Changes of Mating types and Sensitivity to Metalaxyl in populations of *Phytophthora infestans* in Gangwon Area in Korea.

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Abstract

Isolates of *Phytophthora infestans* obtained from several locations of Gangwon area in 1998-2000 were examined for their mating types and sensitivities to metalaxyl. Both A1 and A2 mating type isolates were isolated in 1998, 1999, and 2000. The majority of the *P. infestans* isolates were A1 mating type. About 64.3% of the isolates collected in 1998, 99.0% in 1999 and 85.2% in 2000 were determined as A1 mating type. Sensitivity of the *P. infestans* to metalaxyl was examined by mycelial growth on V8 juice agar amended with matalaxyl. About 44.6% of the isolates examined in 1998 were resistant to metalaxyl, 55.4% of the isolates were intermediate resistant, but none of the isolates tested were sensitive. In 1999 and 2000, 10.5 and 59.3% of the isolates examined were sensitive, 88.6 and 25.9% of the isolates were intermediate resistant, and 0.9 and 14.8% of the isolates were resistant to metalaxyl, respectively. Also resistance to dimethomoph and ethaboxam was examined on V8-juice agar containing 1 and 10 §¶/§¢ dimethomorph and ethaboxam. Above 1 §¶/§¢ concentration, collected isolates were highly sensitive to dimethomorph and ethaboxam. In addition, metalaxyl effectively controlled potato late blight in two field tests in Gangwon area in 2001. Therefore, it is possible to assume that metalaxyl sensitivity of the *P. infestans* isolates of Gangwon area is increasing. These results are quite different from those of early 1990s.

Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) de bary is remarkably explosive disease which can destroy potato fields in a few days. *P. infestans* is a heterothallic fungus that reproduces sexually by means of two mating types, designated A1 and A2. Until the mid-1980s A2 mating type strains had been considered to be restricted to central Mexico, While only A1 strains were distributed worldwide. A2 isolates outside Mexico were first reported from Switzerland in 1984 (Hohl and Iselin, 1984). Since then a number of reports revealed that A2 mating type strains occur in most parts of the world (Carlisle et al. 2001; Marshall-Farrar et al., 1998; Mosa et al., 1989). In Korea, *P. infestans* strains collected from various geographical locations from 1991 to 1993 were detected A2 mating types. The majority of the isolates were A2 mating type (Koh et al., 1994; So and lee, 1993).

Because of the variability of this pathogen, race-specific oligogenic resistance has not been useful for control. Potato cultivars now grown commercially in the Korea do not have high levels of general resistance to late blight. Consequently, growers have relied heavily on periodic application of fungicides and have used large amounts of fungicide for potato late blight control. As you can see in Table 1, 19 fungicide items for potato late blight control were used in Korea at 2000.

Phenylamide fungicides, e.g. metalaxyl, oxadixyl, benalaxyl and ofurace, are single-site inhibitors with a high specific efficacy against fungi of the order Peronosporales (Bruck et al. 1980; Schwinn and Staub, 1988). Their high efficacy and specificity, and consequent widespread use in agriculture, has selected for phenylamide-resistant individuals with pathogen populations (Delp, 1988). Early in 1980, about twe years after metalaxyl entered the market, the first cases of resistance under grower conditions were experienced. These occurred in glasshouses in Israel with *Pseudoperonospora cubensis* on cucumbers. The first cases of resistance in field-grown crop were in mid-1980 in Holland and ireland with *P. infestans* on potatoes (Daggett et al., 1993; Davidse et al., 1981; Delp, 1988; Dowley and O'sullivan, 1981). Occurrence of metalaxyl-resistant isolates of *P. infestans* in potato field in Korea was reported by Choi et al. (1992). Due to the seriousness of the resistance situations the manufacturer voluntarily withdrew metalaxyl single use from the market in Korea. However, mixtures (metalaxy+ethaboxam, metalaxyl+dimethomorph, and metalaxy+mancozeb) were used in the late blight control. This disease is a very serious disease in the Gangwon area where most of korea's seed potatoes are produced. Late blight caused premature vine maturation and tube rots and results in very low yields in certain years. Fry et al. (1993) wrote that to establish the most effective control strategies for late blight, we need to know much more about dispersal and genetic variation in local populations of *P. infestans.*

The goal of this investigation was to determine whether phenotyptic change have occurred among populations of *P. infestans* in Gangwon areas in Korea. Thus, isolates collected from 1988 to 2000 were analyzed for response to metalaxyl and mating type.

Table 1. The lists of fungicides used for control of potato late blight in Korea (2000)

Fungicide	Formulation (a. i. %)	Application rate (g/20L)	Spectrum of fungicidal activity
Dimethomorph	Wp (25)	20	Oomycetes (not *Pythium*)
Dimethomorph + mancozeb	Wp (7.5 + 66.7)	40	Oomycetes, anthracnose
Dimethomorph + copper oxychloride	Wp (15 + 35)	40	Oomycetes
Dimethomorph + copper hydroxide	Wp (20 + 30)	20	Oomycetes
Dimethomorph + tribasic copper sulfate	Sc (7 + 9)	20	Oomycetes
Dimethomorph + dithianon	Wp (8 + 30)	40	Oomycetes, anthracnose
Dimethomorph + metalaxyl	Wp (12.5 + 12.5)	40	Oomycetes
Ethaboxam + dimethomorph	Sc (10 + 15)	20	Oomycetes
Ethaboxam + metalaxyl	Wp (10 + 15)	20	Oomycetes
Ethaboxam + triflumizole	Wp (20 + 10)	20	Oomycetes, powdery mildew
Metalaxyl + mancozeb	Wp (7.5 + 56)	40	Oomycetes
Oxadixyl + copper hydroxide	Wp (8 + 62)	40	Oomycetes
Oxadixyl + mancozeb	Wp (8 + 56)	27	Oomycetes
Cymoxanil + mancozeb	Wp (6 + 50)	40	Oomycetes
Propamocarb + chlorothalonil	Sc (31 + 31)	20	Oomycetes
Mancozeb	Wp (75)	33	Wide
Fluoromide	Wp (75)	20	Wide
Chlorothalonil	Wp (75)	33	Wide
Fluazinam	Wp (50)	10	Wide

[a] Wp=Wettable powder, Sc=Suspension concentrate.

Material & Methods

Sample collection and Isolation. Sampling of blighted leaflets was done in Gangwon area, the major potato growing areas in Korea from 1998 to 2000. Samples of blighted leaflets were collected from commercial fields, private gardens and research plots of potato (Table 2).

P. infestans was isolated from diseased samples by placing blighted leaf fregments on Phytophthora selective medium (V-8 juice 200 ml, ampicillin 500 ppm, vancomycin 200 ppm, rifampicin 50 ppm, pimaricin 100 ppm, benomyl 10 ppm, $CaCO_3$ 4.5 g, agar 20 g, D. W. 800 §¢). Purified isolates were maintained on V-8 rye agar at 18¡É in the dark.

Table 2. Origins of *Phytophthora infestans* isolates collected from Gangwan area in 1998-2000

	Location	Sample Size
1998	Gangnung	40
	Jinbu	14
	Wangsan	5
	Hoenggae	24
	Subtotal	83
1999	Gangnung	78
	Jinbu	7
	Wangsan	10
	Hoenggae	10
	Subtotal	105
2000	Gangnung	18
	Injae	2
	Wangsan	10
	Hoenggae	9
	Yangyang	15
	Subtotal	54
Total		242

Mating type determination. To determine the mating type of an isolate, mycelial agar disk (8mm in diameter) was cut from the advancing edge of a 7-10 day-old colony growing on V-8 rye agar. An agar disk

containing mycelia of an isolate of unknown mating type was placed at the center of a clarified V-8 agar and a standard A1 isolate and A2 isolate of *P. infestans* were placed on opposite sides at a distance of about 3 cm from the unknown isolate. If oospores were observed in the contact zone of colonies between the unknown isolate and the A1 isolate, the unknown isolate was rated as an A2 mating type. If oospores were produced between the unknown isolate and the A2 isolate, the unknown isolate was rated as an A1 mating type.

Resistance to metalaxyl, dimethomorph, and ethaboxam. The responses to metalaxyl, dimethomorph, and ethaboxam were determined by an *in vitro* growth test. Radial growth of an isolate on V-8 juice agar with metalaxyl at final concentrations of 5 and 50 §¶/§¢ was compared to the growth in the absence of metalaxyl. After 6 days of growth, the mean radial growth of two replicates of each isolate was calculated. Sensitive, intermediate, and resistant ratings to metalaxyl were assigned to isolates exhibiting <10%, 10 to 60%, and >60% growth, respectively, on metalaxyl-amended agar compared to growth on unamended agar (Fig. 1). Radial growth of an isolate on V-8 agar amended with dimethomorph and ethaboxam at 1 and 10 §¶/§¢, respectively was compared to growth on unamended agar.

Fig. 1. Responses to metalaxyl of 3 *Phytophthora infestans* isolates.

Results

Mating type of *P. infestans* isolates.

Isolates of *Phytophthora infestans* obtained from several locations of Gangwon area in 1998-2000 were examined for their mating types and sensitivities to metalaxyl. Both A1 and A2 mating type isolates were isolated in 1998, 1999, and 2000. The majority of the *P. infestans* isolates were A1 mating type. About 64.3% of the isolates collected in 1998, 99.1% in 1999 and 85.2% in 2000 were determined as A1 mating type (Table 3).

Table 3. Frequency of mating types of *Phytophthora infestans* isolates obtained from various locations in Gangwon areas in 1998-2000

Year (Location)	No. of isolates tested	Mating type[a]	
		A1	A2
1998			
Gangnung	16	14(87.5)[b]	2(12.5)
Jinbu	11	8(72.7)	3(27.3)
Wangsan	4	3(75.0)	1(25.0)
Hoenggae	11	2(18.2)	9(81.8)
Subtotal	42	27(64.3)	15(35.7)
1999			
Gangnung	78	78(100)[b]	0(0)
Jinbu	7	7(100)	0(0)
Wangsan	10	10(100)	0(0)
Hoenggae	10	9(90.0)	1(10.0)
Subtotal	105	104(99.0)	1(1.0)
2000			
Gangnung	18	18(100)	0(0)
Injae	2	2(100)	0(0)
Wangsan	10	10(100)	0(0)
Hoenggae	9	1(11.1)	8(88.9)
Yangyang	15	15(100)	0(0)
Subtotal	54	46(85.2)	8(14.8)
Total	201	177(88.1)	24(11.9)

[a]Mating type was determined by pairing an unknown isolate with known A1 and A2 mating type isolates.
[b]Percentage of mating type isolates in parentheses.

Metalaxyl resistance

Sensitivity of the *P. infestans* to metalaxyl was examined by mycelial growth on V8 juice agar amended with matalaxyl. About 44.6% of the isolates examined in 1988 were resistant to metalaxyl, 55.4% of the isolates were intermediate resistant, but none of the isolates tested were sensitive. In 1999 and 2000, 10.5 and 59.3% of the isolates examined were sensitive, 88.6 and 25.9% of the isolates were intermediate resistant, and 0.9 and 14.8% of the isolates were resistant to metalaxyl, respectively (Table 4). Variation on metalaxyl sensitivity among isolates was not correlated within and between mating type.

Table 4. Responses to metalaxyl of *Phytophthora infestans* isolates obtained from various locations in Gangwon areas in 1998-2000

Year	No of	The number of isolate[a]

(Location)	isolates tested	Sensitive	Intermediate	Resistant
1998				
Gangnung	40	0(0)[b]	26(65.0)	14(35.0)
Jinbu	14	0(0)	4(28.6)	10(71.4)
Wangsan	5	0(0)	3(60.0)	2(40.0)
Hoenggae	24	0(0)	3(54.2)	11(45.8)
Subtotal	83	0(0)	46(55.4)	37(44.6)
1999				
Gangnung	78	5(6.4)	73(93.6)	0(0)
Jinbu	7	2(28.6)	5(71.4)	0(0)
Wangsan	10	0(0)	10(100)	0(0)
Hoenggae	10	4(40.0)	5(50.0)	1(10.0)
Subtotal	105	11(10.5)	93(88.6)	1(0.9)
2000				
Gangnung	18	17(94.4)	1(5.6)	0(0)
Injae	2	0(0)	2(100)	0(0)
Wangsan	10	0(0)	10(100)	0(0)
Hoenggae	9	0(40.0)	1(11.1)	8(88.9)
Yangyang	15`	15(100)	0(0)	0(0)
Subtotal	54	32(59.3)	14(25.9)	8(14.8)
Total	242	43(17.8)	153(63.2)	46(19.0)

[a]Resistance of the *Phytophthora infestans* isolates to metalaxyl was examined on V-8 juice agar medium containing 5 and 50 §¶/§¢ metalaxyl following an incubation of the fungus at 20¡É for 7 days.

[b]Percentage of resistant isolates in parentheses.

In addition, metalaxyl effectively controlled potato late blight in two field tests in Gangwon area in 2001 (Fig. 2). Therefore, it is possible to assume that metalaxyl sensitivity of the *P. infestans* isolates of Gangwon area is increasing. These results are quite different from those of early 1990s.

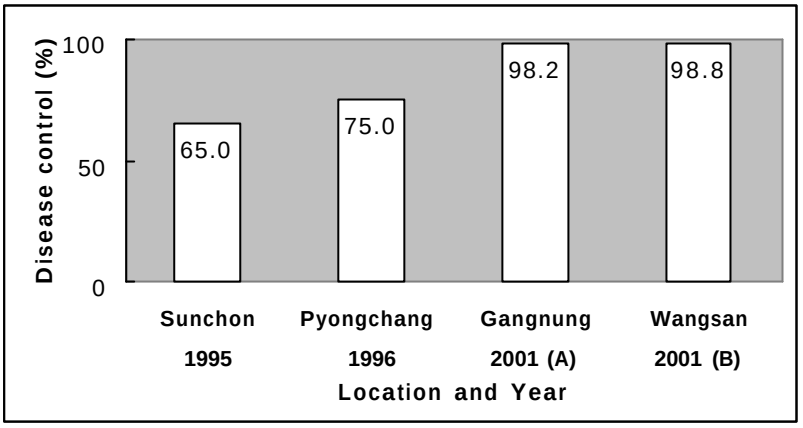


Fig. 2. Control of potato late blight by metalaxyl in field from 1995 to 2001.

Also resistance to dimethomoph and ethaboxam was examined on V8-juice agar containing 1 and 10 §¶/§¢ dimethomorph and ethaboxam. Above 1 §¶/§¢ concentration, collected isolates were highly sensitive to dimethomorph and ethaboxam (Table 5-6).

Table 5. Responses to dimethomorph of *Phytophthora infestans* isolates collected from Gangwon area in 2000

Location	Isolate	The number of isolate	
		1 §¶/§¢	10 §¶/§¢
Gangnung	3	0	0
			0
Hoenggae	3	0	0
Wangsan	4	1	0
Total	20	3 (15.0)	0 (0.0)

[a] Resistance of the *Phytophthora infestans* isolates to dimethomorph was examined on V8-juice agar medium containing 1 and 10 §¶/§¢ dimethomorph following an incubation of the fungus at 20¡É for 7 days.

Table 6. Responses to ethaboxam of *Phytophthora infestans* isolates collected from Gangwon area in 2000

Location	Isolate	The number of isolate	
		1 §¶/§¢	10 §¶/§¢
Gangnung	3	0	0
			0
Hoenggae	3	0	0
Wangsan	4	0	0
Total	20	0 (0.0)	0 (0.0)

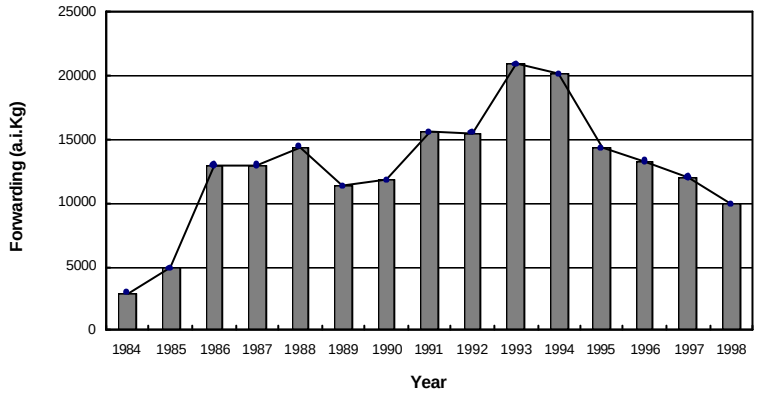
[a] Resistance of the *Phytophthora infestans* isolates to ethoboxam was examined on V8-juice agar medium containing 1 and 10 §¶/§¢ ethoboxam following an incubation of the fungus at 20¡É for 7 days.

Discussion

Until the mid-1980s A2 mating type strains had been considered to be restricted to central Mexico, While only A1 strains were distributed worldwide. A2 isolates outside Mexico were first reported from Switzerland in 1984 (Hohl and Iselin, 1984). Since then a number of reports revealed that A2 mating type strains occur in most parts of the world (Carlisle et al. 2001; Cohen et al., 1997; Kato et al., 1998). In Korea, *P. infestans* strains collected from various geographical locations from 1991 to 1993 were detected A2 mating types. The majority of the isolates were A2 mating type (Koh et al., 1994; So and Lee, 1993). Results of Koh et al. (1994) and So and Lee (1993) were consistent with a point of view of displacement of

the A1 mating type strain by the A2 mating type reported by most of countries (Daggett et al., 1993; Fry et al., 1992; 1993; Spielman et al., 1991).

However, isolates of *Phytophthora infestans* obtained from several locations of Gangwon area in 1998-2000 were not consistent with trends above (Kim et al., 2000). Both A1 and A2 mating type isolates were isolated in 1998, 1999, and 2000. The majority of the *P. infestans* isolates were A1 mating type. About 64.3% of the isolates collected in 1998, 99.1% in 1999 and 81.4% in 2000 were determined as A1 mating type. The reason for returning from A2 to A1 mating type is not clear. There was one change in agricultural practice, withdraw of metalaxyl single use for potato late blight control. In fact, increasing



application of metalxyl in early 1980s were gradually decreased from 1994 in Korea (Fig. 3). Mating type change from A1 to A2 and *vice versa* has been reported to occur by exposure to metalaxyl (Chang and Ko, 1990; Ko, 1994). Chabane et al. (1993) reported on selection and characterization of *Phytophthora parasitica* mutants with ultraviolet-induced resistance to dimethomorph or metalaxyl. Pestic Sci. 39:325-329.

Fig. 3. Metalaxyl forwarding according to year.

Sensitivity to metalaxyl of the *P. infestans* isolates collected from 1988 to 2000 was not agree with isolates collected early 1990s. Metalaxyl effectively controlled potato late blight in two field tests in Gangwon area in 2001. Therefore, it is possible to assume that metalaxyl sensitivity of the *P. infestans* isolates of Gangwon area is increasing. These results are quite different from those of early 1990s (Koh et al., 1994; Lee et al., 1994). Relationship between increasing sensitivity to matalaxyl and returning from A2 to A1 mating type is not clear. It is unclear whether increasing sensitivity to matalaxyl and returning from A2 to A1 mating type in Korea occur. Anyway, decreasing A2 mating type populations with metalaxyl resistance increased A1 mating type populations with metalaxyl sensitivity.

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