Synergistic Interactions of Cymoxanil Mixtures in the Control of Metalaxyl-Resistant *Phytophthora infestans* of Potato

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ABSTRACT

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Cymoxanil mixtures with either mancozeb (Mancur) or with mancozeb and oxadixyl (Pulsan, Sandocur-M) were effective in controlling both metalaxyl-sensitive (MS) and -resistant (MR) *Phytophthora infestans* in potatoes (cv. Alpha) in growth chambers. Metalaxyl-mancozeb (Ridomil-MZ) and oxadixyl-mancozeb (SAN-518) mixtures were effective against the MS fungus but not against the MR fungus. Mancur, Pulsan, and Sandocur-M were more effective in controlling both MS and MR isolates than were their individual ingredients. Increased efficacy of mixtures relative to their components combined (synergy factor) was calculated using both the Abbott-Colby and Wadely methods. Synergy factor values for the control of MS *P. infestans* were highest for Sandocur-M and lowest for SAN-518. We concluded that cymoxanil mixtures are suitable for late blight control in growing areas of Israel where MR *P. infestans* populations prevail.

Additional key words: acylanilides, chemical control, fungicide interaction, oomycetes, phenylamides.

Late blight incited by *Phytophthora infestans* (Mont.) de Bary is a major disease of potato in Israel. Disease incidence and severity have greatly increased since 1982 in all growing areas, especially in the spring (January planting) and autumn (September planting), causing severe reduction in yield and quality. Tuber export has been adversely affected due to infection. In the spring of 1986, 65 out of 88 yield samples inspected in our laboratory from different farms in the coastal plain were infected to an extent of 5–25% (Cohen, *unpublished data*). Since the first appearance of resistance to metalaxyl in *P. infestans* in 1982 (3), the occurrence of field isolates resistant to the fungicide has remained consistently high (1). Out of 41, 69, 30, and 45 isolates collected in 1983, 1984, 1985, and 1986, respectively, 61, 52, 47, and 84% were resistant to the fungicide. Resistant isolates were also frequent in fields not treated with phenylamide (acylanilide) fungicides (1).

Knowing that metalaxyl-resistance is abundant in the local population of *P. infestans*, most farmers avoid using the standard Ridomil-MZ (metalaxyl + mancozeb) fungicide unless they have their fungal population tested for sensitivity to metalaxyl. When resistance is found, the dilemma becomes worse, since mancozeb and a variety of other protectant fungicides provide only partial control of the disease, especially when rows in the field are closed by the haulm.

Recently, we have shown that the two-way fungicidal mixture Mancur (mancozeb + cymoxanil at 4:1 ratio) and the three-way mixture Sandocur-M (mancozeb + cymoxanil + oxadixyl at 7:2:1 ratio) were efficient in controlling late blight in field-grown potatoes (1).

The aim of this study was to critically examine the efficacy of these fungicide mixtures in controlling either metalaxyl-sensitive (MS) or -resistant (MR) field isolates of *P. infestans* in potted potato plants and to compare it to that of other two- and three-way mixtures.

MATERIALS AND METHODS

Plant material. All experiments were conducted with the potato (*Solanum tuberosum* L.) cultivar Alpha. Tubers of about 100 g each were sown in 1.5-L pots filled with sandy loam and grown in the greenhouse (19–28 C). Plants were fertilized with a 1% solution of N, P, and K (20:20:20) twice a week. Three pots with three to four shoots each (10 leaves with 5–7 leaflets per leaf in a shoot, 6–7 wk after sowing) were used for each inoculation test.

Fungal isolates. One MS field isolate (collected from a blighted potato field in Nir-Eliyahu, May 1984) and one MR isolate (collected from a blighted potato field in Mishmereth, April 1986) of *P. infestans* were used. Isolates were maintained on detached potato leaflets floating on water (MS) or 100 μg/ml of metalaxyl (MR) in petri dishes at 12 C and propagated on potato-tuber slices at 20 C in a moist atmosphere in the dark.

Fungicides and fungicide application. The following fungicides were used: oxadixyl 25 WP (2-methoxy-N-(2-oxo-1,3-oxazolidin-3yl) acet-2′, 6′-xylidide; Sandoz, Switzerland), cymoxanil 50 WP (Curzate, 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino) acetamide; Du Pont de Nemours), mancozeb 80 WP, SAN-518 64 WP (oxadixyl and mancozeb mixed at a 1:7 ratio, Sandoz), Mancur 71.4 WP (cymoxanil and mancozeb mixed at 1:4 ratio, Milchan Bros., Israel), Pulsan 67 WP (oxadixyl, cymoxanil, and mancozeb mixed at a 1:0.4:7 ratio, Sandoz), Sandocur-M 69.1 WP (oxadixyl, cymoxanil, and mancozeb mixed at a 1:2:7 ratio), and Ridomil-MZ 67.5 WP (metalaxyl and mancozeb mixed at a ratio of 1:7.5). All fungicides were suspended in water (concentration is given in milligram active ingredient per liter) and sprayed on plants 24 hr before inoculation. Sprays were applied to initial runoff on both leaf surfaces at an air pressure of 0.98 bar (about 5 ml per shoot).

Inoculation. Sporangial suspension in cold (4 C) tap water (about 2×10^4 sporangia per milliliter) of the appropriate isolate were inoculated onto both leaf surfaces of the plants with the aid of a glass atomizer at an air pressure of 0.2 bar. Inoculated plants were placed in a moist atmosphere at 18 C in the dark for about 20 hr to ensure infection and then transferred to growth cabinets (20 ± 1 C, 12 hr of light per day, about 120 μE per square meter per second) for symptom production.

Disease assessment. Disease severity was assessed 7 days after inoculation using the key described in Table 1. A score was given to each shoot. Leaflets showing a hypersensitive response (lesions less than 2 mm) and stems showing less than 1-mm lesions were considered healthy.

Control efficacy determinations. Three experiments were conducted, each with seven fungicides and two fungal isolates. In experiments 1 and 2 (Tables 2 and 3) each fungicide was applied at concentration levels of 10, 20, 40, 80, and 160 mg/L (three pots per treatment) except for oxadixyl, which was applied at 80, 160, 320, 640, and 1,280 mg/L in plants inoculated with the MR isolate. Fungicide concentration required to control the disease by 90%

relative to fungicide-free inoculated plants (ED_{90} observed) was determined with the aid of PROBIT (11). The expected ED_{90} values of fungicide mixtures (ED_{90} expected) were calculated according to Wadely in the manner described previously (10). Increased control efficacy of fungicide mixtures (synergy factor, SF) was calculated using formula 1:

$$SF = \frac{\text{expected effective dose } (ED_{90} \text{ exp})}{\text{observed effective dose } (ED_{90} \text{ obs})}$$

in which ED_{90} expected is the expected dose required for 90% control of the disease and ED_{90} observed is the experimental dose obtained for such a control.

In experiment 3 (Tables 4–6, Fig. 1) fungicide mixtures (SAN-518, Mancur, Pulsan, and Sandocur-M) were applied at concentrations of 10, 20, 40, 80, and 160 mg/L (Fig. 1), and their components were applied individually at matching concentrations to another series of plants (Table 4). Thus, for example, when Sandocur-M was applied at 160 mg/L, oxadixyl, cymoxanil, and mancozeb were applied (1:2:7 ratio) at 16, 32, and 112 mg/L, respectively, each to a different group of three potted plants. Fungicide concentration required to control the blight by 90% was determined for a mixture (Table 5) and for each of its components (Table 4) with the aid of PROBIT (11) (ED_{90} obs). The expected control efficacy of a mixture (Table 6) was calculated with the aid of the Abbott formula (10) for two-way mixtures (Mancur, SAN-518) and with the aid of the Colby formula (6) for three-way mixtures (Pulsan, Sandocur-M). Increased control efficacy of mixtures (SF) was calculated using formula 2:

$$SF = \frac{\text{observed control efficacy (OCE)}}{\text{expected control efficacy (ECE)}}$$

in which OCE is the experimental (observed) percentage control efficacy of a certain dose of the mixture and ECE is the expected percentage control efficacy of that dose. In experiment 3, SF was also calculated according to Wadely (10) (Table 5).

Results from the three experiments (Table 7) are for comparison purposes. SF values greater than 1 suggested synergistic interaction between components of a mixture (7,10).

TABLE 1. A key for the asessment of disease severity of potato late blight on the haulm of potato (cv. Alpha) caused by *Phytophthora infestans*

Score	Plant's organs infected[a]
0	None
0.1	One leaflet
0.25	2–3 leaflets
0.5	Most or all leaflets and petiole of single leaf
0.75	Most or all leaflets and petioles of 2 leaves
1	Most or all leaflets and petioles of 3 leaves and 1/10 of stem
1.5	Most or all leaflets and petioles of 4 leaves and 1/5 of stem
2	Most or all leaflets and petioles of 5 leaves and 1/4 of stem
3	Most or all leaflets and petioles of 6 leaves and 1/3 of stem
4	Most or all leaflets and petioles of 7 leaves and 2/3 of stem
5	Plant fully blighted

[a] Allowance is made to interchange one infected leaf with one-tenth of the stem infected.

TABLE 2. Concentration of fungicides (mg/L) required for 90% control of late blight of potatoes (cv. Alpha) incited by a metalaxyl-sensitive (MS) field isolate of *P. infestans*[a]

	Experiment 1				Experiment 2			
Fungicide	Observed	P[b]	Expected	SF	Observed	P[b]	Expected	SF
Oxadixyl A	41	0.98	...	...	53	0.98	...	...
Cymoxanil B	177	0.96	...	...	199	0.83	...	...
Mancozeb C	204	0.84	...	...	749	0.94	...	...
SAN-518 A+C, 1+7	23	0.98	137	5.96	29	0.99	282	9.75
Mancur B+C, 1+4	68	0.96	198	2.91	48	0.98	483	10.06
Pulsan A+B+C 1+0, 4+7	25	0.94	138	5.52	36	0.99	278	7.72
Sandocur-M A+B+C 1+2+7	28	0.99	143	5.10	32	0.98	261	8.16

[a] Plants were treated with five fungicide concentrations (see Materials and Methods).
[b] P = fit of dose-response data to the PROBIT model (chi-square test); SF = synergy factor.

RESULTS

Experiments 1 and 2, MS isolate. Of the three fungicides applied singly against the MS isolate, oxadixyl was the most effective, mancozeb the least effective, and cymoxanil intermediate (Table 2). The performance of oxadixyl and cymoxanil was similar in the two experiments, whereas that of mancozeb varied greatly. Large variation in the efficacy of mancozeb was noted in our other experiments (see below), as well as by other researchers (15) who used similar spraying and inoculation techniques.

Two- and three-way mixtures were more effective than expected, thus exhibiting strong (three- to 10-fold) synergistic interaction (Table 2). Synergy factor values in experiment 2 were larger than in experiment 1 due to the reduced efficacy of mancozeb in experiment 2. SAN-518 was the most effective mixture per unit active ingredient (ED_{90}: 23–29 mg/L).

Experiments 1 and 2, MR isolate. Oxadixyl at 1,280 mg/L showed a very poor efficacy with only 10% control. A relatively high concentration of cymoxanil was required (336–358 mg/L) for 90% control (Table 3).

Isolates of *P. infestans* were found to differ greatly in sensitivity to cymoxanil applied to potato foliage regardless of their sensitivity to metalaxyl (2). Of all the mixtures used, SAN-518 was the least effective (ED_{90}: 936–967 mg/L), while Sandocur-M was the most effective (ED_{90}: 54–55 mg/L). Its efficacy was about 9–16 times greater than expected (Table 3).

Experiment 3. The design of this experiment enabled calculating synergistic interactions according to Abbott (or Colby, 6) and Wadely (10). Dosage-response curves for control of the MS and the MR isolates by four fungicidal mixtures are presented in Figure 1, A and B, respectively. Dose-response data for the three individual fungicides composing these mixtures are presented in

TABLE 3. Concentration of fungicides (mg/L) required for 90% control of late blight of potatoes (cv. Alpha) incited by a metalaxyl-resistant (MR) field isolate of *P. infestans*

	Experiment 1				Experiment 2			
Fungicide	Observed	P[a]	Expected	SF	Observed	P[a]	Expected	SF
A+B+C 1+2+7								
Oxadixyl[b] A	1,280	...	20,000[b]	...	1,280	0.95	20,000[b]	...
Cymoxanil B	358	0.98	...	...	336	0.91	...	...
Mancozeb C	456	0.99	...	...	1,297	0.98	...	...
SAN-518 A+C, 1+7	936	0.98	519	0.55	967	0.97	1,469	1.52
Mancur B+C, 1+4	61	0.99	454	7.44	80	0.98	394	4.94
Pulsan A+B+C 1+0, 4+7	82	0.94	509	6.20	112	0.99	1,265	11.25
Sandocur-M	55	0.96	476	8.66	54	0.99	887	16.30

[a] P = fit of dose-response data to the PROBIT model (chi-square test); SF = synergy factor.
[b] Oxadixyl at 1,280 mg/L controlled the disease by only 10%. Therefore, 20,000 was chosen as an expected value for oxadixyl.

TABLE 4. Dosage-response data of cymoxanil, oxadixyl, and mancozeb for the control of late blight of potatoes incited by a MS and a MR field isolates of *P. infestans* (experiment 3)

Disease control (% ± SD)[a]								
Cymoxanil			Oxadixyl			Mancozeb		
mg/L	MS	MR	mg/L	MS	MR	mg/L	MS	MR
0.5	0	0	1.0	10 ± 6	0	7.0	21 ± 6	11 ± 10
1.0	0	0	1.2	11 ± 6	0	8.0	25 ± 0	15 ± 11
2.0	0	4 ± 5	1.25	11 ± 6	0	8.3	25 ± 0	16 ± 11
4.0	7 ± 11	11 ± 10	2.0	21 ± 12	0	8.75	25 ± 0	16 ± 12
8.0	14 ± 20	18 ± 6	2.4	25 ± 10	0	14	36 ± 10	18 ± 15
16	32 ± 6	41 ± 6	2.5	25 ± 10	0	16	39 ± 6	19 ± 6
32	36 ± 20	44 ± 0	4.0	31 ± 8	0	16.6	39 ± 5	17 ± 6
			4.8	36 ± 0	0	17.5	36 ± 10	27 ± 12
			5.0	36 ± 0	0	28	43 ± 6	22 ± 10
			8.0	48 ± 10	0	32	50 ± 15	26 ± 6
			9.6	57 ± 10	0	33.2	50 ± 15	26 ± 6
			10.0	57 ± 10	0	35	50 ± 11	33 ± 10
			16	68 ± 14	0	56	54 ± 12	30 ± 15
			19.2	73 ± 13	0	64	57 ± 10	33 ± 10
			20	73 ± 12	0	66.4	61 ± 11	33 ± 17
						70	68 ± 10	50 ± 9
						112	79 ± 9	56 ± 10
						128	84 ± 0	59 ± 6
						132.8	86 ± 6	69 ± 13
						140	86 ± 6	70 ± 6
ED_{90}[b]	496	284		41	20,000		273	1,010
P[c]	0.99	0.98		0.99	...		1.0	0.99

[a]SD = standard deviation of the mean.
[b]Fungicide concentration (mg/L) required for 90% control of the disease.
[c]P = fit of the dose-response data to the PROBIT model (chi-square test) (11).

TABLE 5. Observed and expected control efficacies of four fungicidal mixtures against late blight in potatoes incited by a MS and a MR field isolates of *P. infestans*

Fungicide	ED_{90} observed				ED_{90} expected		SF[a]	
	MS	P[b]	MR	P[b]	MS	MR	MS	MR
SAN-518	19	0.99	805	0.91	165	1,147	8.7	1.4
Mancur	44	0.97	53	0.99	300	668	6.8	12.6
Pulsan	46	0.98	92	0.99	166	1,002	3.6	10.9
Sandocur-M	38	0.99	50	0.99	185	713	4.9	14.3

[a]Values obtained by dividing ED_{90} expected/ED_{90} observed; SF = synergy factor.
[b]P = fit of the dose-response data to the PROBIT model (chi-square test) (11).

TABLE 6. Synergism among fungicidal mixtures in controlling late blight of potatoes incited by a MS and a MR field isolates of *P. infestans*

Concentration (mg/L)	Synergy factor							
	SAN-518		Mancur		Pulsan		Sandocur-M	
	MS	MR	MS	MR	MS	MR	MS	MR
10	2.4	...	2.6	2.0	1.4	2.6	2.0	3.8
20	1.7	0.2	1.8	2.3	1.3	2.9	1.4	2.6
40	1.4	0.7	1.5	2.1	1.2	2.6	1.3	2.5
80	1.2	0.6	1.4	1.6	1.2	2.2	1.2	1.6
160	1.0	0.7	1.1	1.3	1.0	1.3	1.0	1.3

Table 4. PROBIT analyses of the data in Table 4 and Figure 1 supplied the numerical information tabulated in Table 5. Of the four mixtures used, SAN-518 was most efficient against the MS isolate, in terms of ED_{90} and SF values (Fig. 1A, Table 5), but least efficient against the MR isolate (Fig. 1B, Table 5). Sandocur-M and Mancur were highly effective, but not significantly different from Pulsan in controlling the MR isolate (Fig. 1B, Table 5).

Data in Figure 1 and Table 4 were also used for calculating synergism according to Abbott (10) and Colby (6). Synergy factor values against the MS isolate were highest for Mancur, and against the MR isolate, highest for Sandocur-M (Table 6).

TABLE 7. Fungicides' concentrations required for 90% control of late blight of potato incited by a MS and a MR field isolates of *P. infestans*[a]

Fungicide	ED_{90} ± SD[b], mg/L			MR/MS
	MS	MR	*t* test	
Oxadixyl	45 ± 6 b	≫1280 a	$P<0.001$	28.44
Cymoxanil	291 ± 145 a	326 ± 31 c	$P>0.1$	1.12
Mancozeb	408 ± 242 a	921 ± 349 b	$P>0.1$	2.26
SAN-518	24 ± 4 b	903 ± 70 b	$P<0.001$	37.63
Mancur	53 ± 10 b	65 ± 11 d	$P>0.1$	1.23
Pulsan	36 ± 9 b	95 ± 12 d	$0.001<P<0.01$	2.64
Sandocur-M	33 ± 4 b	53 ± 2 d	$0.001<P<0.01$	1.61
Ridomil-MZ	26 ± 16 b	240 ± 23 c	$P<0.001$	9.23

[a]Average values of three experiments (except for Ridomil-MZ with two experiments) for each isolate with three pots per treatment-concentration. Each fungicide was tested with five fungicide concentrations (see Materials and Methods) totaling 720 plants. Means followed by the same letter in columns are not significantly different according to Waller-Duncan *K*-ratio *t* test.
[b]SD = Standard deviation of the mean.

ED_{90} values derived from experiments 1–3 after the PROBIT procedure (11) were used to calculate the ratio between the dose of each fungicide required for 90% control of the MR isolate and the dose required for 90% control of the MS isolate. Table 7 shows that among mixtures, the largest ratio of about 38 was obtained for SAN-518, whereas the smallest values of about 1.2 and 1.6 were obtained for Mancur and Sandocur-M, respectively. Cymoxanil mixtures were significantly more effective than Ridomil-MZ in controlling the MR isolate but not in controlling the MS isolate.

DISCUSSION

Control of late blight incited by metalaxyl-resistant isolates of *P. infestans* is a continuing and major problem in potato production in Israel. While protectant (surface) fungicides may provide an adequate control early in the season when inoculum pressure and haulm density are low, they fail to do so later in the season when inoculum pressure and haulm densities increase.

This study shows that the two-way fungicide mixture Mancur

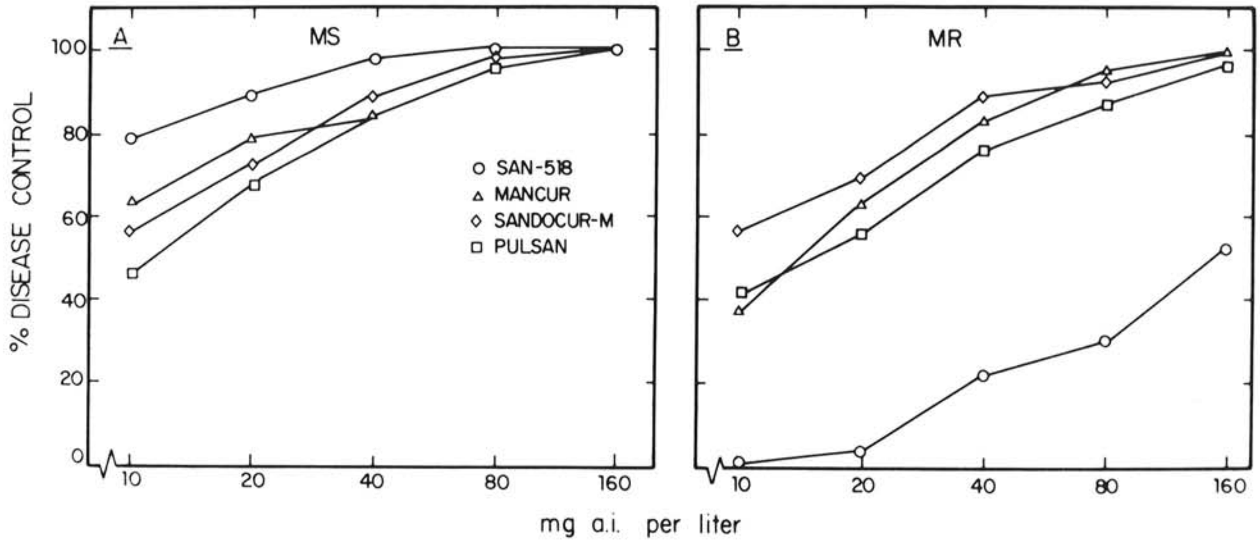

Fig. 1. Efficacy of four fungicidal mixtures in controlling late blight in potatoes incited by metalaxyl-sensitive (A) and metalaxyl-resistant (B) field isolates of *Phytophthora infestans*. Disease severities in fungicide-free plants were 4.7 (MS) and 4.5 (MR) on the 0–5 visual scale (Table 1). Plants were sprayed with fungicides and inoculated thereafter on both leaf surfaces. Least significant differences ($P < 0.05$) for the metalaxly-sensitive isolate were 16, 18, 17, 5, and 0%, and for the metalaxyl-resistant isolate, 30, 25, 18, 8, and 9% in fungicides concentrations of 10, 20, 40, 80, and 160 mg/L, respectively (experiment 3).

and the three-way fungicide mixtures Pulsan and Sandocur-M provided acceptable control of both MS and MR isolates of the fungus in potted plants under growth-chamber conditions. The two-way mixtures SAN-518 and Ridomil-MZ were potent in controlling the MS but not the MR isolate of the fungus.

The effectiveness of Mancur, Pulsan, and Sandocur-M was reflected not only in the relatively low dosages required to achieve 90% control of the disease incited by the MR isolate, but also in the low ratio between dosages required to achieve 90% control of the MR and the MS isolates. Dosages required for 90% control of the MR isolate were far smaller than those expected based on the efficacy of the ingredients in these mixtures. This finding indicated that strong synergistic interactions occur between ingredients of Mancur (mancozeb and cymoxanil) and between those of Pulsan and Sandocur-M (mancozeb, cymoxanil, and oxadixyl). Our field observations showed that these fungicidal mixtures effectively controlled late blight in potatoes in Israel incited by an MR isolate in 1985 (1) and in 1986 (Cohen and Samoucha, *unpublished data*).

Synergistic interactions in the two-way mixtures mancozeb + cymoxanil, mancozeb + oxadixyl, and cymoxanil + oxadixyl, and in the three-way mixture mancozeb + cymoxanil + oxadixyl were reported by Gisi et al (7) and Grabski and Gisi (8) in the tomato *P. infestans* and the grape *Plasmopara viticola* pathosystems in the greenhouse. The latter authors reported higher synergistic factors in MR than in MS isolates of *P. infestans* on tomato. Samoucha and Gisi (16) demonstrated that cymoxanil + oxadixyl mixed at 0.4:1 ratio were twice as synergistic toward MR than toward MS isolate of *P. infestans* in tomato.

Our previous study showed strong synergistic interactions between either metalaxyl or fosetyl-Al and five protectant (surface) fungicides in controlling late blight in potatoes. Synergism was greater toward MR than toward MS isolates of *P. infestans* (13). Similar results were obtained in both greenhouse and field conditions with MS and MR isolates of *Pseudoperonospora cubensis* in cucumbers (12).

This study shows that phenylamide-mancozeb mixtures (SAN-518 and Ridomil-MZ) were inefficient in controlling MR *P. infestans*, but addition of cymoxanil to SAN-518 greatly improved its efficacy. This and our previous studies (1) indicate that for the control of Israeli MR isolates of *P. infestans*, the addition of 20% cymoxanil to SAN-518 (Sandocur-M) is preferable to an addition of 4.76% cymoxanil (Pulsan). This difference probably resulted from the higher dosages of cymoxanil required for the control of Israeli isolates (2,4), compared to European isolates (9) of the fungus.

In a recent study we found that Mancur, Sandocur-M, and Pulsan were effective not only in controlling MR isolates of *P. infestans*, but also in suppressing the buildup of MR isolates in mixed MS + MR pathogen populations (5). Other experiments revealed longer residual activities of these three fungicides in controlling MR isolates of *P. infestans* in the field than any of the other seven fungicides tested (14). Field experiments are currently being conducted to establish an effective spray schedule in which cymoxanil mixtures will be used to combat late blight in potato.

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