Plant Pathol. J. 22(1) : 1-6 (2006)

The Plant Pathology Journal

Responses of Peach Blossom Blight and Brown Rot Fungus *Monilinia fructicola* to Benzimidazole and Diethofencarb in Korea

Tae Heon Lim[1], Jin Ho Kim[2] and Byeongjin Cha[3]*

[1]Technology Innovation Center and [2]Department of Plant Resources, Sangju National University, Sangju 742-711, Korea
[3]Department of Plant Medicine, Chungbuk National University, Cheongju 361-763, Korea

(Received on August 30, 2005; Accepted on November 21, 2005)

The population shifts of *Monilinia fructicola* isolates which were resistant to the fungicide benzimidazoles were investigated in four regions of Korea from 1998 to 2000. The isolation frequency of benzimidazole-resistant isolates ranged from 18.8% to 29.6% in Chongdo and from 22.0% to 26.8% in Gyeongsan during the same period. However, the frequency of benzimidazole-resistant isolates was less than 4.0% in Chochiwon and Youngduk during the same period. Benzimidazole-resistant isolates showed cross-resistance among benzimidazoles. On the other hand, none of the isolates showed cross-resistance to diethofencarb and carbendazim. Regardless of the year, the benzimidazole-resistant isolates of EC_{50} higher than 500 μg a.i./ml were isolated more frequently in mid and late season than in early season. In an orchard of Gyeongsan that had not been exposed to any fungicides for several years, the population of benzimidazole-resistant isolate had persisted without much fluctuation for three years. These results suggest that benzimidazole resistance of *M. fructicola* is becoming a problem in controlling brown rot and blossom blight of peach in regions like Chongdo and Gyeongsan.

Keywords : brown rot, *Monilinia fructicola*, peach blossom blight

Brown rot and blossom blight, caused by *Monilinia fructicola,* are the most destructive diseases of stone fruits including peach, plum, and apricot in Korea (Lim et al., 1998). The diseases are controlled by application of a fungicide spray during blossom and the fruit-ripening period (Agrios, 2005; Ogawa et al., 1985), and by cultural practices (Agrios, 2005). Prior to the introduction of systemic fungicides, control of brown rot had depended on the use of protectant fungicides such as captan and thiram (Ogawa et al., 1985). From 1980s, the advent of many effective fungicides has resulted in reasonable control efficacies (Bus et al., 1991; Elmer and Gaunt, 1994; Kim et al., 1995). Benzimidazole fungicides were introduced in the 1970s, to control various diseases caused by plant pathogenic fungi. However, a few years after introducing, fungal resistance to benzimidazole fungicides were reported from several studies. In the past two decades, fungal resistance to the fungicides has become an increasingly important problem (Bus et al., 1991; McGrath, 2001). Plant pathogens, such as *Botrytis cinerea, Penicillium digitatium, P. italicum, Venturia inaequalis, Uncinula necator, Coccomyces hiemalis*, and *Didymella bryoniae,* have been reported for their resistance to benzimidazole fungicides throughout the world (Bus et al., 1991; Delp, 1988; Jones and Ehret, 1980; Keinath and Zitter, 1998; Koenraadt et al., 1992; Köller and Wilcox, 2001; McGrath, 2001; Pearson and Taschenberg, 1980).

In Korea, the control of the brown rot depends mainly on the use of fungicides including benzimidazoles, dicarboximides, and ergosterol biosynthesis inhibitors. Among the fungicides, the use of benzimidazoles was limited because the target fungi rapidly developed resistance and the control activity to the pathogen was reduced (Elmer and Gaunt, 1994; Jones and Ehret, 1980; Lim et al., 1998; 1999, Ogawa et al., 1984, Szkolnik and Gilpatrick, 1977; Wild and Eckert, 1982). However, benzimidazoles are still favored by stone fruits farmers in Korea to control brown rot due to their low cost, experience in cultural practices, and pesticide dealer's advice. There have been only a few reports on the resistances of fungal pathogens against benzimidazoles in Korea. Most of these reports were limited to *Botrytis cinerea* that causes gray mold on several plants (Kim et al., 1995). Benzimidazole resistance of *M. fructicola* was first reported in Korea in 1998 (Lim et al., 1998).

The objectives of this study were to 1) investigate sensitivity of *M. fructicola* isolates to benzimidazoles, 2) evaluate resistance levels of various isolates to these fungicides, and 3) determine cross–resistance of benzimidazole resistant isolates to diethofencarb and carbendazim fungicides.

*Corresponding author.
Phone) +82-43-261-2557, FAX) +82-43-271-4414
E-mail) bjcha@cbnu.ac.kr

Materials and Methods

Fungal isolates. Plant parts with brown rot or blight symptom were collected from peach orchards of customary fungicide-application in Chongdo (CD), Gyeongsan (GY), and Youngduk (YO) of Kyeongsangbuk-do and Chochiwon (CH) of Chungcheongnam-do. Samples were collected three times a year from 1998 to 2000: early- (February to March), mid- (July to August), and post- (December) season. Fresh and mummified fruits, stems, and flowers collected from trees were kept individually in polyethylene bags and incubated at 25°C for 5 days and under 95% of relative humidity for sporulation. To obtain monoconidial isolates, conidia on the lesions were collected with a transfer loop and suspended in sterile distilled water. A small drop of conidial suspension was placed on water agar and incubated at 25°C for 8 h. An agar block containing single germinated conidium was cut with a sterile needle under a dissecting microscope (× 20) and transferred to potato dextrose agar (PDA). After incubation at 25°C for 5 days, monoconidial cultures of each isolate were used for fungicide sensitivity tests. Changes in the frequency of benzimidazole-resistant isolates in orchards of no-fungicide application was investigated with isolates collected from the GY area.

Sensitivity test to benzimidazoles. Benomyl [50% wettable powder (WP)], carbendazim (60% WP), and thiophanate-methyl (70% WP) were used in this experiment. Each fungicide was diluted in sterile water and added to PDA at 50 to 60°C to prepare the fungicide-amended medium (Zehr et al., 1991). Sensitivity to benzimidazoles was evaluated by growth of *M. fructicola* isolates on PDA amended with 0, 0.1, 1.0, 10, 100, or 1,000 μg a.i./ml of benomyl, carbendazim, or thiophanate-methyl. A 5-mm-diameter mycelium plug was taken from the actively growing margin of 7-day-old PDA culture and transferred to the test media. The longest and the shortest diameters of the colony were measured after incubation at 25°C for 7 days. Resistance to benzimidazoles was determined by the growth of the isolate at 1.0 μg a.i./ml of fungicide, according to previous studies (Keinath and Zitter, 1998; Lim et al., 1999).

Evaluation of fungal resistance to fungicide. The concentration to decide resistance to benzimidazoles was 1.0 μg a.i./ml based on previous studies (Delp, 1998; Keinath and Zitter, 1998; Köller and Wilcox, 2001; Ogawa et al., 1985). In 1998, 417 isolates were collected during early-growing season.

Cross-resistance between carbendazim and diethofencarb. Cross-resistance of carbendazim-resistant isolates to diethofencarb was tested on PDA amended with 0, 0.1, 1.0, 10, and 100 μg a.i./ml of carbendazim or diethofencarb. Other methods were accorded with sensitivity test to benzimidazoles.

Inoculation test. Several isolates from orchards of extensive benzimidazoles application and exhibiting high EC_{50} value were selected for the inoculation test. The check was an isolate from an orchards of no fungicide application for several years. Peaches (cv. Yoomyung) were surface-disinfested by immersing in 70% ethanol for 10 sec and air-dried for 1 h under shade. Then the peaches were soaked with benzimidazole suspensions of either 10 or 100 μg a.i./ml for 30 min, and inoculated by placing an agar plug of *M. fructicola* on the peace surface. The agar plug was prepared from the margin of a 7-day-old culture of *M. fructicola* isolate as mentioned earlier. Inoculated peaches were kept in covered trays and incubated at 25°C for 7 days, under 95% of relative humidity. The diameter of the decay lesion was measured after 7 days.

All the data were analyzed statistically with PROC GLM of SAS (version 8.1, SAS Inc., USA). Dose-responses of tested fungicides were compared with linear regression.

Results

Sensitivity to benzimidazoles. Among eight isolates of *M. fructicola*, only four (D758, K745, K765, and K7106) of those did not grow on PDA amended with 1.0 μg a.i./ml of each fungicide and were determined to be sensitive isolates. The other four isolates (D795, D726, K740, and K760) were resistant and grew on PDA amended with up to 1,000 μg a.i./ml of each fungicide (Fig. 1). The response of resistant isolates to benomyl was expressed as $y=0.838-0.13x$ and $R^2=0.67$ in linear regression, where y is the relative colony diameter to control (mycelial growth on PDA amended with each concentration of fungicide divided by that of no-fungicide-PDA) and x is $\log_{10}$ of benomyl concentration (μg a.i./ml). The resistant isolates showed good growth at all concentrations of benzimidazoles, but the sensitive isolates did not grow at 1 μg a.i./ml. About 12% of the 417 isolates were resistant to benzimidazoles. Most of the resistant isolates originated from CD and GY (Fig. 2), and showed cross-resistance to other benzimidazoles used in this study (Table 1). Similar responses to fungicides were observed throughout 3 years. In 1998, at the beginning of this study, frequency of resistant isolates was quite low in CH and YO (data not shown). The frequency of carbendazim - resistant isolates in CD and GY was greater than 20% in every study (data not shown). In GY, the subpopulation of benzimidazole-resistant isolates

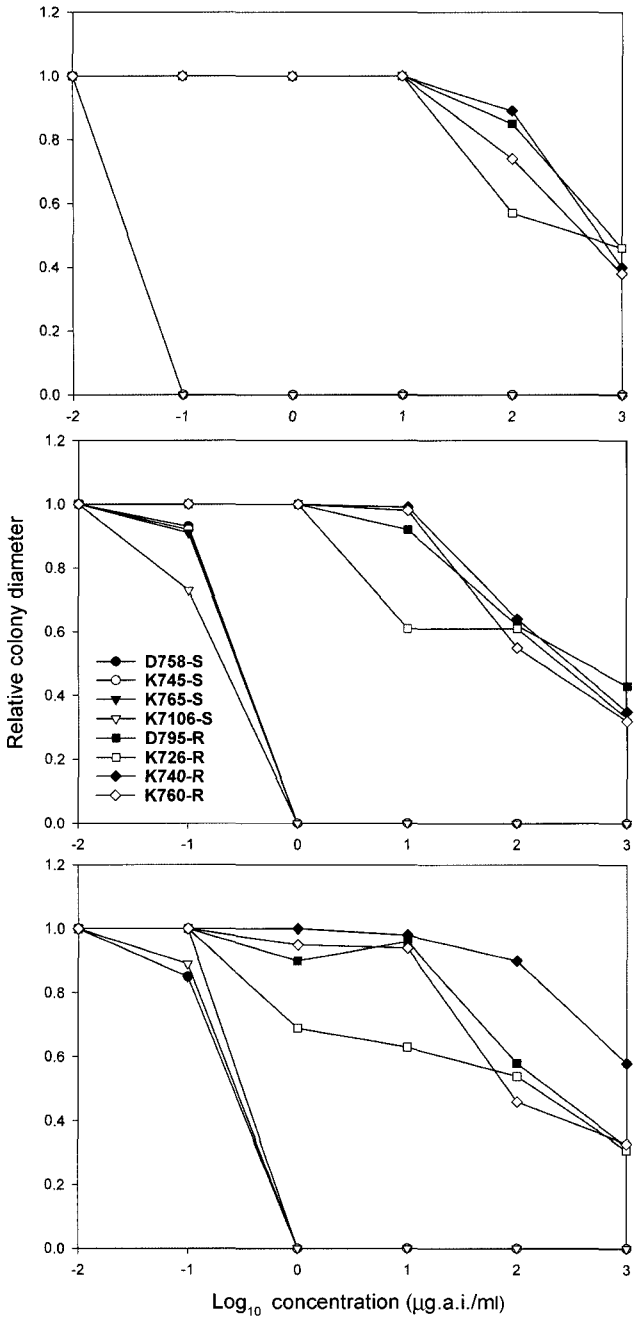

Fig. 1. Relative growth of benzimidazole- sensitive and - resistant isolates of *Monilinia fructicola* on PDA amended with 0.01, 0.1, 1.0, 10, 100, and 1,000 μg a.i./ml of carbendazim, benomyl, and thiophanate-methyl.

occupied 18.8, 35.2, and 29.6% in 1998, 1999, and 2000, respectively. The frequency of resistant isolates in CD recorded 22.0, 25.3, and 26.8% during the same period (Fig. 2).

On the other hand, the rate of carbendazim-resistant isolates ranged from 37.8 to 39.3% in GY throughout this study (data not shown). In CH, the frequency of resistant isolates was very low (Fig. 2). However, in orchards of no fungicide, carbendazim-resistant isolate was never detected during the study.

Resistance level. All the isolates that grew well at 1 μg a.i./ml of carbendazim were separated into six groups with their EC_{50} values at 100 μg a.i./ml concentration intervals. In the early-growing season of each year, EC_{50} values for most isolates ranged from 201 to 300 μg a.i./ml (Fig. 3). However, more than 50% of isolates collected during mid- and late-growing season showed EC_{50} values of greater than 401 μg a.i./ml (Fig. 3). Regardless of sampling time, only a few isolates had EC_{50} values less than 100 μg a.i./ml. The resistance factor (RF = EC_{50} values of resistant isolate divided by the EC_{50} values of sensitive isolate) was over 100 (data not shown).

Cross-resistance between carbendazim and diethofencarb. Most of the carbendazim-resistant isolates collected in this experiment were sensitive to diethofencarb. In mid-season of 1998, one isolate exhibited resistance to both diethofencarb and carbendazim (Table 2). However, this isolate lost its resistance to the fungicides after several times of sub-culturing (data not shown).

Inoculation test. Both 10 and 100 μg a.i./ml of carbendazim provided 98 to 100% control of brown rot caused by the sensitive isolates (D758, K745, K765, and K7106) of *M. fructicola* (Table 3). On the other hand, the same fungicide and concentration provided only 25 to 39% control efficacy to the disease caused by the resistant isolates D795, D726, K740, and K760. Therefore, the fungicide carbendazim is no longer effective for brown rot of peaches in some cases.

Discussion

The isolates of *M. fructicola* collected from Korean peach orchards from 1998 to 2000 could be separated into sensitive and resistant groups at a discriminatory concentration (0.1 μg a.i./ml) of benomyl, carbendazim, or thiophanate-methyl (Fig. 1). In preliminary experiments, it was determined that carbendazim was more inhibitory to *M. fructicola* than benomyl and thiophanate-methyl. Keinath and Zitter (1998) reported that benzimidazoles had different activities against *Didymella bryoniae*. In CH and YO, benzimidazole-resistant isolates (BRI) were isolated only at the beginning of 1998 and the frequency was lower than 4 and 2%, respectively (Fig. 2). On the other hand, in GY and CD, BRI were detected in each year of the study and the frequency was greater than 20% except in 1998 in CD. According to Ogawa et al. (1985), benomyl-resistant

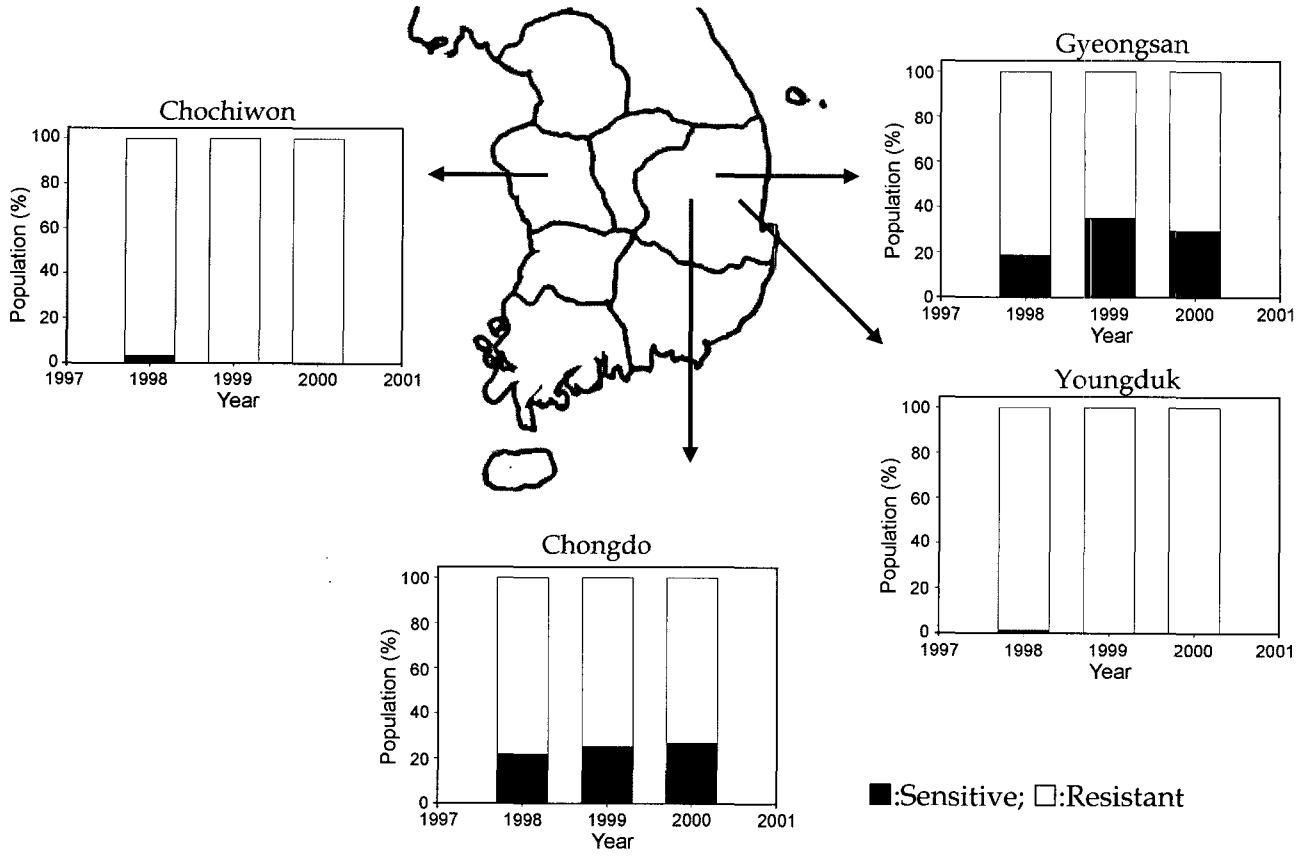

Fig. 2. Incidence of carbendazim-resistant *Monilinia fructicola* isolates from peach orchards in four regions of Korea in 1998. The resistance was tested on PDA amended with 1.0 μg a.i./ml of carbendazim.

Table 1. Cross-resistance of *Monilinia fructicola* to benzimidazoles[a]

Year	Benomyl-resistant isolates (No.)	Benomyl -resistant isolates resistant to benzimidazoles combination (%)		
		Car + Ben	Thi + Ben	Ben + Car + Thi
1998	137	99.3	99.3	99.3
1999	224	100	100	100
2000	83	100	100	100

[a]The resistance was tested with potato dextrose agar amended with 1.0 μg a.i./ml of benomyl (Ben), carbendazim (Car), and thiophanate-methyl (Thi).

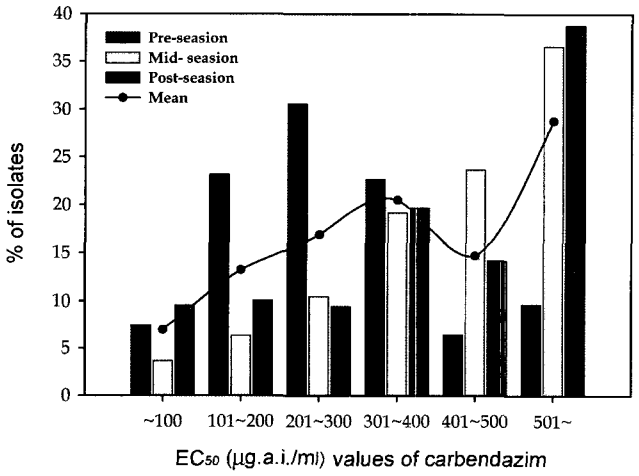

Fig. 3. Resistance levels of carbendazim-resistant *Monilinia fructicola* isolates in orchards with several fungicides application during growing season in 2000. The EC_{50} value of the fungicide was determined on PDA amended with 0, 0.1, 1.0, 10, 100, 1,000 μg a.i./ml of carbendazim. There were significant differences among subpopulations separated by EC_{50} values (F test, $P \leq 0.05$).

isolates of *M. fructicola* could be recovered from the field if the population was over 20%. They also reported that the population of resistant-isolates increased over time by using repeated applications of the same fungicide and combination with non-systemic fungicide such as captan. Based on the results of this research and earlier study (Ogawa et al., 1984), it seems that the use of benzimidazole-fungicides to control brown rot and blossom blight in peach orchards may have to be minimized in GY and CD areas. It has been suggested that fungicide-resistant isolates quickly appear on susceptible hosts in areas with high disease pressure (Keinath and Zitter, 1998). The high population of resistant

Table 2. Cross-resistance of *Monilinia fructicola* to carbendazim and diethofencarb in Korea

Isolation time (year/month)	No. of tested isolates	No. of resistance isolates to	
		Carbendazim (1 μg a.i./ml)	Carbendazim (1 μg a.i./ml) + Diethofencarb (1 μg a.i./ml)
1998/Feb. to Mar.	417	49 (11.8)[a]	0 (0)
1998/Jul. to Aug.	435	65 (14.9)	1 (0.2)
1998/Dec.	179	22 (12.3)	0 (0)
1999/Feb. to Mar.	470	77 (16.4)	0 (0)
1999/Jul. to Aug.	450	80 (17.8)	0 (0)
1999/Dec.	371	67 (18.1)	0 (0)
2000/Feb. to Mar.	387	42 (10.9)	0 (0)
2000/Jul. to Aug.	228	41 (18.0)	0 (0)

[a]Numbers in parentheses represent percentage of resistant isolates to fungicides.

Table 3. EC_{50} values of eight *Monilinia fructicola* isolates and *in vivo* control efficacy of carbendazim on those isolates

Isolates	EC_{50} values (μg a.i./ml)	Control values (%) of carbendazim (μg a.i./ml)[ab]	
		10	100
BS[c]			
D758	0.02	98.9a	100a
K745	0.01	98.0a	100a
K765	0.02	100a	100a
K7106	0.02	99.8a	99.7a
BR[d]			
D795	938.7	24.9b	30.7b
D726	479.6	35.6b	39.4b
K740	841.0	30.6b	34.9b
K760	511.3	33.4b	36.9b

[a]Control values were calculated by the area of decay lesion: 100 (decayed area of no fungicide application – decayed area of fungicide application)/decayed area of no fungicide application.
[b]Means with the same letter in the columns are not significantly different ($P \leq 0.05$).
[c]BS represents benzimidazoles-sensitive isolates.
[d]BR represents benzimidazoles-resistant isolates.

isolates in CD and GY could be associated with the cultivar, harvesting time, planting method, and patterns of fungicide application in these areas (Bus et al., 1991; McGrath, 2001). In fact, for economical reasons, most of peach orchards in GY and CD were for nectarines. Environmental conditions might be favorable for the disease and the pathogen was assured to keep high inoculum density during the growing season in these areas. In addition, repeated applications of the fungicide make the pathogen easy to develop resistance to the fungicides used (Bus et al., 1991; McGrath, 2001). However, in an orchard in GY where no fungicide had been applied, the population of the resistant isolates remained stable during the experiment. These results suggest that it will be difficult to manage populations of the resistant isolates in the field once they reached to above a certain level. Mid-season spray of benzimidazoles at CD and GY is thought to have increased the rate of resistant isolates, and resulted in dispersal of them rather than control *M. fructicola*. Fitness of resistnat isolates is strongly associated with the persistence of them in the field (Lim et al., 1999; McGrath, 2001). There have been several reports that the fitness of BRI was not much different from that of sensitive isolates (Elmer and Gaunt, 1994; Keinath and Zitter, 1998; Lim et al., 1999). Based on these reports, it can be considered that BRI can persist in peach orchards in Korea.

The fungicide diethofencarb is known to control BRIs in the field. Kim et al. (1995) reported multiple resistance of *Botrytis cinerea* to benzimidazoles, diethofencarb, and dicarboximide in Korea. In this study, none of the tested isolates showed any cross-resistance against carbendazim and diethofencarb (Table 2). It is necessary to continue monitoring the incidence of cross-resistant isolates to benzimidazoles and diethofencarb. Population shifts of *M. fructicola* in the field should be monitored, too (Bus et al., 1991; Lim et al., 1999; McGrath, 2001). However, benzimidazole application in CD and GY may generate very dangerous situation because of preferential selection by benzimidazoles-resistant isolates (Köller and Wilcox, 2001). For effective control of diseases caused by *M. fructicola* in these areas, it is necessary to avoid benzimidazole applications and to monitor sensitivity shift of all fungicides applied. Other means to reduce selection pressure by fungicides should be established, too.

According to EC_{50} values (Fig. 3), it was analyzed that the rate of highly resistant isolates increased as the growing season goes on. This result suggests that the resistance level of *M. fructicola* may increase even by single application of the fungicide in CD and GY areas. If the shifts of carbendazim-resistant population are monitored periodically in the field, and the fungicide for brown rot control is chosen carefully, development of carbendazim-resistance could be delayed and minimized.

Acknowledgments

This work was supported by the research grant of the Chungbuk National University in 2005.

References

Agrios, G. N. 2005. Plant Pathology. 5th ed. Elsevier, Inc., New York.

Bus, V. G., Bongers, A. J. and Risse, L. A. 1991. Occurrence of *Penicillium digitatum* and *P. italicum* resistant to benomyl, thiabendazole, and imazail on citrus fruit from different geographic origins. *Plant Dis.* 75:1098-1100.

Delp, C. J. 1988. Fungicide resistance in North America. The American Phytopathological Society, St. Paul, MN.

Elmer, P. G. A. and Gaunt, R. E. 1994. The biological characteristics of dicarboximide-resistant isolates of *Monilinia fructicola* from New Zealand stone-fruit orchards. *Plant Pathol.* 43:130-137.

Jones, A. L. and Ehret, G. R. 1976. Isolation and characterization of benomyl-tolerant strains of *Monilinia fructicola*. *Plant Dis. Rep.* 60:765-769.

Jones, A. L. and Ehret, G. R. 1980. Resistance of *Coccomyces hiemalis* to benzimidazole fungicides. *Plant Dis.* 64:767-769.

Koenraadt, H., Somervill, S. C. and Jones, A. L. 1992. Characterization of mutations in the beta-tubulin gene of benomyl-resistant field strains of *Venturia inaequalis* and other plant pathogenic fungi. *Phytopathology* 82:1348-1354.

Keinath, A. P. and Zitter, T. A. 1998. Resistance to benomyl and thiophanate-methyl in *Didymella bryoniae* from South Carolina and New York. *Plant Dis.* 82:479-484.

Kim, B. S., Lim, T. H., Park. E. W. and Cho, K. Y. 1995. Occurrence of multiple resistant isolates of *Botrytis cinerea* to benzimidazole and *N*-phenylcarbamate fungicide. *Korean J. Plant Pathol.* 11:146-150.

Köller, W. and Wilcox, W. F. 2001. Evidence for the predisposition of fungicide-resistant phenotype of *Venturia inaequalis* to a preferential selection for resistance to other fungicides. *Phytopathology* 91:776-781.

Lim, T. H., Chang, T. H. and Cha, B. J. 1998. Incidence of benzimidazole – and - dicarboximide resistant isolates of *Monilinia fructicola* from overwintering mummies and peduncles on peach trees. *Korean J. Plant Pathol.* 14:367-370.

Lim, T. H., Chang, T. H. and Cha, B. J. 1999. Biological characteristics of benzimidazole-resistant and -sensitive isolates of *Monilinia fructicola* from peach fruits in Korea. *Korean J. Plant Pathol.* 15:340-344.

McGrath, M. T. 2001. Fungicide resistance in cucurbit powdery mildew: experiences and challenge. *Plant Dis.* 85:236-245.

Ogawa, J. M., Manji, B. T., Bostock, R. M., Canez, V. M. and Bose, E. A. 1984. Detection and characterization of benomyl-resistant *Monilinia laxa* on apricots. *Plant Dis.* 68:29-31.

Ogawa, J. M., Manji, B. T. and Sonoda, R. M. 1985. Management of the brown rot disease on stone fruits and almonds in California. In: *Proceedings of brown rot of stone fruit workshop*, pp 8-15. New York State Agricultural Experiment Station.

Pearson, R. C. and Taschenberg, E. F. 1980. Benomyl-resistant strains of *Uncinula necator* on grapes. *Plant Dis.* 64:677-680.

Tate, K. G., Ogawa, M. J., Manji, B. T. and Bose, E. A. 1974. Survey for benomyl tolerant isolates of *Monilinia fructicola* and *Monilinia laxa* in stone fruit orchards of California. *Plant Dis. Rep.* 58:663-665.

Sanamuang, N. and Gaunt, R. E. 1995. Persistent and fitness of carbendazim- and dicarboximide-resistant isolates of *Monilinia fructicola* (Wint.) Honey in flowers, shoots and fruit of stone fruit. *Plant Pathol.* 44:448-457.

Sonada, R. M. and Ogawa, J. M. 1982. Growth rate of *Monilinia fructicola* resistant and sensitive to benomyl on potato dextrose agar and on peach fruit. *Plant Dis.* 66:1155-1156.

Szkolnik, M. and Gilpatrick, J. D. 1977. Tolerance of *Monilinia fructicola* to benomyl in Western New York State orchards. *Plant Dis. Rep.* 60:654-657.

Wild, B. L. and Eckert, J. W. 1982. Synergy between a benzimidazole-sensitive and benzimidazole-resistance isolates of *Penicillum digitatum*. *Phytopathology* 72:1329-1332.

Zehr, E. I., Toler, J. E. and Luszcz, L. A. 1991. Spread and persistence of benomyl-resistant *Monilinia fructicola* in South Carolina peach orchards. *Plant Dis.* 75:590-593.