Overseasoning of Metalaxyl-Sensitive and Metalaxyl-Resistant Isolates of *Phytophthora infestans* in Potato Tubers

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ABSTRACT

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The ability of *Phytophthora infestans* to overseason in infected potato tubers, cultivar Alpha, was studied. Recovery of infective sporangia from tubers 20 wk after inoculation with one of 10 metalaxyl-sensitive (MS) or with one of 10 metalaxyl-resistant (MR) isolates was significantly higher for the MS than for the MR isolates. Recovery tests with tubers infected with three pairs of mixed MS and MR isolates (1:1 sporangia) showed the predominance of MS isolates in two pairs and equal recovery of MS and MR isolates in a third pair. Lesions collected from the first late blight foci in commercial potato fields in 1987–1991 (autumn sowing) contained mostly MS isolates, although MR isolates predominated later in the season. The significantly higher recovery of MS isolates of *P. infestans* from potato tubers than of MR isolates explains the predominance of MS isolates in first foci of late blight in commercial potato fields in the autumn sowing in Israel.

Additional keywords: fitness, fungicide resistance, initial inoculum, phenylamides.

Resistance to phenylamide fungicides (e.g., metalaxyl and oxadixyl) in oomycetous plant pathogens is a major problem worldwide (2,15). The use of such fungicides for disease control was severely limited because of the rapid buildup of resistance in sprayed crops (2,15). In Israel, epidemics of downy mildew in cucurbits (caused by *Pseudoperonospora cubensis*) and late blight in potatoes (caused by *Phytophthora infestans* (Mont.) de Bary) became more frequent and more severe since the appearance of resistant strains in 1979 (12) and 1982 (3), respectively. Studies in our laboratory revealed that in both fungi, the resistant isolates possessed higher fitness to their respective hosts than the wild-type, sensitive isolates (1,4,7,8,10,11,13). Metalaxyl-resistant (MR) isolates of *P. infestans* germinated faster (1), infected potato leaves within shorter dew periods (1), and produced larger lesions in potato leaves than metalaxyl-sensitive (MS) isolates (7). MR isolates of either fungus were also capable of competing successfully with sensitive isolates in mixed isolate induced epidemics in the field (4,8,9). However, MS isolates of *P. infestans* had a longer infectious period on potato plants than did MR isolates (9). In mixed isolate epidemics, MS isolates sporulated on almost fully blighted crops, whereas no sporulation of the MR isolates could be detected (8,9). Tuber inoculation studies (11) showed that MR isolates of *P. infestans* produced larger and deeper lesions than did MS isolates, although the proportion of infected tubers was similar.

In this paper, we provide evidence that the higher fitness of MR isolates of *P. infestans* on foliage and tubers of potato is not necessarily an advantage during the overseasoning phase of the life cycle. The overseasoning data presented here explain the appearance of MS isolates in first blight foci in the field.

MATERIALS AND METHODS

Overseasoning in single-isolate-infected tubers. Tubers of potato (*Solanum tuberosum* L.) cultivar Alpha produced locally (with no phenylamide fungicides applied) were used in all experiments. Tubers (80 ± 20 g each) were taken from storage (4 C) 1 mo after harvest, washed, blotted dry, and dipped for 1 min in a sporangial suspension (1,250 sporangia per milliliter) of *P. infestans*. Twenty isolates of *P. infestans* were used for inoculation: 10 sensitive to metalaxyl (MS1–MS10) and 10 resistant to metalaxyl (MR1–MR10). The origin, sensitivity to metalaxyl, mating type, and virulence factors of the isolates are given elsewhere (5,7,10). Inoculated tubers were placed in a moisture-saturated atmosphere at 18 C in the dark for 24 h; then, tubers were blotted dry and placed in open trays as a single layer of tubers at 20 C in the dark for 2 wk so that disease would develop. The visibly infected tubers were selected (about 80% of the inoculated tubers), divided into four groups, and incubated (as above) at 5, 12, 20, and 25 C in the dark. After 20 wk, the proportion of tubers showing soft rot (bacterial and secondary fungal contaminations), dry rot due to late blight, and mummification (shrunken and dried due to late blight) was determined. To induce fungal sporulation, we cut in half tubers showing dry rot and placed them in a moisture-saturated atmosphere at 18 C in the dark for 24 h. The proportion of tubers supporting sporulation of *P. infestans* (as confirmed by a microscopic examination) was determined.

Overseasoning in double-isolate-infected tubers. Tubers of Alpha were inoculated by a momentary dipping in a sporangial suspension (1,250 sporangia per milliliter) made of an MS and an MR isolate at 1:1 (based on number of sporangia). To ensure that the fungus was in the tubers, we incubated tubers in a moisture-saturated atmosphere at 18 C for 24 h in the dark, then blotted them dry, and incubated them at 20 C in the dark for 2 wks. Starting at 2 wks after inoculation, a random sample of 10 tubers was withdrawn every 4 wk (for 20 wk); tubers were cut in half and incubated in a moisture-saturated atmosphere at 18 C for 24 h in the dark so that fungal sporulation would occur. Sporangia produced were removed from the cut surfaces and lenticels with a camel's hair brush, placed in distilled water, and used for determination of the percentage of MR (or percentage of MS) sporangia. The potato tuber disk bioassay developed by Kadish and Cohen (6) was used.

Experimental design. Single-isolate inoculation experiments were conducted twice with the 10 MS and the 10 MR isolates. In each experiment, 100 infected tubers per isolate were randomly selected and incubated at each of the four temperatures. All 400 tubers were assayed for fungal sporulation at the end of the 20-wk incubation period.

Double-isolate inoculation experiments were conducted twice with three pairs of MS and MR isolates. In each experiment, 100 tubers were inoculated with each isolate pair, of which 70 were suitable for the bioassays. At each of the five sampling dates, 10 tubers were randomly selected and used for the determination of percentage of MR sporangia (6). Sporangia were collected

TABLE 1. Mean proportion of inoculated potato tubers (cultivar Alpha) supporting sporulation of metalaxyl-sensitive (MS) and -resistant (MR) isolates of *Phytophthora infestans* and mean proportion of tubers showing soft rot or mummification after 20 wk of storage at four temperatures

Temperature at storage (C)	Proportion of tubers (%)[z]					
	MS isolates			MR isolates		
	Sporulation	Soft rot	Mummification	Sporulation	Soft rot	Mummification
5	98.8 aA	0 C	0 D	98.6 aA	0 D	0 D
12	83.6 aB	0 C	15.5 C	77.0 bB	4.5 C	18.0 C
20	60.4 aC	11.0 B	28.0 B	42.5 bC	24.5 B	32.0 B
25	46.3 aD	19.5 A	33.5 A	26.2 bD	36.0 A	36.5 A

[z] Figures represent means for 10 MS or 10 MR isolates. Means in rows followed by the same small letter or means within columns followed by the same capital letter are not significantly different at $P = 0.05$, according to the Waller-Duncan multiple range test.

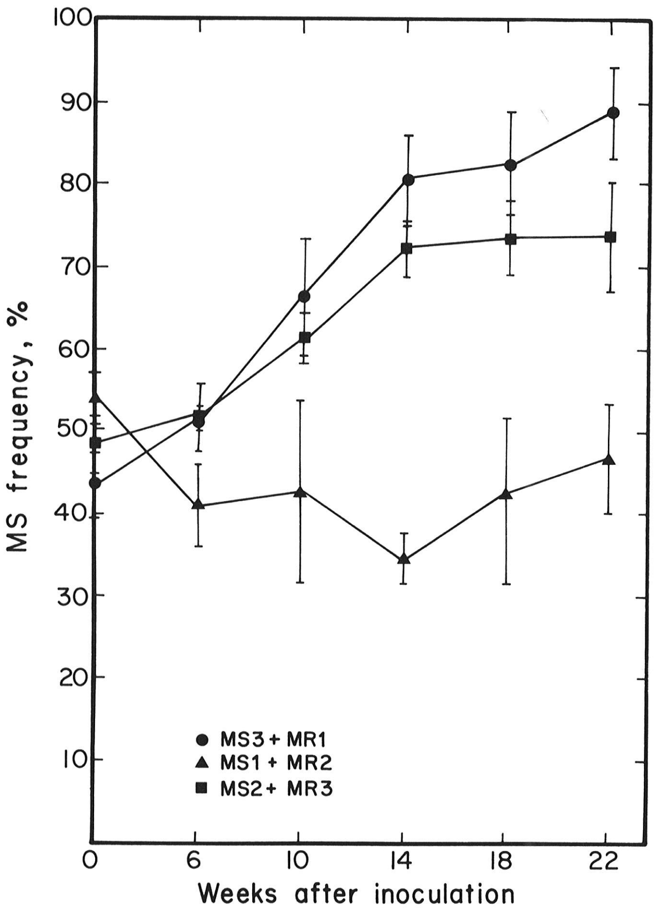

Fig. 1. Mean frequency of metalaxyl-sensitive (MS) sporangia recovered from potato tubers (cultivar Alpha) inoculated with mixed MS and metalaxyl-resistant (MR) isolates (1:1) of *Phytophthora infestans* at various time intervals after inoculation (see text for methods). Bars indicate standard deviations of the mean from the two experiments.

from each tuber and inoculated onto potato tuber disks treated with water on metalaxyl as described before (6). Because the results from the first experiment did not significantly differ (pair Student's *t* test was performed to each treatment) from the results of the second experiment, means of the 20 tubers (and standard deviation of the mean) were used.

Overseasoning in nature. In Israel, potato crops are sown in either the autumn with local seed tubers harvested the previous spring or in the late winter with seeds imported from Europe (autumn harvest there). First foci of late blight are usually seen in mid-November in the first season and in mid-March in the second season. Whereas first foci in mid-November may represent the fungus harbored by the seed tubers sown, first foci in mid-March may originate from either the seed tubers or from neighboring infected crops that are not yet harvested. Late blight lesions (usually a single blighted stem) were collected in commercial potato fields from first foci observed by county agents, brought to the laboratory, washed, blotted dry, and placed in humid conditions at 18 C in the dark for 24 h to induce fungal sporulation. To determine the frequency of MR and MS sporangia in the sporangial population, we inoculated the sporangia produced onto untreated potato tuber disks or onto those treated with 100 μg/ml of metalaxyl in the method previously described (6).

RESULTS

Overseasoning in single-isolate inoculations. Proportions of tubers supporting sporulation of *P. infestans* after 20 wk of storage are given in Table 1. Percentage ranges for MS isolates were 97.7–100%, 68.8–92.7%, 53.1–72.5%, and 34.9–52.7% for tubers incubated at 5, 12, 20 and 25 C, respectively. The respective ranges for MR isolates were 96.5–100%, 71.2–83.4%, 28.6–49.7%, and 9.4–41.0%. The temperature of incubation had a significant effect on recovery of *P. infestans* from tubers (as assessed by sporulation) for both groups of isolates (Table 1). Recovery decreased as temperature increased. Although nearly all tubers supported sporulation of *P. infestans* after incubation at 5 C, only about 25% and 50% of the tubers supported sporulation of MR and MS isolates, respectively, after incubation at 25 C (Table 1). Recovery of MS and MR isolates did not significantly differ in tubers incubated at 5 or 12 C. However, MS isolates supported sporulation in significantly ($P \leq 0.05$) larger proportions of tubers than MR isolates at 20 and 25 C (Table 1). The proportions of tubers showing soft rot or turning into mummies increased significantly ($P \leq 0.05$) with increase in temperature regardless of the isolate used for inoculation (Table 1). No sporangia of *P. infestans* could be recovered from soft-rotten tubers or mummified tubers.

Overseasoning in double-isolate inoculations. Figure 1 presents means from the two recovery experiments of percentage of MS (as calculated from respective percentage of MR) sporangia from tubers inoculated with 1:1 mixed MS and MR isolate pairs. The data show that MS isolates either keep their original proportion in tubers (MS1 + MR2) or increase their proportion with time (MS2 + MR3; MS3 + MR1). MS3 increased from its original proportion of 43.3 ± 3.9% to 88.5 ± 5.7% in two experiments of the sporangia recovered from infected tubers after 22 wk of incubation at 20 C. MS2 increased from 49.4 ± 5.2% to 74.0 ± 7.1%. MS1 changed from 53.8 ± 3.7% to 47.6 ± 6.9% (Fig. 1).

Overseasoning in nature. Although MR isolates of *P. infestans* predominated in potato fields in Israel since they were discovered in 1982 (3) and until 1991, MS isolates predominated (71%) in lesions from first foci collected in November to December (autumn season) of 1987–1991 (Table 2). Lesions collected from first foci in the spring (February to March) showed a predominance of MR isolates, as did the lesions collected during the rest of either season (viz., January to February [autumn sowing] or April to June [spring sowing]) (Table 2).

DISCUSSION

The long-standing dilemma of why MR isolates of *P. infestans*, which possess higher fitness to potato crops than MS isolates, did not develop in nature before metalaxyl was introduced to agriculture has been partly solved in this study. The data presented

TABLE 2. Sensitivity of *Phytophthora infestans* to metalaxyl in lesions collected from first foci or during the growing season in commercial potato fields in Israel during the years 1983–1991

	Number of isolates[z]							
	First foci autumn		First foci spring		Remainder of each season		Total	
Year	MS	MR	MS	MR	MS	MR	MS	MR
1983	...	...	...	...	...	...	16	27
1984	...	...	...	...	...	...	33	34
1985	...	...	...	...	...	...	16	14
1986	...	...	...	...	...	...	8	40
1986–1987	3	2	3	5	7	19	13	26
1987–1988	2	2	7	9	22	31	31	42
1988–1989	3	0	5	10	4	50	12	60
1989–1990	7	3	3	18	7	47	17	68
1990–1991	5	1	8	11	12	53	25	65

[z] MS, metalaxyl-sensitive; MR, metalaxyl-resistant.

show that the capacity of MR isolates to overseason in potato tubers is inferior to that of MS isolates. The test we chose for studying overseasoning was the recovery of sporangia from infected tubers 20 wk after storage. MR isolates, because of their excessive aggressive colonization of potato tubers (11), cause one of two processes. They either mummify the tubers, producing dry, black, shrunken tissue from which no viable sporangia could be recovered, or they enable severe soft rotting of the tubers by secondary fungi and pectolytic bacteria so that viable sporangia of *P. infestans* could not be recovered. MS isolates, on the other hand, developed smaller and shallower lesions (11) in the tuber tissue, producing much less soft rot and almost no mummified tubers, thus enabling the recovery of viable sporangia from a relatively high percentage of tubers. If overseasoning in potato tubers is the ability of the fungus to produce infective sporangia in tubers 20 wk after inoculation and storage, then we may conclude that the MS isolates of *P. infestans* that we tested have a higher rate of overseasoning in potato tubers than do the MR isolates tested (i.e., higher fitness at this stage of the disease cycle).

The contribution of infected tubers to the formation of primary lesions in the field is not clear (14). We did not detect even a single late blight lesion in potato (Bintje) plants from 1,200 tubers infected with either one of six isolates (MS1–MS3 and MR1–MR3) in a contained plastic house during November 1991 to February 1992 (*unpublished data*).

Our observations agree with those of Walker and Cooke (16,17) who showed that Irish MR isolates of *P. infestans* grew faster than MS isolates on tuber slices at 5 C but not at 15 C. Also, MR isolates infected more inoculated whole tubers than did MS isolates. However, fewer visibly infected tubers inoculated with MR isolates than with MS isolates survived to produce plants the following season, because many were invaded by pectolytic bacteria (16,17).

Our laboratory studies explain the observations made in nature. Over the last 5 yr, we have analyzed the sensitivity to metalaxyl of *P. infestans* isolates collected from the first late blight lesions seen by county agents in commercial potato fields. Potato crops are sown in Israel in two seasons: late August to early October (autumn season) from local seed tubers harvested in June and mid-December to February (spring season) from seed tubers imported from Europe. First lesions on the autumn and spring crops occur about 8 wk after sowing. The proportion of MS isolates in the first visible foci exceeded that of MR isolates in collections made in November to December of 4 out of 5 yr (in 1 yr, proportions were equal). This reflects the high survivability of MS versus MR isolates, which we found in our laboratory experiments. However, the opposite was seen in the first visible foci collected in February to March from crops of the spring season as well as in the later stages of the epidemic in both seasons; this implicates the higher fitness of MR isolates over MS isolates on the foliage of potato (7,10).

The following scenario takes place in potato crops in Israel. Tubers stored for seed are likely to be infected with both MR and MS strains; tubers infected with MR are more frequent. During storage, most tubers infected with MR strains disintegrate because of the enhanced aggressiveness of MR strains. Tubers infected with MS strains survive better, and a higher proportion of first lesions caused by MS strains results. During the season, MR strains compete better relative to MS strains and, thus, predominate in the fungal population during the logarithmic phase of the epidemic (9). First foci in the spring season are largely caused by MR strains probably because of a carryover of MR sporangia from the mature autumn crops.

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