although not investigated systematically, a certain selection of races (pathotypes) may be imposed on the population of *P. infestans* by commercial potato cultivars. *Solanum demissum* was one of the most important sources for late blight resistance in potato, particularly the dominant race-specific (vertical) resistance. Most of the current commercial potato cultivars have some R-gene background. Breeding for quantitatively inherited (horizontal, non race-specific) resistance free of R-genes has only recently started at IPC in Peru (55). Finally, alternative hosts for *P. infestans* may play a role in the epidemic and overwintering phases of the pathogen. Recently, Deahl & Inglas (19) discovered isolates of *P. infestans* in northwestern Washington on *Solanum sarachoides* that were pathogenic to tomato and potato (complex race T1, R 1.2.3.4.7.10); all isolates were of A1 mating type and resistant to metalaxyl.

EPILOGUE

When used in mixture with other antifungal compounds, phenylamide fungicides (PAFs) are still potent inhibitors of diseases caused by Peronosporales, e.g. late blight of potato and tomato caused by *P. infestans*. The proportion of resistant isolates is high and has gradually been stabilized in Europe (at 50–70%). Sensitive isolates are still present; they decrease during the epidemics and recover between seasons. Resistance to PAFs is not linked to the mating type of *P. infestans*. The proportion of A2 isolates in Europe is low (0–20%), with A1 isolates being mostly resistant and A2 sensitive to PAFs. Repeated selection through PAFs enhanced the fitness of resistant subpopulations. Sexual recombination favoring genetic diversity and segregation may re-establish sensitive subpopulations with enhanced fitness. We should closely watch the future behavior of both the blight and its causal agent, *P. infestans* and deploy the most appropriate measures for their control.

ACKNOWLEDGMENTS

Our sincere thanks go to Dr. B Schöber-Butin, BBA Braunschweig, for having made available to us some of her original data on *P. infestans* from Germany. We acknowledge technical support and stimulating discussions provided by R Gees, L Ohl, E Rimbach, and D Edel of Sandoz Agro, and A Baider, S Farkas, and Z Reshit of Bar-Ilan University.

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