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Sensitivity of *Phytophthora infestans* Isolates to Fungicides Metalaxyl and Ethaboxam in Korea

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Sensitivity of *Phytophthora infestans* isolates to fungicides metalaxyl and ethaboxam in Korea was examined with 260 isolates for 3 years (9 isolates in 2000, 93 isolates in 2001, and 158 isolates in 2002). Both A1 and A2 mating types were found from the isolates collected for 3 years. A1 mating type was dominant in the population with 8 isolates (88.9%) in 2000, 84 isolates (89.4%) in 2001, and 138 isolates (87.3%) in 2002. Only some isolates from diseased tomatoes in Buyergun and diseased potatoes in Pyeongchanggun were of the A2 mating type. As for metalaxyl sensitivity, 77.0% of the isolates were moderately resistant with 8 isolates (88.9%) in 2000, 73 isolates (77.7%) in 2001, and 120 isolates (75.9%) in 2002. Meanwhile, those found resistant were 1 isolate (11.1%) in 2000, 16 isolates (17.0%) in 2001, and 33 isolates (20.9%) in 2002. Only 5 isolates (3.2%) were sensitive to metalaxyl in 2002. There was no significant difference in the sensitivity among years. As for ethaboxam, no isolate was able to grow at 5.0 μg/ml, and only four isolates (1.5%) grew at 1.0 μg/ml with heavy retardation compared with the untreated control. Based on these 3-year results, the minimum inhibitory concentration (MIC) of ethaboxam to *P. infestans* was determined to be 0.2-1.0 μg/ml. Results indicate that resistance development by *P. infestans* to ethaboxam is not likely to occur in the natural condition. Furthermore, 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.

Keywords **:** ethaboxam, cross resistance, metalaxyl, mating type, *Phytophthora infestans*, resistance

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is an extremely destructive disease which can destroy potato fields in just a few days. *P. infestans* is heterothallic with two mating types, designated as A1 and A2. While only A1 mating type was found worldwide, A2 mating type had been restricted to central Mexico until the mid-1980s. Isolates with A2 mating type outside Mexico were first reported from Switzerland in 1984 (Hohl and Iselin, 1984). Since then, a number of reports have revealed that A2 mating types occurred in many countries (Carlisle et al., 2001; Mosa et al., 1989). In Korea, *P. infestans* isolates collected from various geographical locations from 1991 to 1993 were mainly of the A2 mating type (Koh et al., 1994; So and Lee, 1993).

Because of the genetic variability of *P. infestans*, the race-specific oligogenic resistance has not been useful for the control of late blight. 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 such as mancozeb and metalaxyl.

Phenylamide fungicides including metalaxyl, oxadixyl, benalaxyl, and ofurace have a biochemical action site to pathogens belonging 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 to phenylamide-resistant isolates within the pathogen populations (Daggett et al., 1993; Davidse et al., 1981; Delp, 1988; Dowley and O'sullivan, 1981). Occurrence of *P. infestans* resistant to metalaxyl in Korean potato fields was reported by Choi et al. (1992). These resistant strains often have higher fitness than sensitive ones in the natural habitat (Kadish and Cohen, 1988; Kim et al., 1993). This kind of resistance problem continuously requires new fungicides with different modes of action as a solution. In this respect, ethaboxam is a valuable addition to the group of fungicides specific to diseases caused by Oomycetes. Ra et al. (1995) first discovered this 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; Kim et al., 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. This indicates a low risk of resistance problem with ethaboxam.

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Before extensive use of a fungicide, however, sensitivity monitoring in the natural condition is fundamental for the management of any potential risk from resistance in the future. In view of these, this study was conducted to: 1) monitor any phenotypic change on sensitivity of *P. infestans* to metalaxyl; 2) evaluate their sensitivity to ethaboxam; and 3) find out any relationship in sensitivity between metalaxyl and ethaboxam. To attain these objectives, isolates of *P. infestans* collected from natural fields in several locations in Korea in 2000-2002 were analyzed.

Materials and Methods

Sampling and isolation. From 2000 to 2002, blighted potato leaflets were sampled from commercial fields and research stations in various locations throughout Korea (Table 1). *P. infestans* was isolated by placing fragments of a blighted leaf on a selective medium of V-8 juice 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, and 10 mg/l of benomyl. Purified isolates were maintained at 18°C on V-8 juice agar.

Determination of mating type. To determine the mating type of an isolate, a mycelial agar disk (8 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 was designated by observing oospores in the contact zone

Table 1. Frequency of mating type occurrence and metalaxyl resistance of *Phytophthora infestans* isolates obtained from various locations in 2000-2002

Year (Location)	Host plant	Isolates	Mating type		Response to metalaxyl		
			A1	A2	S	MR	R
2000							
Gangneungshi	Potato	4	4(100)	0	0	4(100)	0
Gangneungshi	Tomato	3	3(100)	0	0	3(100)	0
Jungsunggun	Potato	1	1(100)	0	0	1(100)	0
Pyoengchanggun	Potato	1	0	1(100)	0	0	1(100)
Subtotal		9	8(88.9)	1(11.1)	0	8(88.9)	1(11.1)
2001							
Gangneungshi	Potato	56	56(100)	0	3(5.4)	50(89.2)	3(5.4)
Heongsunggun	Potato	1	1(100)	0	0	1(100)	0
Hongchengun	Potato	3	3(100)	0	1(33.3)	2(66.7)	0
Pyongchanggun	Potato	4	4(100)	0	0	4(100)	0
Kimchengun	Potato	3	3(100)	0	0	0	3(100)
Kimjegun	Potato	7	7(100)	0	1(14.3)	6(85.7)	0
Bosunggun	Potato	4	4(100)	0	0	4(100)	0
Muhangun	Potato	2	2(100)	0	0	2(100)	0
Buyergun	Tomato	10	0	10(100)	0	0	10(100)
Unknown	Potato	4	4(100)	0	0	4(100)	0
Subtotal		94	84(89.4)	10(10.6)	5(5.3)	73(77.7)	16(17)
2002							
Gangneungshi	Potato	46	46(100)	0	2(4.3)	44(95.7)	0
Heongsunggun	Potato	6	6(100)	0	0	6(100)	0
Hongchengun	Potato	2	2(100)	0	0	2(100)	0
Jungsungun	Potato	11	11(100)	0	1(9.1)	10(90.9)	0
Pyoengchanggun	Potato	63	43(68.3)	20(31.7)	1(1.6)	34(54)	28(44.4)
Samcheokshi	Potato	9	9(100)	0	1(11.1)	8(88.9)	0
Bosunggun	Potato	5	5(100)	0	0	3(60)	2(40)
Muhangun	Potato	3	3(100)	0	0	0	3(100)
Jejudo	Potato	13	13(100)	0	0	13(100)	0
Subtotal		158	138(87.3)	20(12.7)	5(3.2)	120(75.9)	33(20.9)
Total		261	230(88.1)	31(11.9)	10(3.8)	201(77.0)	50(19.2)

between each standard and unknown isolates. The standard strain of A1 mating type, KA-2, was kindly obtained from the Korea Research Institute of Chemical Technology, and that of A2 mating type, BC-3, from the Chungnam National University.

Resistance to metalaxyl and ethaboxam. Responses to metalaxyl and ethaboxam were determined by *in vitro* growth test. One isolate of *P. infestans* with two replicates was measured for radial growth 6 days after incubation, and the colony diameter on V-8 juice agar amended with metalaxyl at 10 μg/ml was compared with that without metalaxyl. An isolate was assigned as "sensitive" when the radial growth in the presence of metalaxyl is less than 10% of the radial growth in the absence of metalaxyl, "moderately resistant" with 10 to 60% radial growth, and "resistant" with greater than 60% radial growth (Koh et al., 1994). As for ethaboxam, V-8 juice agar was amended with ethaboxam at 0.5, 1, and 5 μg/ml. The radial growth was measured similarly as described above and used to determine minimum inhibition concentration (MIC).

Results and Discussion

Isolates of *P. infestans*. *P. infestans* was isolated from diseased plant leaflets throughout Korea: 9 isolates from four locations in 2000; 94 isolates from 9 locations in 2001; and 158 isolates from 9 locations in 2002 (Table 1). These locations are major potato production areas in Korea. Of the 261 isolates, 13 isolates were obtained from tomato.

Mating type of *P. infestans* isolates. Both A1 and A2 mating type isolates were isolated in 2000, 2001, and 2002. About 88.9% of the isolates collected in 2000, 89.4% in 2001, and 87.3% in 2002 were of the A1 mating type (Table 1). Only isolates from leaves of diseased tomato in Buyergun and those of diseased potatoes in Pyeongchanggun were of the A2 mating type. 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 (Carlisle et al., 2001; Kato et al., 1998). In Korea, *P. infestans* strains collected from various locations from 1991 to 1993 were firstly reported to be of A2 mating type with the major population (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 (Daggett et al., 1993; Fry et al., 1993; Spielman et al., 1991).

However, isolates of *P. infestans* obtained from Gangwon area in 1998-2000 were different (Kim et al., 2000) with the Al mating type. Reasons for the return from A2 to A1 mating type is not clearly understood yet. Meanwhile, there was one agricultural practice, the application withdrawal of metalaxyl alone for potato late blight control, which could have caused the shift in mating type. In fact, metalaxyl application gradually decreased from 1994 in Korea (Kim et al., 2000). Although relationship between increased sensitivity to metalaxyl and shifting of mating type is not clearly understood, it is becoming clear that isolates of A2 mating type with metalaxyl resistance have decreased and that those of A1 mating type have increased with metalaxyl sensitivity.

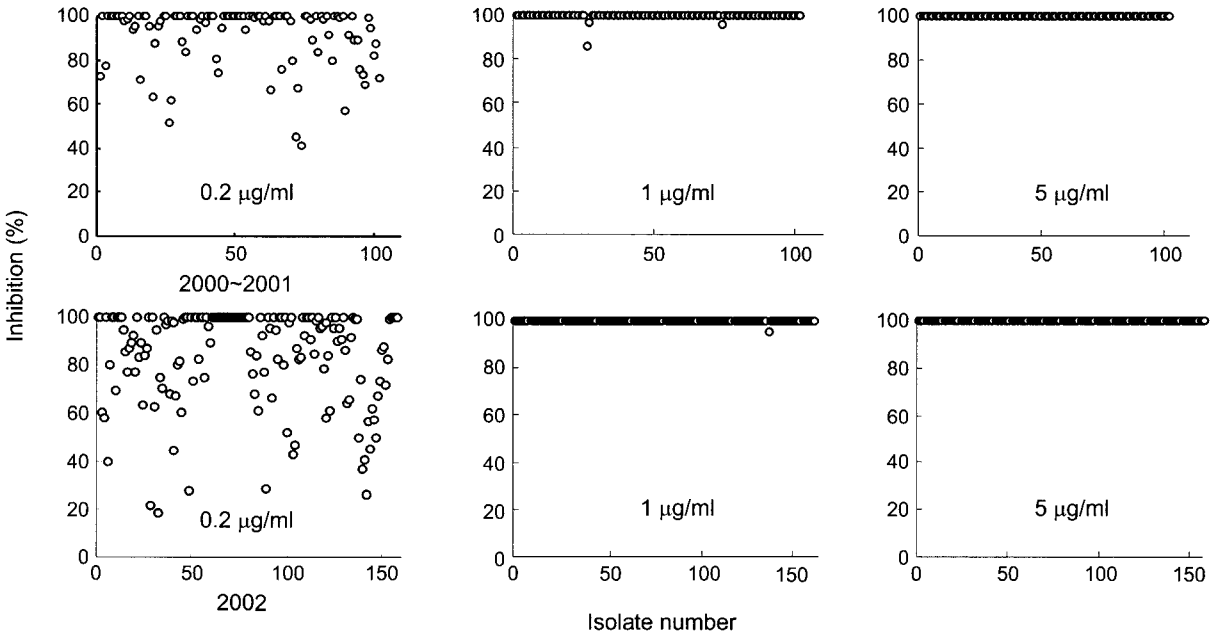

Fig. 1. Sensitivity levels of *Phytophthora infestans* isolates obtained in 2000-2002 to ethaboxam. Sensitivity was measured in terms of percent inhibition of mycelial growth on V-8 medium containing 0.2, 1.0, and 5 μg/ml ethaboxam.

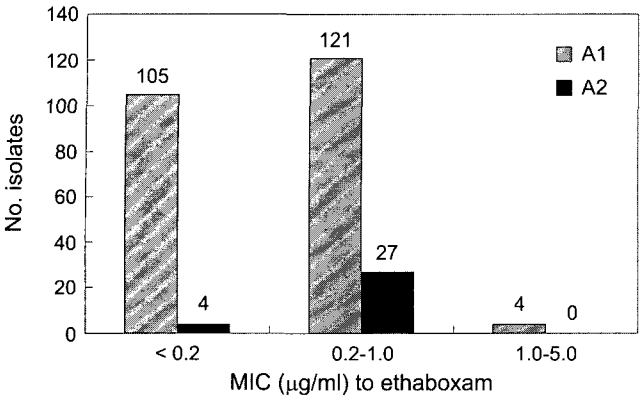

Fig. 2. Sensitivity of *P. infestans* by mating type (A1 and A2) to ethaboxam at 0.2, 1.0, and 5.0 μg/ml.

Sensitivity of *P. infestans* to metalaxyl. Sensitivity of *P. infestans* to metalaxyl was classified by the radial growth on V-8 agar amended with metalaxyl. In 2000-2001, most isolates were moderately resistant (78.6%) and resistant (16.5%), while sensitive fungus appeared as 4.9% (Table 1). Similarly in 2002, most isolates (75.9%) were moderately resistant, 20.9% resistant, and 3.2% sensitive.

Sensitivity of *P. infestans* to ethaboxam. Ethaboxam sensitivity was examined on V-8 agar containing 0.2, 1, and 5 μg/ml ethaboxam. All *P. infestans* isolates obtained in 2000-2001 did not grow at all in ethaboxam at 5 μg/ml concentration, and only three isolates (2.9%) were able to grow at 1.0 μg/ml concentration. Although not completely inhibited, these three isolates were severely retarded with 14.1% growth compared with those without ethaboxam. All isolates were highly sensitive to ethaboxam at concentrations higher than 1.0 μg/ml (Fig. 1) and the MIC range was 0.2-1.0 μg/ml for 260 isolates tested. In 2002, similar results were obtained, and only one isolate grew at 1.0 μg/ml. There was no difference in sensitivity to ethaboxam during the periods. These results indicate that ethaboxam has not been faced with any resistance yet. Furthermore, both mating types were sensitive to ethaboxam at 1.0 μg/ml (Fig. 2). This study shows that genetic factors related with mating type have no influence on resistance of *P. infestans* to ethaboxam.

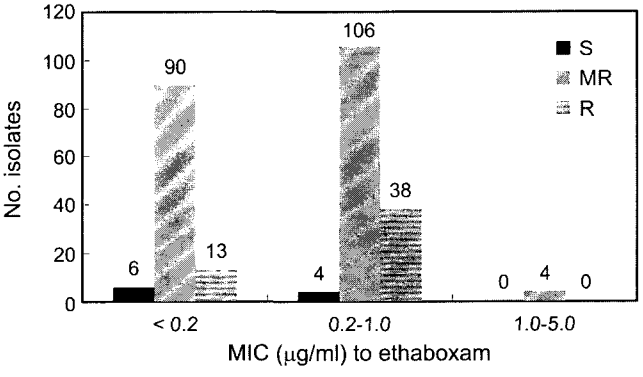

Fig. 3. Sensitivity of *P. infestans* by metalaxyl resistance to ethaboxam at 0.2, 1.0, and 5.0 μg/ml. S=sensitive; MR=moderately resistant; R=resistant.

Sensitivity of metalaxyl-resistant isolates to ethaboxam. Regardless of metalaxyl sensitivity, all isolates of *P. infestans* were sensitive to ethaboxam. Even for the isolates with moderate resistance and resistance to metalaxyl, MICs for ethaboxam were also 0.2-1.0 μg/ml (Fig. 3). These results indicate that ethaboxam has no cross resistance to metalaxyl. Furthermore, Kim et al. (2002) indicated that ethaboxam has no cross resistance to strobilurin fungicides. Based on the results, ethaboxam might have an advantage over other oomycetes fungicides in terms of resistance management and could become an effective alternative for disease control by fungicide application.

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