Plant Pathol. J. 21(1) : 33-38 (2005)

The Plant Pathology Journal

Changes in the Sensitivity to Metalaxyl, Dimethomorph and Ethaboxam of *Phytophthora infestans* in Korea

Xuan-Zhe Zhang, Kyoung-Yul Ryu[1], Jeom-Soon Kim[1], Jung-Uk Cheon[1] and Byung-Sup Kim*

Department of Applied Plant Science, Kangnung National University, Gangneung 210-702, Korea

[1]Laboratory of Crop Protection, National Institute of Highland Agriculture, RDA, Suwon 441-707, Korea

(Received on January 14, 2005; Accepted on February 18, 2005)

Changes of control efficacy of chemical to potato late blight caused by *Phytophthora infestans* in potato fields from 2001 to 2004 were examined. Control efficacy of metalaxyl was suddenly decreased from 100% in 2002 to 50% in 2004 and that of dimethomorph also was similar to those of metalaxyl. However, the control efficacy of ethaboxam no great change. Both A1 and A2 mating type isolates were isolated from 2001 to 2004 in several areas in Korea. The majority of the *P. infestans* isolates were A1 mating type. Total 939 isolates of *P. infestans* obtained from several areas in Korea from 2001 to 2004 were examined for changes of sensitivity to metalaxyl. Frequencies of metalaxyl resistance isolates were gradually increased from 17% in 2001 to 84.2% in 2004, but isolation frequencies of metalaxyl sensitive and intermediate resistant isolate were decreased. Cause of decreasing control efficacy of metalaxyl was thought by increase of resistance isolates in A1 mating type population according to increasing metalaxyl use. Most isolates were grown at 0.5 μg/ml of dimethomorph and isolates grown at 1 μg/ml of dimethomorph were approximately 10.2-22.9%. However, no isolate was able to grow at 5.0 μg/ml. Based on these results, minimum inhibitory concentrations (MIC) of dimethomorph to *P. infestans* were determined to be 0.5-1.0 μg/ml. Our results indicated that the reason decreasing control efficacy of dimethomorph was not caused by occurrence of resistant isolates. About 5% and 12.1% isolates among the total isolates collected in 2003 and 2004 were grown on V-8 juice rye agar containing 1.0 μg/ml ethaboxam. The 2.1 and 25.4% isolates had MICs of 0.2-0.4 μg/ml, and MIC values of 87.9% and 74.3% isolates were less than 0.2 μg/ml concentrations of ethaboxam. Therefore, resistance development by *P. infestans* to ethaboxam is not likely to occur in the natural condition.

Keywords : dimethomorph, ethaboxam, metalaxyl, *Phytophthora infestans*, resistance monitoring

*Corresponding author
Phone) +82-33-640-2353, FAX) +82-33-647-9535
E-mail) bskim@kangnung.ac.kr

The oomycete *Phytophthora infestans*, the cause of potato (*Solanum tuberosum*) and tomato (*Lycopersicon essulentum*) late blight, is an extremely destructive disease which can destroy potato fields in just a few day. Since the 1980s, the occurrence of mating type A2 and metalaxyl- resistant strains outside Mexico indicate that the population structure of fungus has changed (Fry et al., 1992). Recently, potato cultivars now grown commercially in Korea, do not have high levels of general resistance to late blight. Consequently, growers have relied heavily on periodic application of fungicides.

Chemical control strategies for potato late blight changed with the migration of phenylamide resistant isolates of *P. infestans* into the United States (Goodwin et al., 1994; Johnson et al., 2000). Phenylamide fungicides including metalaxyl have a biochemical action site to pathogens belong to the order Peronosporales (Bruck et al., 1980; Schwinn and Staub, 1988). However, their high efficacy and specificity consequently leading to widespread use in agriculture have resulted in phenylamide-resistant isolates within the pathogen populations (Davidse et al., 1981; Delp, 1998; Dowley and O'sullivan, 1981). Occurrence of *P. infestans* resistant to metalaxyl in Korean potato fields was reported by Choi et al. (1992).

This kind of resistance problem continuously requires new fungicides with different modes of action as solution. In the early 1990s, several new fungicides were released, including dimethomorph (BASF Corporation), a cinnamic acid derivative. Dimethomorph was highly selective towards certain members of the Peronosporales (Albert et al., 1991), had a moderate level of apoplastic systemicity (Cohen et al., 1995), and inhibited many stages of the life cycle of *P. infestans* via the disruption of cell wall formation (Kuhn et al., 1991). In 1993, dimethomorph was registered with a wettable powder in Korea. To reduce the risk of resistance development, dimethomorph was initially released for emergency use aganist potato late blight as a pre-mixed wettable powder consisting of 66.7% mancozeb and 7.5% dimethomorph by weight in Korea.

Ra et al. (1995) first discovered ethaboxam chemical in 1993, and thereafter it was registered in 1998 and commercialized as the first fungicide domestically developed in 1999 in Korea (Kim et al., 1999, 2002). Kim et al. (2002) reported that ethaboxam-resistant mutants of *P. infestans* and *P. capsici* were not inducible by UV irradiation and NMNG treatment with repeated attempts. Kim et al. (2003) reported that there was no indication of cross resistance between metalaxyl and ethaboxam because all the isolates, regardless of classification for their sensitivity to metalaxyl, were not able to grow at 5.0 μg/ml of ethaboxam. This indicates a low risk of resistance problem with ethaboxam.

The objectives of this study were to evaluate and analyze of control efficacy of above three chemicals against potato late blight and monitor on changes in sensitivity of *P. infestans* to metalaxyl, dimethomorph and ethaboxam.

Materials and Methods

Sampling and isolation. From 2001 to 2004, blighted potato and tomato leaflets were sampled from commercial fields and research stations in various locations of Korea (Table 1). Isolation was made by placing blighted leaf fragments on potato tuber slices in a Petri dish, and then incubating them at 22°C. After 5-7 days, the fungus grew on the surface of the tuber slice. Each isolates derived by transferring a hyphal tips on a selective medium of V-8juice agar (V-8 juice 200 ml, calcium carbonate 4.5 g, agar 20 g, distilled water 800 ml) amended with antibiotics at 500 mg/l of ampicillin, 200 mg/l of vancomycin, 50 mg/l of rifampicin, 100 mg/l of pimaricin, 35 mg/l of PCNB and 10 mg/l of benomyl. Purified isolates were maintained at 18 on V-8 juice agar.

Determination of mating type. To determine the mating type of an isolate, a mycelial agar disk (7 mm in diameter) was cut from the colony edge of *P. infestans* grown for 7-10 days on V-8 juice agar. An agar disk of unknown mating type was placed at the center, and both standard A1 and A2 isolates of *P. infestans* were placed 3 cm apart from the center on opposite sides. The mating type designated by observing oospores in the contact zone between each standard and unknown isolates.

Changes of control efficacy of chemical in field. Field experiment was investigated in potato field located in Gangneungshi Wangsan in 2001-2004. Potato cultivar, Sumi was planted in a randomized complete block design arranged in replicated 3 times. Chemicals used in this experiment were metalxyl, dimethomorph and ethaboxam. Chemical spraying begun when discovered first symptom in the potato fields, and sprayed 4 times with ten day interval.

Responses to chemical. Responses to metalaxyl, dimethomorph and ethaboxam were determined by *in vitro* growth test. One isolate of *P. infestans* with two replicates was measured for radial growth 7 days after incubation, and the colony diameters on V-8 juice rye agar amended with metalaxyl at 5 μg/ml, 10 μg/ml and 100 μg/ml were compared with that without metalaxyl. Radial growth as a percentage of the control was determined for each isolate at each concentration. Sensitive isolates were those with growth < 10% of the control at 5 μg/ml; intermediate resistant isolates had a growth ≥ 10% of the control at 5 μg/ml but less than 40% of the control at 100 μg/ml; resistant isolates had growth ≥ 40% of the control at 100 μg/ml. As for dimethomorph and ethaboxam, V-8 juice rye agar was amended with dimethomorph at 0.5, 1 and 5 μg/ml, and with ethaboxam at 0.2, 0.4 and 1 μg/ml. Radial growth as a percentage of the control was determined for each isolate at each concentration.

Results and Discussion

Isolates of *P. infestans*. *P. infestans* was isolated from diseased plant leaflets throughout Korea: 94 isolates from nine areas and unknown areas in 2001, 158 isolates from nine areas in 2002, 465 isolates from eight locations in 2003, and 222 isolates from four locations in 2004 (Table 1). These locations are major potato production areas in Korea. Of the 939 isolates, 30 isolates were obtained from tomato.

Mating type of *P. infestans* isolates. Both A1 and A2 mating type isolates were isolated in 2003 and 2004. The majority of the *P. infestans* isolates were A1 mating type. About 94% of the isolates collected in 2003 were determined as A1 mating type, and A2 mating type appeared in fungus isolated from leaves of diseased tomato in Gangneungshi and those of diseased potatoes in Bosunggun and Muhangun (Table 1). About 97.3% of the isolates collected in 2004 were determined as A1 mating type, and A2 mating type only discovered in isolats isolated from leaves of diseased tomato in Gangnuengshi. Prior to the 1980s, only the A1 mating type occurred in worldwide populations of *P. infestans* outside of Mexico (Goodwin et al., 1991; Hohl et al., 1984). A2 mating type outside Mexico was first reported in Switzerland in 1984 (Hohl and Iselin, 1984). Thereafter, A2 mating type strains have been reported to occur in most parts of the world (Kato et al., 1998). In Korea, *P. infestans* isolates collected from various geographical areas from 1991 to 1993 were mainly of the A2 mating type (Koh et al., 1994; So and Lee, 1993). These observations were consistent with the assumption on the displacement of A1 population by A2 mating type as reported in many countries (Fry et al., 1993; Spielman et al., 1991). However, most isolates of *P. infestans* collected from 2003 to 2004 in Korea were A1 mating type. This

Table 1. Frequency of mating type occurrence and metalaxyl resistance of *Phytophthora infestans* isolates obtained from various locations in 2001-2004

Year (Location)	Host plant	Isolates	Mating type		Response to metalaxyl		
			A1	A2	S	MR	R
2001							
Gangneungshi	Potato	56	56	0	3	50	3
Heongsunggun	Potato	1	1	0	0	1	0
Hongchengun	Potato	3	3	0	1	2	0
Pyongchanggun	Potato	4	4	0	0	4	0
Kimchengun	Potato	3	3	0	0	0	3
Kimjegun	Potato	7	7	0	1	6	0
Bosunggun	Potato	4	4	0	0	4	0
Buyergun	Tomato	10	0	10	0	0	10
Muhangun	Potato	2	2	0	0	2	0
Unknown	Potato	4	4	0	0	4	0
Subtotal		94	84(89.4)	10(10.6)	5(5.3)	73(77.7)	16(17)
2002							
Gangneungshi	Potato	46	46	0	2	44	0
Heongsunggun	Potato	6	6	0	0	6	0
Hongchengun	Potato	2	2	0	0	2	0
Jungsungun	Potato	11	11	0	1	10	0
Pyongchanggun	Potato	63	43	20	1	34	28
Samcheokshi	Potato	9	9	0	1	8	0
Bosunggun	Potato	5	5	0	0	3	2
Muhangun	Potato	3	3	0	0	0	3
Jejudo	Potato	13	13	0	0	13	0
Subtotal		158	138(87.3)	20(12.7)	5(3.2)	120(75.9)	33(20.9)
2003							
Gangneungshi	Potato	196	196	0	6	125	65
Gangneungshi	Tomato	14	0	14	0	0	14
Jungsunggun	Potato	31	31	0	1	25	5
Pyongchanggun	Potato	112	112	0	2	44	66
YangYanggun	Potato	57	57	0	2	43	12
Bosunggun	Potato	12	6	6	0	3	9
Kimjegun	Potato	13	13	0	0	0	13
Muhangun	Potato	8	0	8	0	0	8
Namwongun	Potato	22	22	0	0	8	14
Sub total		465	437(94)	28(6.0)	11(2.4)	248(53.3)	206(44.3)
2004							
Gangneungsh	Potato	121	121	0	1	7	113
Gangneungsh	Tomato	6	0	6	0	0	6
Jungsunggun	Potato	3	3	0	0	0	3
Pyongchanggun	Potato	88	88	0	9	17	62
YangYanggun	Potato	4	4	0	0	1	3
Sub total		222	216(97.3)	6(2.7)	10(4.5)	25(11.3)	187(84.2)
Total		939	875(93.2)	64(6.8)	31(3.3)	466(49.6)	442(47.1)

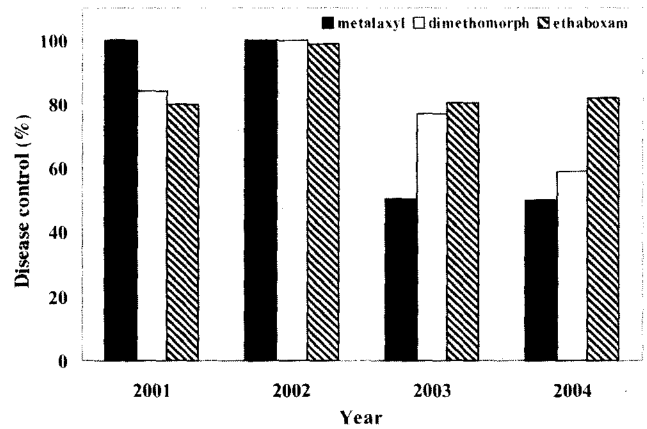

Fig. 1. Changes of control efficacy of metalaxyl, dimethomorph and ethaboxam to potato late blight caused by *Phytophthora infestans* in potato field from 2001 to 2004. Statistic analysis calculated by control efficacy of each fungicide according to year. Data are the everage of 3 replications and they were analyzed using Duncan's multiple range test. The same letters within a column mean no significant differences between the numbers.

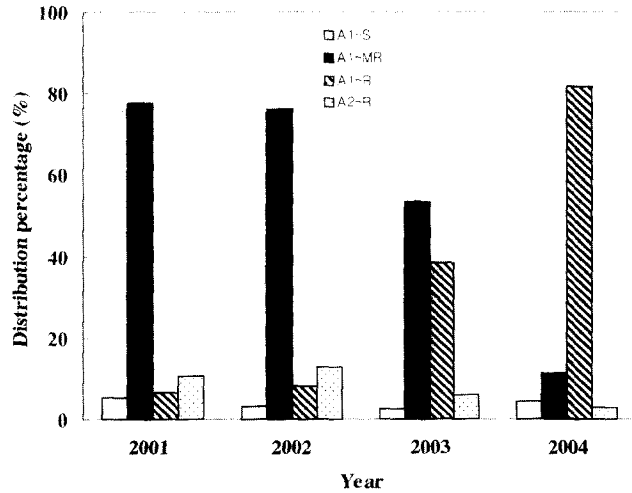

Fig. 2. Distribution of metalaxyl sensitivity patterns according to mating type of *Phytophthora infestans* isolates obtained from Korea in 2001-2004 (A1-S: metalaxyl sensitive isolates with A1 mating type, A1-MR: metalaxyl moderate resistance isolates with A1 mating type, A1-R: metalaxyl resistance isolates with A1 mating type and A2-R: metalaxyl resistance isolates with A2 mating type).

result is identical with trends of return from A2 to A1 mating type (Kim et al., 2000).

Analysis of changes of control efficacy of metalaxyl and the cause. To investigate changes of control efficacy of metalaxyl to potato late blight caused by *P. infestans*, the field experiment was conducted in Gangneungshi Wangsan in 2003 and 2004. Although Zhang et al. (2003) reported that control efficacy of metalaxyl was very outstanding in 2002, control values of metalaxyl were 50.3% and 50% in 2003 and 2004, respectively (Fig. 1).

To investigate decreasing cause of control efficacy of metalaxyl, sensitivity to metalaxyl *in vitro* were investigated. About 44.3% of the isolates obtained from 2003 were resistant to metalaxyl, 53.3% of the isolates were intermediate resistant and only 2.4% of the isolates were sensitive to metalaxyl (Table 1). Among the metalaxyl resistance isolates, A1 mating type was about 39% (Fig. 2). Also, 84.2% of the isolates obtained from 2004 were resistant to metalaxyl, 11.3% of the isolates were intermediate resistant and only 4.5% of the isolates were sensitive to metalaxyl (Table 1). Among the metalaxyl resistance isolates, A1 mating type was 81.5% (Fig. 2).

Metalaxyl is an oomycete-specific fungicide used for effectively controlling potato late blight and is highly inhibitory to mycelial growth and sporangium formation of *P. infestans* even at low concentrations (Bruck et al., 1980). However, heavy spraying of fungicides stimulated resistant to the chemical and forced the appearance of a new strain. Resistance was first recorded in *P. infestans* in Ireland and the Netherlands in 1980 (Davidse et al., 1981; Dowley and O'Sullivan, 1981). Also Koh et al., (1994) and Lee et al., (1994) reported that most Korean isolates was resistant to metalaxyl. However, Kim et al. (2000) and Zhang et al. (2003) reported that density of resistant isolates to metalaxyl was fully decreased. Although most metalaxyl resistance isolates described in reports in Korea were A2 mating type. From now, our results indicated that most metalaxyl resistance isolates were A1 mating type. Thus, it is important to investigate the metalaxyl sensitivity changes in *P. infetans* continuously. Cause of decreasing control efficacy of metalaxyl was thought that increasing of resistance isolates with A1 mating type according to directed selection, increasing metalaxyl use.

Analysis of changes of control efficacy and sensitivity to dimethomorph. Field experiment of control effect of dimethomorph was made in same field of metalaxyl. Control value (%) of dimethomorph was 77.1% in 2003 and 58.9% in 2004, respectively (Fig. 1). Decrease of control efficacy of dimethomarph was differed with conclusion described by Zhang et al. (2003).

To investigate cause of changes of control efficacy, we examined the effect of dimethomorph on the mycelial growth *in vitro*. About 83.6% and 77% of the total isolates MIC value collected from 2003 to 2004 was less than 0.5 and 16.4% and 22.9% isolates were grown at 1.0 μg/ml, but no isolate was able to grow at 5.0 μg/ml (Fig. 3). Based on these results, minimum inhibitory concentration (MIC) of dimethomorph to *P. infestans* was determined to be 0.5-1 μg/ml. Thus, our result indicated that resistant isolate to dimethomorph was not occurred.

Because of optimal weather condition for potato late

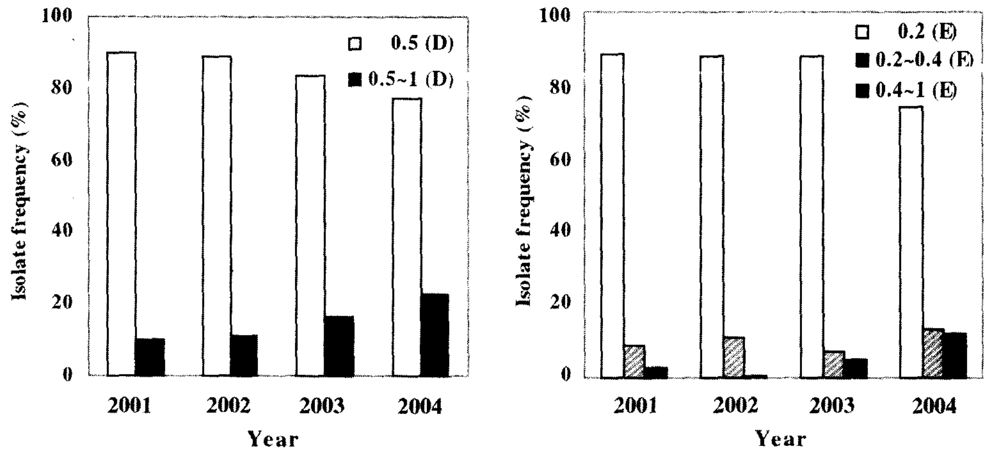

Fig. 3. The frequency of isolates with each minimum inhibitory concentration (MIC) to dimethomorph and ethaboxam of *Phytophthora infestans* obtained from Korea in 2001-2004. < 0.5 (D): the MIC is less than 0.5 ppm with dimethomorph, 0.5~1 (D): the MIC is ranged 0.5~1 ppm with dimethomorph, < 0.2 (E): the MIC is less than 0.2 ppm with ethaboxam, 0.2~0.4 (E): the MIC is ranged 0.2~0.4 ppm with ethaboxam, 0.4~1 (E): the MIC is ranged 0.4~1.0 ppm with ethaboxam.

blight epidemic in 2003, growers had heavily relied on periodic fungicide applications. Stein et al. (2003) reported that application intervals beyond 7 days would not be effective control because late blight epidemics can develop and progress at a high rate under conducive conditions, especially on un-protected foliage. However, application interval was 10 day in our experiment in 2003 and 2004. Cause of decreasing control efficacy was thought because application interval was long. But resistance development by *P. infestans* to dimethomorph is not likely to occur in the natural condition.

Analysis of changes of control efficacy and sensitivity to ethaboxam. Experiment of control effect of ethaboxam also was made in same field. Control efficacy of ethaboxam was examined for 80.4% and 81.9% in 2003 and 2004, respectively (Fig. 1).

Although control efficacy of ethaboxam was not decreased until now, sensitivity monitoring in the natural condition is fundamental for the management of any potential risk from resistance in the future. Ethaboxam sensitivity was examined on V-8 juice rye agar containing 0.2, 0.4 and 1.0 μg/ml ethaboxam. About 5% and 12.1% isolates among the total isolates collected from 2003 to 2004 were grown at 1.0 μg/ml. The 12.1 and 25.4% isolates had MIC of 0.2-0.4 μg/ml, and MIC values of 87.9% and 74.3% isolates were less than 0.2 μg/ml concentrations of ethaboxam (Fig. 3). This result was similar that isolates of *P. infestans* collected in Korea was sensitivity to ethaboxam as described by Kim et al. (2003). Also, resistance development by *P. infestans* to ethaboxam is not likely to occur in the natural condition.

Acknowledgments

This study was supported in part by a grant from Academic Research Program, the Kangnung National University in Korea in 2004.

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