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Baseline Sensitivity to Mandipropamid Among Isolates of *Phytophthora capsici* Causing Phytophthora Blight on Pepper

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Sensitivity to the new carboxylic acid amide fungicide, mandipropamid, of *Phytophthora capsici* causing pepper Phytophthora blight was determined on 187 isolates collected in Korea over 3 years, from 2005 to 2007. All isolates were sensitive to mandipropamid, with EC_{50} values for growth of mycelia ranging from 0.001 to 0.037 μg/ml. Among the isolates, 147 (79.0%) isolates were sensitive to metalaxyl, whereas others were resistant to this fungicide. Mandipropamid had the same effect on mycelium growth of both metalaxyl-sensitive and metalaxyl-resistant isolates, indicating an absence of cross-resistance between these two fungicides. Comparison of the sensitivities of *P. capsici* isolates showed a positive correlation between sensitivity to mandipropamid and dimethomorph (r^2=0.8533). The results of this study indicate that there is no evidence for development of resistance to mandipropamid in this population of *P. capsici* isolates collected in Korea.

Keywords : dimethomorph, fungicide resistance, mandipropamid, metalaxyl, *Phytophthora capsici*

Pepper Phytophthora blight caused by *Phytophthora capsici* is considered one of the most devastating soil-borne diseases of pepper and is a limiting factor for pepper production in Korea (Hwang and Kim, 1995). Although control strategies are limited because of difficulties in effectively controlling this soil-borne disease, chemical application, cultural practices, and crop rotation are considered effective control measures. Recently, resistant pepper cultivars have been developed and are commercially available. These commercial pepper cultivars are only partially resistant, even though they were commercialised as being resistant. Disease management is therefore achieved mainly through a combination of cultural practices, crop rotation, and fungicide use. Among these, fungicide application is regarded as an effective control measure. Phenylamides (e.g. mefenoxam), strobilurins (e.g. trifloxystrobin and kresoxim-methyl), and multi-site inhibitors (e.g. copper) have been used to control Phytophthora blight of pepper, however, resistance has evolved to several classes of fungicides. Recently, mandipropamid, a mandelic acid amide within the carboxylic acid amide (CAA) fungicide group, was developed. The CAA group includes dimethomorph, flumorph, iprovalicarb, and benthiavalicarb. Mandipropamid provides outstanding control of late blight on tomato and potato and of downy mildew on grapes, cucurbits, leafy vegetables, brassicas, bulb vegetables, hops, and tobacco caused by oomycete pathogens (Cohen and Gisi, 2007; Cohen et al., 2008; Harp et al., 2007).

The mode of action of CAA fungicides is not well understood. Morphological studies indicate that dimethomorph, iprovalicarb, and benthiavalicarb inhibit cell wall biosynthesis (Cohen et al., 1995; Jende et al., 2002; Matheron and Porchas, 2000; Reuveni, 2003). Biochemical studies of mandelamide compound SX 623509 on mycelium of *P. infestans* suggest alterations in phospholipid biosynthesis, with inhibition of phosphatidylcholine biosynthesis as the main target (Griffiths et al., 2003). The genetic studies of Gisi et al. (2007) on crosses of CAA-sensitive and -resistant isolates of *Plasmopara viticola* have indicated that all CAA fungicides share a similar mode of action.

Although resistance to CAA fungicides was detected in *P. viticola* (Gisi et al., 2007; Gisi and Sierotzki, 2008), no resistance has been reported in field isolates of *P. infestans*, even though CAA fungicides have been used for more than 15 years. In Korea, mandipropamid was registered under the commercial name REVUS in 2006 to control Phytophthora disease in pepper, potato, and ginseng. However, baseline sensitivity to mandipropamid among field isolates of *P. capsici* causing Phytophthora blight has not been studied. Defining the sensitivity distribution of iso-

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lates of *P. capsici* from pepper plants to mandipropamid will be useful for further monitoring of fungicide sensitivity once the fungicide is widely used by farmers to control pepper Phytophthora blight. Therefore, the main objective of this research was to determine sensitivities of isolates of *P. capsici* obtained from infected pepper plants to mandipropamid, based on mycelium growth *in vitro*, and to determine the cross-sensitivity/resistance patterns between mandipropamid and other fungicides, such as metalaxyl and dimethomorph.

Material and Methods

Media. V-8 juice agar medium (200 ml V-8 juice, 1 g $CaCO_3$, 17 g Agar, and 800 ml distilled water) is routinely used to culture and test for fungicide sensitivity of *P. capsici* isolates. To isolate *P. capsici* from blighted pepper plants, this was used as a selective medium by adding antibiotics (10 mg pimaricin, 10 mg rifampicin, and 100 mg ampicillin) and fungicides (25 mg hymexazol and 50 mg PCNB) to 1 liter of cornmeal agar medium (Difco, Sparks, MD 21152 USA/38800 Le Pont de Claix, France), cooled to 50°C after autoclaving. Sporangia of *P. capsici* were produced on oatmeal agar medium (Difco, Sparks, MD 21152 USA/38800 Le Pont de Claix, France).

Collection of isolates. Over 3 years, from 2005 to 2007, stems, crowns, and roots of pepper plants with wilting symptoms of Phytophthora blight were collected throughout South Korea. Each wilting pepper plant was randomly sampled from various fields separated by up to 10 km. Small pieces of tissue were cut from the margins of lesions on the infected parts of the pepper plants. Tissue pieces were surface-disinfected in 1% NaClO for 2 min, washed in sterile distilled water for 1 min, kept on plates containing the abovementioned selective medium, and subsequently incubated at 20°C for 4 or 5 days.

Single zoospore isolates. Isolates were cultured on V-8 agar for 4 days at 20°C. Mycelium plugs (5 mm in diameter) were taken from the edge of an actively growing colony and incubated on oatmeal agar at 20°C for 7 days (Kim et al., 2007). Following scarification of the mycelial mat, the plates were placed in an incubator at 20°C for 2 days under continuous light to induce sporulation. Sporangia were harvested in sterile distilled water and decanted through two layers of autoclaved cheesecloth to remove hyphal debris. The sporangial suspension was chilled at 4°C for 2 h and then transferred to 25°C for 1 h to induce zoospore release. Zoospore suspensions from each isolate were collected and the concentration in each suspension determined using a hemacytometer. To produce single zoospore isolates, the zoospore suspension was adjusted to 10^4 zoospores/ml, and 5 μL suspension was spread evenly on V-8 juice agar. After 5 h at 20°C, a germinating zoospore from each isolate was transferred to a plate containing V-8 juice agar. Single zoospore isolates were subcultured on new V-8 juice agar and stored at 20°C in 10-ml tubes with screw caps before use in the further experiments.

Fungicide sensitivity test. Three fungicides were used for sensitivity tests: mandipropamid, dimethomorph and metalaxyl. Isolates of *P. capsici* were grown on V-8 juice agar for 4 days at 20°C in the dark. Agar plugs (5 mm diameter) cut from the edge of a colony were transferred to V-8 juice agar amended with fungicide at each indicated concentration. After incubation for 4 days, two perpendicular colony diameters were measured per plate, and the diameter of the agar plug was subtracted from the colony diameter before calculating the inhibition ratio (%) of mycelium growth, expressed as the inhibition percentage of mycelium growth on fungicide-amended V-8 agar medium against that on medium without fungicide. All 187 isolates were tested twice with three replicate plates in each test. The effective concentration inhibiting mycelium growth by 50% (EC_{50}) for each isolate was calculated as the inhibition ratio (%) of mycelium growth against the log fungicide concentration.

Results

Sensitivity of *P. capsici* isolates to mandipropamid. A total of 187 *P. capsici* isolates were assessed for their sensitivity to mandipropamid, and sensitivities over the 3 years were compared (Table 1). The EC_{50} values over these 3 years ranged from 0.001 to 0.037 μg/ml, with mean values of 0.016, 0.013, and 0.013 μg/ml in 2005, 2006, and 2007, respectively. For dimethomorph, the EC_{50} values of all isolates recovered from infected pepper plants ranged from 0.077 to 0.787 μg/ml, with means of 0.061, 0.059, and 0.100 μg/ml in the respective sampling years. The sensitivities of the isolates to mandipropamid and dimethomorph did not change significantly over these 3 years. In particular, there was no geographical variation in the sensitivity to mandipropamid for 2006 to 2007 (Fig. 1). The frequency distribution of the EC_{50} values for the 187 isolates had a unimodal distribution that was skewed to the right (Fig. 2).

Correlation between mandipropamid and metalaxyl or dimethomorph. The sensitivity phenotypes of *P. capsici* isolates to metalaxyl were designated as metalaxyl-sensitive or metalaxyl-resistant phenotypes according to the

Table 1. Baseline sensitivity data for field isolates of *Phytophthora capsici* on mandipropamid-amended V-8 juice agar

Fungicide	Year	Number of isolates	EC_{50} value[a] (μg/ml)			Resistance factor[b]
			Range	Mean	SD	
Mandipropamid	2005	31	0.001-0.032	0.016	0.006	32
	2006	101	0.001-0.037	0.013	0.006	37
	2007	55	0.001-0.029	0.013	0.006	29
Dimethomorph	2005	31	0.077-0.363	0.207	0.061	4.7
	2006	101	0.091-0.415	0.209	0.059	4.6
	2007	55	0.084-0.787	0.266	0.100	9.4

[a] Effective concentration that reduces mycelium growth by 50%.
[b] Resistance factor=highest EC_{50} value divided by lowest EC_{50} value.

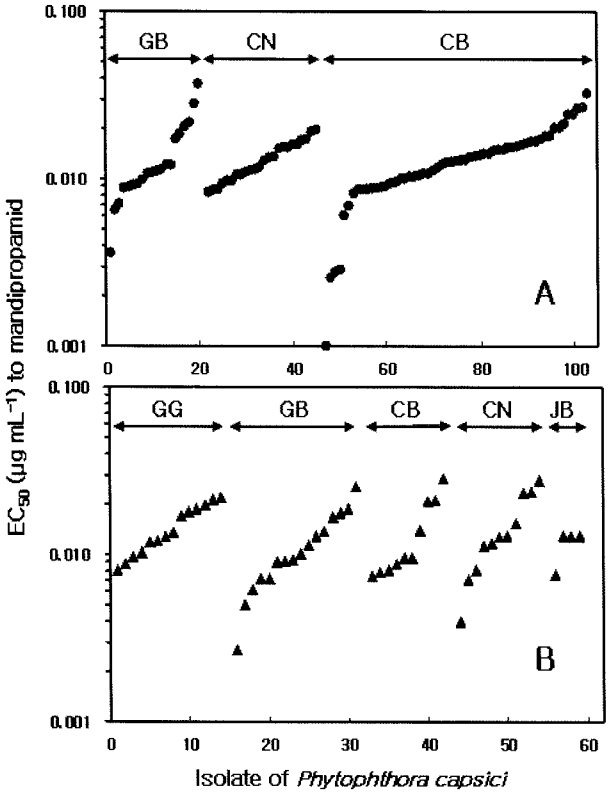

Fig. 1. Sensitivity of the 187 isolates of *Phytophthora capsici* collected from infected pepper plants in Korea from 2006 (A) to 2007 (B). Percentage inhibition of mycelium growth at each concentration of mandipropamid was calculated relative to the untreated control without mandipropamid, and effective concentration for a 50% reduction (EC_{50}) value was calculated by regressing percent-age growth inhibition against the log fungicide concentration. Each abbreviation indicated as follows; GG, Gyeonggi-Do; GB, Gyeongsangbuk-Do; CB, Chungcheongbuk-Do; CN, Chungcheongnam-Do; CB, Jeollabuk-Do.

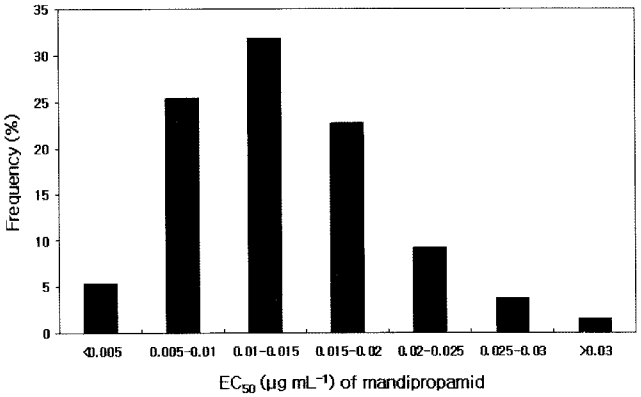

Fig. 2. Frequency distribution of *P. capsici* isolates for each class of effective concentration for a 50% reduction (EC_{50}) in sensitivity tests with growth of mycelia on fungicide-amended medium. Effective concentration for EC_{50} was calculated by regressing percentage growth inhibition against log fungicide concentration.

criteria of Kim et al. (2007). Among the 187 isolates, 40 (21.0%) were resistant to metalaxyl. To investigate cross-resistance between mandipropamid and metalaxyl, the sensitivities to mandipropamid of isolates with different levels of sensitivity to metalaxyl were compared. Sensitivity to mandipropamid was not associated with the level of sensitivity to metalaxyl (Table 2). Mandipropamid had almost equal activity against the mycelium growth of both metalaxyl-sensitive and metalaxyl-resistant isolates, indicating an absence of cross-resistance between these two fungicides. However, the sensitivities of the isolates showed a high correlation between sensitivity to mandipropamid and to dimethomorph (r^2=0.8533), indicating that there was positive cross-resistance between these two fungicides (Fig. 3).

Discussion

In Korea, pepper production is threatened by pepper Phytophthora blight caused by *P. capsici* (Hwang and Kim, 1995), for which many fungicides have been developed. A novel fungicide, mandipropamid, has recently been registered and commercialised in Korea. In the present study, 187 isolates of *P. capsici* recovered from infected pepper plants were tested to determine baseline sensitivity to mandipropamid using mycelium growth as an indicator, because CAA fungicides show dominant activity on mycelium growth and spore germination (Cohen et al., 2008). Establishment

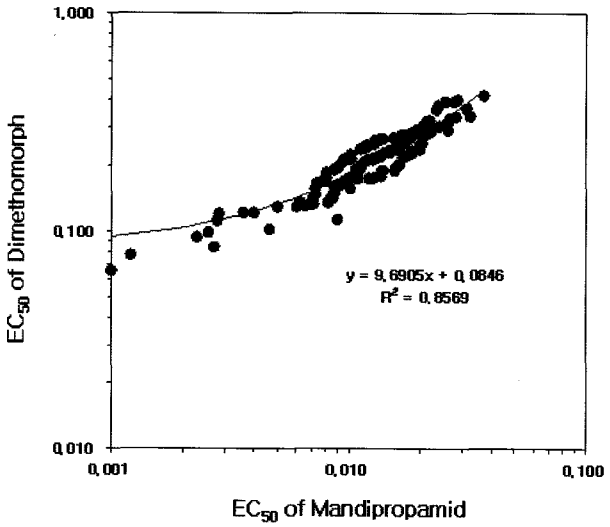

Fig. 3. Relationship between the effective concentration of mandipropamid and dimethomorph needed to reduce mycelium growth by 50% (EC_{50}) for 187 isolates of *P. capsici*. R^2 is the correlation value for the relationship.

of baseline sensitivity data is of great importance in fungicide resistance management, as these data are essential to allow detection of shifts in pathogen sensitivity and resistance in the pathogen population, which can be correlated with future control failures (Jutsum et al., 1998; Russell, 2004). The sensitivity distribution of *P. capsici* to mandipropamid showed a unimodal distribution skewed to the right, indicating that there was no resistant subpopulation among the isolates. The EC_{50} values of all isolates ranged from 0.001 to 0.037 μg/ml (mean=0.014 μg/ml) over 3 years from 2005 to 2007. These data can be used as a baseline for monitoring the occurrence of isolates of *P. capsici* causing pepper Phytophthora blight in fields resistant to mandipropamid.

According to the species of *Phytophthora*, mandipropamid may show variable activity against mycelium growth. Cohen et al. (2007) reported that EC_{50} values of *P. infestans* isolates collected from Israeli fields ranged from 0.007 to 1.17 μg/ml (mean=0.29 μg/ml), and the mean EC_{50} value in European isolates for 2004 was 0.609 μg/ml. However, our results showed that *P. capsici* isolates collected from 2005 to 2007 in Korea had EC_{50} values from 0.001 to 0.037 μg/ml, which is much lower than those of *P. infestans*. Therefore, the effect of mandipropamid on mycelium growth varies according to species of *Phytophthora*. Because of the difference in response to mandipropamid in *P. capsici* and *P. infestans*, it is necessary to investigate the activity of mandipropamid against other species of *Phytophthora* that cause crop diseases to extend the application spectrum of mandipropamid to other plant diseases caused by other species of *Phytophthora*.

Table 2. Sensitivity of metalaxyl-sensitive and -resistant phenotypes of *P. capsici* to mandipropamid *in vitro*

Metalaxyl sensitivity	Number of isolates	Frequency (%)	EC_{50} (μg/ml) values for mandipropamid		
			Range	Mean	SD
Sensitive	148	79.0	0.001-0.037	0.013	0.006
Resistant	40	21.0	0.002-0.027	0.014	0.004

As shown in Table 2, mandipropamid was equally effective on isolates sensitive and resistant to metalaxyl, which inhibits RNA polymerisation in ribosomal RNA synthesis (Davidse, 1995). Given these data, we conclude that because mandipropamid did not have cross-resistance with metalaxyl, it can be used to control populations of *P. capsici* resistant to metalaxyl. As shown in Fig. 3, however, there was a high correlation between mandipropamid and dimethomorph, both of which are in the CAA fungicide group and act not only to inhibit cell wall biosynthesis/assembly (Matheron and Porchas, 2000; Reuveni, 2003) but also to alter phospholipid biosynthesis (Griffiths et al., 2003). Also, in *Plasmopara viticola*, cross-resistance was observed between mandipropamid and the CAA fungicides iprovalicarb and benthiavalicarb (valinamide sub-group) and dimethomorph and flumorph (cinnamic acid amide sub-group) (Gisi et al., 2007). All five of these fungicides are therefore classified as CAA fungicides. To investigate the risk of resistance development in *P. infestans*, several researchers have tried to produce mutants resistant to fungicides (Rubin et al., 2008; Stein and Kirk, 2004; Yuan et al., 2006). Many mutants resistant to mandipropamid developed after initial mutagenesis, but all showed unstable resistance and failed to grow on media with mandipropamid after one to eight asexual infection cycles (Rubin et al., 2008). Field experiments conducted from 2001 to 2006 showed that selection pressure imposed by repeated spraying of sublethal or excess doses of mandipropamid on potato or tomato crops infected with mixed field isolates of *P. infestans* produced no resistant isolates against this fungicide (Cohen et al., 2007). These results indicate that, as well as low field fitness, the probability of a buildup of a population of *P. infestans* resistant to mandipropamid in the field is low. No field isolates of *P. capsici* resistant to mandipropamid were detected in our study over the 3 years. In general, *Phytophthora* species have been presented to be medium-risk pathogens. Thus, resistance monitoring of mandipropamid and resistance prevention strategies should be considered with regard to its continued use in the chemical control strategy for pepper Phytophthora disease, although our results suggest that resistant isolates of *P. capsici* have not yet appeared in pepper fields in Korea.

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References

Cohen, Y., Baidr, A. and Cohen, B. T. 1995. Dimethomorph activity against oomycete fungal plant pathogens. *Phytopathology* 85:1500-1506.

Cohen, Y. and Gisi, U. 2007. Differential activity of carboxylic acid amide fungicides against various developmental stages of *Phytophthora infestans*. *Phytopathology* 97:1274-1283.

Cohen, Y., Rubin, A. and Gotlieb, D. 2008. Activity of carboxylic acid amide (CAA) fungicides against *Bremia lactucae*. *European J. Plant Pathol.* 122:169-183.

Cohen, Y., Rubin, E., Hadad, T., Gotlieb, D., Sierotzki, H. and Gisi, U. 2007. Sensitivity of *Phytophthora infestans* to mandipropamid and the effect of enforced selsction pressure in the field. *Plant Pathol.* 56:836-842.

Davids, L. C. 1995. Phenylamide fungicides: biochemical action and resistance. In *Modern Selective Fungicides: Properties, Applications, Mechanisms of Action*. ed. by H. Lyr, 2nd ed., pp. 347-354. Gustav Fischer Verlag, Villengang, Germany, and New York.

Gisi, U. and Sierotzki, H. 2008. Fungicide modes of action and resistance in downy mildews. *Eur. J. Plant Pathol.* 122:157-167.

Gisi, U., Waldner, M., Kraus, N., Dubuis, P. H. and Sierotzki, H. 2007. Inheritance of resistance to carboxylic acid amide (CAA) fungicides in *Plasmopara viticola*. *Plant Pathol.* 56:199-208.

Griffiths, R. G., Dancer, J., O'Neill, E. and Harwood, J. L. 2003. A mandelamide pesticide alters lipid metabolism in *Phytophthora infestans*. *New Phytol.* 158:345-353.

Harp, T., Cochran, A., Tory, D., Kuhn, P., Payan, L., Laird, D. and Tally, A. 2007. Development of Revus 2.09SC (a.i. mandipropamid) in the U.S. for control of downy mildews on leafy vegetables. *Phytopathology* 97:S45.

Hwang, B. K. and Kim, C. H. 1995. Phytophthora blight of pepper and its control in Korea. *Plant Dis.* 79:221-227.

Jende, G., Steiner, U. and Dehne, H. W. 2002. Microscopical characterization of fungicidal effects on infection structures and cell wall formation of *Phytophthora infestans*. In: *Modern fungicide and antifungal compounds III*. ed. by H. W. Dehae, K. H. Kuck, P. E. Russell, and H. Lyr, pp. 83-90. Agroconcept, Bohn, Germany.

Jutsum, A. R., Heaney, S. P., Perrin, B. M. and Wege, P. J. 1998. Pesticide resistance: Assessment of risk and implementation of effective management strategies. *Pesticide Sci.* 54:435-446.

Kim, S. B., Lee, S. M., Min, G. Y. and Kim, H. T. 2007. Response to metalaxyl of *Phytophthora capsici* isolates collected in 2005 and 2006. *Kor. J. Pesticide Sci.* 11:305-312. (in Korean)

Matheron, M. E. and Porchas, M. 2000. Impact of azoxystrobin, dimethomorph, fluazinam, festyl-Al, and metalaxyl on growth, and zoospore cyst germination of three *Phytophthora* spp. *Plant Dis.* 84:454-458.

Reuveni, M. 2003. Activity of the new fungicide bethiavalicarb against *Plasmopara viticola* and its efficacy in controlling downy mildew in grapevines. *Eur. J. Plant Pathol.* 109:243-251.

Rubin, A., Gotlieb, D., Gisi, U. and Cohen, Y. 2008. Mutagenesis of *Phytophthora infestans* for resistance against carboxylic acid amide and phenylamide fungicides. *Plant Dis.* 92:675-683.

Russell, P. E. 2004. Sensitivity baselines in fungicide resistance research and management. FRAC, Monogr. No. 3, Brussels.

Stein, J. M. and Kirk, W. W. 2004. The generation and quantification of resistance to dimethomorph in *Phytophthora infestans*. *Plant Dis.* 88:930-934.

Yuan, S. K., Liu, X. L., Si, N. G., Dong, J., Gu, B. G. and Jiang, H. 2006. Semsitivity of *Phytophthora infestans* to flumorph: *in vitro* determination of baseline sensitivity and the risk of resistance. *Plant Pathol.* 55:258-263.