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Distribution of *Monilinia fructicola* Isolates Resistant to Dicarboximide or to both Procymidone and Carbendazim in Korea

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To evaluate the sensitivity of *Monilinia fructicola* to dicarboximides used in controlling brown rot of peach, the fungus was isolated from commercial peach orchards in Chochiwon (CH), Chongdo (CD), Gyeongsan (GY), and Youngduk (YO) in Korea. The population shift of dicarboximide-resistant isolates of *M. fructicola* was investigated for 3 years starting 1998. The frequency of procymidone-resistant isolates (PRI) was higher in CD and GY than in CH and YO. The frequency of PRI was higher in the mid season (July-August) than in the rest of the year. Cross-resistance rate of PRI to iprodione was over 87.8% during the investigation, and double-resistance to both procymidone and carbendazim was less than 10%. However, the rate of cross-resistant isolates to vinclozolin was low. In the orchards in GY and CH without any fungicide spray, the PRI population was persistent and did not vary for 3 years. The results suggest that dicarboximide resistance of *M. fructicola* could be a problem in controlling brown rot and blossom blight on peach trees because it may take a long time to recover the population with sensitive isolates even in the absence of these fungicides.

Keywords **:** brown rot, cross-resistance, dicarboximides, *Monilinia fructicola*.

Control of diseases caused by *Monilinia fructicola* is globally imperative, particularly in the production of commercially important stone fruits such as peach, nectarin, apricot, and plum. Although biocontrol and cultural practices have been recommended and studied, fungicide applications such as benzimidazoles, dicarboximides, and ergosterol biosynthesis inhibitors are most widely practiced to control blossom blight and brown rot (Byrde and Willetts, 1997). However, frequent fungicide applications are costly and may lead to the development of fungicide resistance (Delp, 1988). Sensitivity shift to various fungicides against phytopathogenic fungi has been reported in both field and laboratory experiments (Delp, 1988; Michaildes et al., 1987; Penrose et al., 1985; Sanoamuang and Gaunt, 1995; Staub, 1991).

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Fungicide resistance in plant pathogens became a problem in the mid 1960s and has increased over the last 20 years with the use of systemic fungicides such as benzimidazoles, dicarboximides, ergosterol biosynthesis inhibitors (EBIs), and phenylamide (Dewaard et al., 1993; Sanoamuang and Gaunt, 1995; Staub, 1991). Several fungicides have been used to control blossom blight, twig blight, and brown rot caused by *Monilinia* spp. on stone fruit trees in Korea. The fungicides include benzimidazoles (carbendazim and thiophanate-methyl), dicarboximides (iprodione, procymidone, and vinclozolin), EBIs (bitertanol, difenoconazole, and hexaconazole), and strobilurin (azoxystrobilurin). These fungicides have been used for possibility of appearance of fungicide-resistant isolates by decreased binding affinity, reduced uptake, and increased non-specificity (Baldwin and Rathmell, 1988).

In Korea, the study on resistance to these fungicides has been limited to *Botrytis* diseases (Kim and Kwon, 1993; Kim et al., 1995, 1996; Lim et al., 1996; Park et al., 1992). The benzimidazole- and dicarboximide-resistant isolates of *M. fructicola* were initially reported from peduncles, mummified fruits, and blossom blight in spring (Lim et al., 1998).

The objectives of this study were to survey population shift of procymidone-resistant isolates and double-resistant isolates to both procymidone and carbendazim, and to investigate the sensitivity of *M. fructicola* isolates to vinclozolin.

Materials and Methods

Fungal isolates. Fresh and mummified fruits, stems, and flowers with brown rot or blighted blossoms were collected from peach orchards in Chochiwon (CH), Chongdo (CD), Gyeongsan (GY), and Youngduk (YO) that has been sprayed with fungicide for several years. Samples were collected three times (early: February-March; mid: July-August; post: December) per year from 1998 to 2000. All the plant parts taken from the trees were placed in separate polyethylene bags and were incubated to sporulation at 25°C for 5 days under >95% relative humidity. To obtain monoconidial isolates, an agar block containing a single germinated conidium was cut with a sterile needle and transferred on potato dextrose agar (PDA). After incubation at 25°C for 5 days, mono-

conidial cultures were used for fungicide sensitivity tests. The fluctuation of population of dicarboximide-resistant isolates without spray of any fungicides for several years was investigated with isolates from CH and GY.

Fungicide and sensitivity tests. Fungicides used were carbendazim (60% WP), iprodione (50% WP), procymidone (50% WP), and vinclozolin (50% WP). Each fungicide was diluted in sterile water at appropriate concentrations and added onto PDA. Based on a previous study (Lim at al., 1999), response to iprodione, procymidone, and vinclozolin was evaluated by mycelial growth of *M. fructicola* isolates on fungicide-free PDA and PDA amended with 0.033, 0.33, 3.3, and 33 μg a.i./ml for iprodione; 0.0028, 0.028, 0.28, 2.8, and 28 μg a.i./ml for procymidone; and 0.0029, 0.029, 0.29, 2.9, and 29 μg a.i./ml for vinclozolin, respectively. The 5-mm diameter mycelium plugs were taken from the margin of 7-day-old culture on PDA and transferred to the test media. The colony diameters were measured after incubation at 25°C for 7 days.

Double-resistance to both carbendazim and procymidone. Fungal isolates from GY and CD were tested for double-resistance. Isolates were cultured on fungicide-free PDA and PDA amended with 1.0 μg a.i./ml of carbendazim and 25 μg a.i./ml of procymidone. Mycelial growth of the isolates was measured with the same method as described above.

Sensitivity to vinclozolin of *M. fructicola* isolates. Sensitivity to vinclozolin was examined with *M. fructicola* isolates starting mid season for 3 years. The sensitivity was observed based on the ratio of growth on PDA with vinclozolin (2.5 μg a.i./ml) to the growth on PDA without the fungicide.

Results

Sub-population of procymidone-resistant isolates. The responses of isolates were tested on PDA with 25 μg a.i./ml of procymidone. In CH, 22.4% of early season isolates grew on fungicide-PDA. For 3 years, the population of procymidone-resistant isolates was 22.4% of the total isolates. The growth rate of resistant isolates reached its highest in the mid season (Fig. 1). These results were confirmed for 3 years of experiment. In CD, the growth rate of dicarboximide-resistant isolates was 28.2% for 3 years. The rate of resistant isolates was lowest in the post season. In the early and mid seasons, the rate of fungicide-resistant isolates was 30.7% and 31.7%, respectively, and was higher than that in the other regions. In GY, the rate of resistant isolates was 27.1% for 3 years and the lowest was in the post season with 23.8%. In YO, the rate of resistant isolates was 21.6% for 3 years and highest in the mid season. The rates of the early and post season isolates were 19.8% and 18.7%, respectively. In CH and YO, procymidone-resistant isolates had lower growth rates than those in the other regions, but there was no significant difference in isolation time (Fig. 2).

Double-resistance to both carbendazim and procymidone.

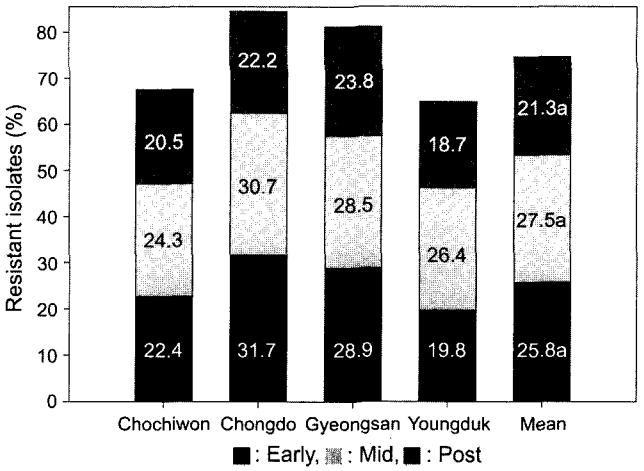

Fig. 1. Fluctuation of population of procymidone-resistant *Monilinia fructicola* isolates from peach orchards with season from 1998 to 2000. The same letters in mean bar are not significantly different at 5% level by L.S.D test.

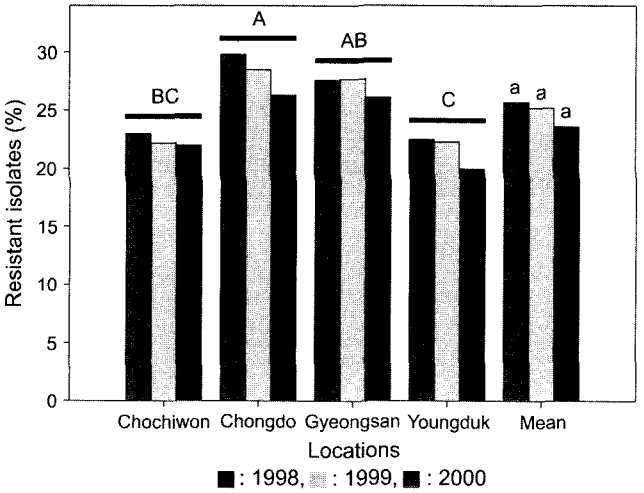

Fig. 2. Rate of procymidone-resistant *Monilinia fructicola* isolates with isolation years. Resistance to the fungicide was tested on PDA amended with procymidone 25 μg a.i./ml. Capital and small letters on the bar show significant difference (P=0.05) among locations and years, respectively.

In CD, 9.9% of carbendazim-resistant isolates showed resistance to procymidone for 3 years. In GY, 7.8% of carbendazim-resistant isolates showed resistance to procymidone for 3 years (Fig. 3).

Cross-resistance among dicarboximides. In 1998, 259 isolates (87.2%) of procymidone-resistant isolates grew on PDA with 25 μg a.i./ml of procymidone and iprodione, while 19 isolates (6.4%) grew on PDA with 25 μg a.i./ml of procymidone and vinclozolin. The rate of cross-resistance to iprodione was the highest in first test (Table 1). In 1999, 286 isolates (87.2%) of procymidone-resistant isolates grew on PDA with 25 μg a.i./ml of procymidone and iprodione,

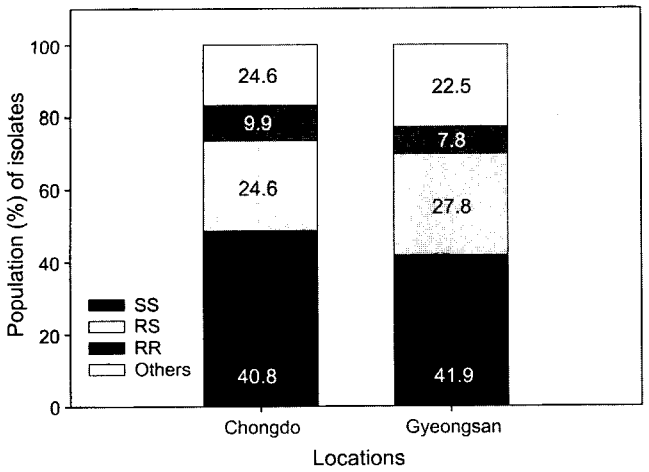

Fig. 3. Population of double-resistant *M. fructicola* isolates to procymidone and carbendazim. The response was tested on PDA amended with carbendazim at 1 μg a.i./ml and procymidone at 25 μg a.i./ml. SS=carbendazim- and procymidone- sensitive isolates; RS=carbendazim-resistant and procymidone-sensitive isolates; RR=carbendazim- and procymidone-resistant isolates.

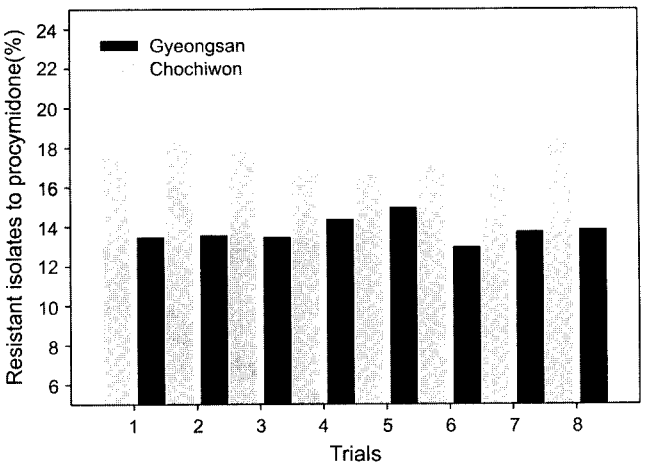

Fig. 4. Shift of population of procymidone-resistant isolates in the orchards without fungicide spray for a few years at Chochiwon and Gyeongsan. Resistance was determined by mycelial growth of *M. fructicola* on PDA amended with procymidone at 25 μg a.i./ml.

Table 1. Cross-resistance of procymidone-resistant *Monilinia fructicola* isolates from four locations to dicarboximides[a]

Year	Time isolated	No. of resistant-isolates to procymidone/ tested isolates	Cross-resistant isolates to	
			Iprodione	Vinclozolin
1998	Early	124 / 430	117 (94.4)[b]	7 (5.6)[c]
	Mid	124 / 430	103 (83.6)	9 (7.3)
	Post	48 / 261	39 (81.3)	3 (6.3)
	Total	296 / 1,121	259 (87.2)	19 (6.4)
1999	Early	127 / 455	114 (89.8)	8 (5.4)
	Mid	122 / 443	105 (86.1)	9 (7.4)
	Post	78 / 384	66 (84.6)	5 (6.4)
	Total	327 / 1282	285 (87.2)	22 (6.7)
2000	Early	77 / 420	66 (85.7)	8 (10.4)
	Mid	110 / 403	100 (90.9)	8 (7.2)
	Total	187 / 823	166 (88.8)	16 (8.6)

[a]The response was tested with PDA mixed with fungicide suspension containing 25 μg a.i./ml of procymidone, iprodione, and vinclozolin.
[b]The number in parenthesis represents rate of resistant isolates to procymidone and iprodione.
[c]The number in parenthesis represents rate of resistant to procymidone and vinclozolin.

and 22 isolates (6.7%) grew on PDA with 25 μg a.i./ml of procymidone and vinclozolin. In 2000, 166 isolates (88.8%) of procymidone-resistant isolates grew on PDA with 25 μg a.i./ml of procymidone and iprodione, and 16 isolates (8.6%) grew on PDA with 25 μg a.i./ml of procymidone and vinclozolin. The isolates from the four locations showed high cross-resistance between procymidone and iprodione. However, they showed very low cross-resistance to vinclozolin.

Shift of population of resistant isolates in orchard without any fungicide spray. In GY, the rate of procymidone-resistant isolates was 13.0-15.0% throughout this study. In CH, the rate of procymidone-resistant isolates in the orchard without fungicide application ranged from 16.8 to 18.7%, lower than that in commercial orchards. There was no shift of population between the two locations (Fig. 4).

Sensitivity to vinclozolin of *M. fructicola* isolates. Vinclozolin sensitivity of *M. fructicola* isolates was investigated, and most of the tested isolates in 1998 did not grow on PDA amended with vinclozolin. Only 11 isolates (11.0%) showed growth at 2.5 μg a.i./ml of vinclozolin. There was no isolate showing growth over 50% to mycelial growth on PDA without fungicide. In 1999, 15 isolates (15.0%) grew on PDA with the fungicide, while 3 isolates (3.0%) grew over 50% to mycelial growth on PDA without fungicide. In

Table 2. Sensitivity of *Monilinia fructicola* isolates from four locations to vinclozolin[a]

Year	No. of tested isolates	Growth rate (%)[b]				
		10-20	21-30	31-40	41-50	51
1998	100	8 (8.0)	3 (3.0)	0 (0.0)	0 (0.0)	0 (0.0)
1999	100	10 (10.0)	2 (2.0)	0 (0.0)	1 (1.0)	2 (2.0)
2000	120	11 (9.2)	3 (2.5)	2 (1.7)	1 (0.8)	0 (0.0)
Total	320	29 (9.1)	8 (2.5)	2 (0.6)	2 (0.6)	2 (0.6)

[a]The growth of isolates was tested on PDA amended with and without vinclozolin at 25 μg a.i./ml.
[b]Growth rate=(diameter of mycelial growth on PDA amended with vinclozolin/diameter of mycelial growth on PDA without fungicide) × 100.

2000, 17 isolates (14.2%) grew on PDA amended with fungicide, while 1 isolate showed mycelial growth over 50% to mycelial growth on PDA without fungicide (Table 2).

Discussion

Isolates of *M. fructicola* collected from 1998 to 2000 could be grouped by sensitivity at certain doses of fungicides based on a previous study (Lim et al., 1999). In the investigated regions, the rate of procymidone-resistant isolates was 24.8% for 3 years, and reached to the highest in mid season (July-August). In addition, the rate was higher in GY and CD than in CH and YO. Higher procymidone-resistant population in GY and CD could be associated with the responses to the other fungicides such as benzimidazoles, application history of fungicide, responses to previously sprayed fungicides, and disease severity (Delp, 1988; Köller and Wilcox, 2001). The high population of the resistant isolates in the mid season could be associated with intensive spray of dicarboximide fungicides such as procymidone, iprodione, and vinclozolin, and the environmental condition favorable to disease development (Baldwin and Rathmell, 1988; Delp, 1988). These results and those of previous studies (Delp, 1988; Ogawa et al., 1985) imply that the use of dicarboximide fungicides in controlling brown rot and blossom blight might be restricted in the observed regions.

In Korea, compared to benzimidazole-resistance, the problem of dicarboximide-resistance in peach and nectarine could be more serious. For example, the benzimidazole-resistant isolates appeared in GY and CD (data not shown). In the orchards without use of fungicide in GY and CD, the population of procymidoneresistant isolates was lower than that in the commercial orchards and did not shift distinctly during the experiment. These results suggest that it is difficult to reduce the resistant isolates in the fields, and that the maintenance of resistant population was associated with fitness of resistant isolates and practice of farmer (Delp, 1988). Environmental conditions like high disease pressure during growing season could lead farmers to spray fungicides more frequently, which eventually leads to resistance (Baldwin and Rathmell, 1988; Delp, 1988; Dewaard et al., 1993; McGrath, 2001; Stub, 1991). However, resistant isolates were known to be inferior in fitness compared with the wild type (Delp, 1988; Elmer and Gaunt, 1994). Dicarboximide-resistant population in the orchards without fungicide spray could be associated with disease pressure from surrounding orchards, original population of resistant-isolates, and application history of the fungicides (Delp, 1988).

Throughout this study, the resistant isolates to procymidone showed cross-resistance to iprodione at high rate. However, the cross-resistance to vinclozolin remained low for 3 years. This observation is not consistent with previous studies (Delp, 1988) and may be associated with fungicide-application history and practices of farmers. The results also suggest that vinclozolin may be useful to control brown rot and blossom blight caused by *M. fructicola* (Delp, 1988; Elmer and Gaunt 1993; Köller and Wilcox, 2001; Osorio et al., 1993).

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