Chapter

Emergence of Benzimidazole- and Strobilurin-Quinone Outside Inhibitor-Resistant Strains of *Colletotrichum gloeosporioides* sensu lato, the Causal Fungus of Japanese Pear Anthracnose, and Alternative Fungicides to Resistant Strains

Nobuya Tashiro, Youichi Ide, Mayumi Noguchi,
Hisayoshi Watanabe and Mizuho Nita

Abstract

Japanese pear anthracnose (JPA) can cause severe tree defoliation during the growing season. Infected trees become weak and produce fewer flower buds the following spring. This economically serious fungal plant disease has affected cultivated pears in Japan since 1910. Initially, JPA was controlled by benzimidazole fungicides. However, benzimidazole-resistant pathogen strains emerged in the late 1990s, and the range of JPA has expanded in Japan. Since then strobilurin-quinone outside inhibitors (ST-QoIs) such as azoxystrobin and kresoxim-methyl became popular, but ST-QoI-resistant pathogen strains appeared. By 2005, JPA control became difficult once again. In this chapter, we outline the history of JPA fungicide resistance problems, assess advantages and disadvantages of available fungicide options, and develop JPA management strategies based on evidences we obtained from a series of field and lab studies.

Keywords: anthracnose, benzimidazole, deciduous disease, Japanese pear, *Pyrus pyrifolia*, ST-QoI

1. Introduction

A sudden and severe outbreak of Japanese pear anthracnose (JPA) occurred in July 1999 on the Japanese pear cultivars "Housui" and "Niitaka" (*Pyrus pyrifolia* Nakai var. culta Nakai) in Saga prefecture on Kyushu Island, which is one of the major Japanese pear-producing regions located in southwestern Japan [1, 2].

At first, phytotoxicity was suspected owing to extensive and rapid symptom development throughout the orchard. Subsequent investigation revealed that it was JPA caused by *Colletotrichum gloeosporioides* sensu lato (Cgsl) [1, 2].

JPA was first reported in Japan by Kurosawa in 1910 [3]. He observed JPA in Fukuoka prefecture which is adjacent to Saga prefecture in June 1910. The infection caused black spots on the leaves and severe defoliation. Disease incidence and severity differed among varieties. It was severe on "Doitsu," moderate on "Nijusseiki," and mild on "Chojuro." Morphological analyses indicated that the causal organism was *C. gloeosporioides*. Kurosawa stated that Bordeaux mixture could be an effective treatment and damaged leaves should be incinerated to prevent the spread of the disease. Based on Kurosawa's research, Hara introduced JPA in his textbook entitled *Fruit Tree Disease Theory* [4]. Ikata reported that JPA was uncommon and caused no severe damage except for an outbreak in Fukuoka prefecture in 1910 [5]. There were no further reports on JPA until 1974.

In 1974, severe JPA infestations on the "Yakumo" cultivar were reported in Fukushima prefecture of the Tohoku region, which is located in northeastern Japan. The outbreak caused severe defoliation. Ochiai et al. monitored the progress of the outbreak and isolated the causal organism [6]. Ochiai and Hayashi discussed the pathogenicity of isolated *Colletotrichum* sp. and indicated that disease severity differed among host cultivars [7]. They also mentioned the effect of the infection timing (the number of days elapsed after leaf expansion) [8], temperature and leaf wetness on infection and disease incidence [9], and growth medium and temperature on pathogen growth [10]. However, there were no published reports on pathogen control methods.

In 1987 and 1998, severe incidences of JPA were reported in Kochi prefecture in Shikoku Island, located in southwestern Japan. Morita et al. reported the symptoms and transition of the outbreak. They documented the efficacy of thiophanate-methyl/maneb wettable powder (WP), maneb WP, and benomyl WP at controlling this disease [11]. There was also a report of an outbreak of moderately benzimidazole-resistant strains in 1998 [12].

Probably because JPA happened sporadically over a long time period and in small and isolated geographical areas, there was a very limited effort to identify fungicides that are effective against JPA. Therefore, no registered fungicides were available for JPA when the major JPA outbreak occurred in Saga prefecture in 1999.

2. JPA symptoms and causal organism

2.1 Symptoms

In JPA-affected orchards in Saga prefecture, Japanese pear cultivars "Housui" and "Niitaka" developed minute black spots formed on the leaf laminae and petioles starting in mid-June. The leaves appear as though they have been stabbed with a fine needle. The perforations are visible when the leaves are held up to the sunlight. Since the lesions are very small, it is difficult for the grower to notice the initial disease symptoms unless the leaves are inspected very closely. The initially tiny black dots then expand into small curved black spots 0.5–1 mm in diameter. Certain lesions may develop into large blackish-brown spots ∼2 cm in diameter. By that time, the leaves rapidly turn yellow and abscise (**Figure 1**).

When the JPA outbreaks were observed in 1999, JPA caused a severe defoliation by mid-July and markedly reduced tree vigor. The intense defoliation caused new leaves to emerge soon after the event; however, these new leaves were quickly and fatally infested with JPA. In addition, defoliation triggered flowering in autumn

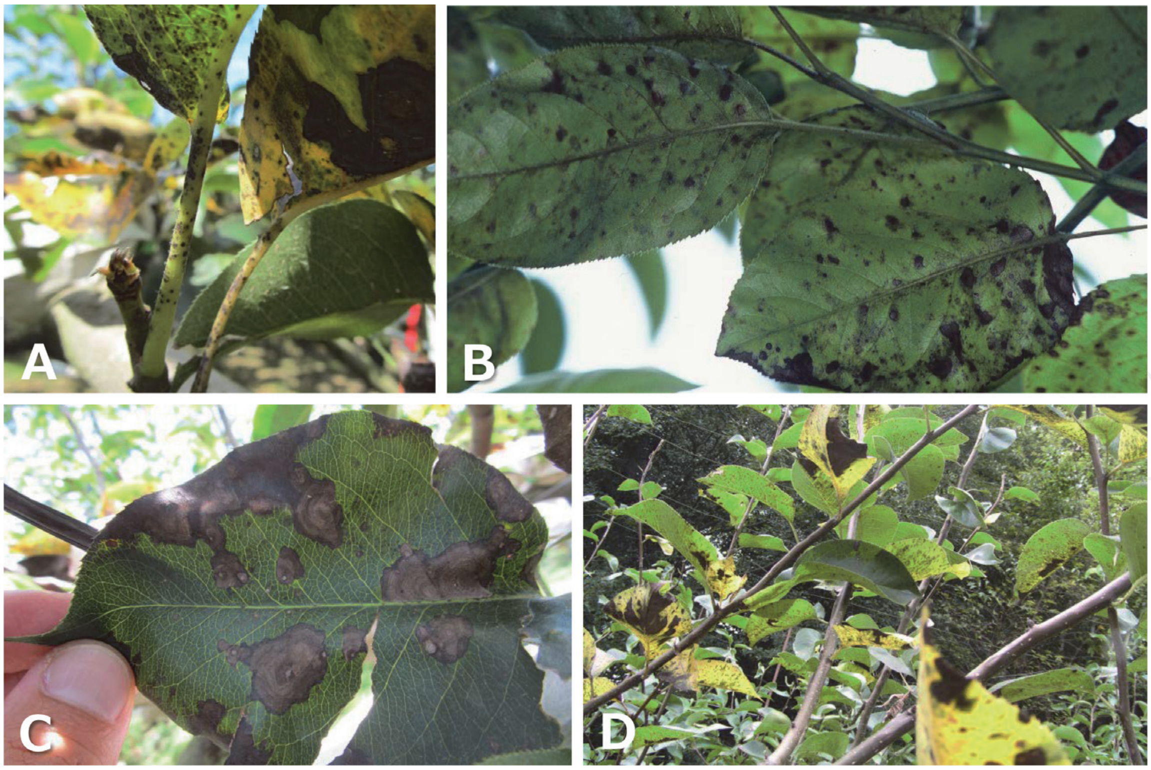

Figure 1.
Black spots on anthracnose-infected petioles and leaves caused by Colletotrichum gloeosporioides *sensu lato observed in Japanese pear cv. Housui (*Pyrus pyrifolia *Nakai var.* culta *Nakai) in the field. (A) Very minute black spots symptoms on the petioles, (B) leaf symptoms showing numerous black spots of various sizes, (C) large blackish brown spots with a diameter of about 1–2 cm, (D) yellowing leaves with large blackish brown spots.*

which leads to a fewer number of flowers in the next spring, which caused serious yield loss in the following year (**Figure 2**).

2.2 Causal organism

Fungal cultures were isolated from the large dark brown lesions on leaves and smaller lesions on petioles of the 'Housui' and "Niitaka" Japanese pear cultivars. Morphologically, these isolates were identical. The isolates formed light salmon flesh-colored conidial masses on spore-inducing media (K_2HPO_4 1 g, $MgSO_4$ 0.5 g, peptone 5 g, lactose 10 g, agar 30 g, and distilled water 1000 mL) (**Figure 3**). Foliar spray inoculation of a conidial suspension (105 mL^{-1}) on 'Housui' reproduced disease symptoms similar to those observed in the orchards (**Figure 3**). The inoculated fungi were re-isolated to confirm Koch's postulates [1, 2].

The conidia are cylindrical with an average size of 15.8 μm × 5.0 μm (**Figure 3**). The mycelia from these isolates grow at 10–35°C with an optimum at 28°C. PCR using primer CgInt [13] to detect Cgsl disclosed a band at ~450 bp similar to that obtained by using Cgsl as a control.

Based on its morphological characteristics, a similar foliar disease observed on "Kousui" in Akita prefecture in the Tohoku region of Japan was thought to be anthracnose caused by *Colletotrichum acutatum* sensu lato [14]. However, DNA-based identification failed to establish *C. acutatum* sensu lato as the cause of JPA in the pear orchards of other regions of Japan [12, 15]. Therefore, most of the JPA pathogens in Japan are probably caused by Cgsl.

In China, *C. fructicola* was reported as an anthracnose pathogen causing leaf black spot in sandy pear (*Pyrus pyrifolia* Nakai) in 2015 [16]. In 2019, 12 species of *Colletotrichum* spp. including *C. fructicola* and *C. gloeosporioides* were reported as

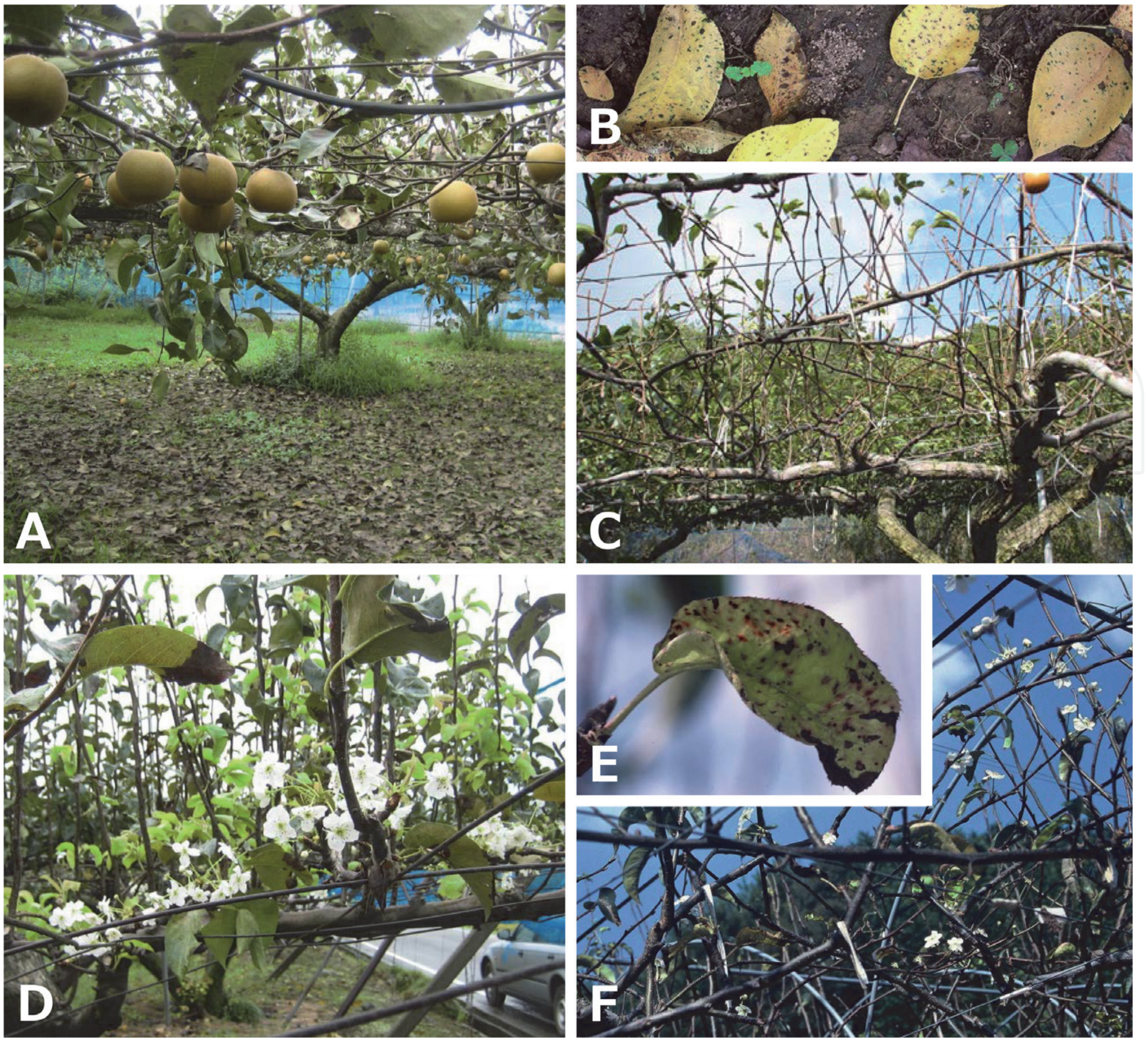

Figure 2.
Defoliation in summer and flowering in autumn caused by Colletotrichum gloeosporioides *sensu lato observed in Japanese pear cv. Housui (*Pyrus pyrifolia *Nakai var.* culta *Nakai) in the fields. (A) Severe defoliation in the field of Japanese pear cv. Housui (*Pyrus pyrifolia *Nakai var.* culta *Nakai) (courtesy M. Suzuki); (B) yellowish discolored fallen leaves with a lot of black spots; (C) twigs with no leaves in summer; (D) emerging of new leaves and flowers soon after intense defoliation, the number of flowers in the next spring was dramatically decreased to cause great yield loss in the following year (courtesy M. Suzuki); (E) black spots symptoms on newly emerged leaves after defoliation, these new leaves quickly withered; (F) defoliation of almost all of the leaves in the summer, leading to the reduced vigor of the trees.*

pathogens causing anthracnose on pear leaves and fruit [17]. In Japan, we did not confirm anthracnose symptoms on Japanese pear fruit caused by Cgsl. A report of JPA outbreak from Akita prefecture, where they suspected *C. acutatum* sensu lato to be the causal agent, did not include anthracnose on fruits. However, *C. fioriniae* destroyed "Niitaka" fruit in Oita prefecture in 2013 [18]. In Korea, two species of *C. gloeosporioides* sensu lato [19] and *C. acutatum* sensu lato [20] were reported as the causal organisms of Asian pear fruit rot.

The *Compendium of Apple and Pear Diseases and Pests* describes apple and pear bitter rot as a common disease and mentions that apple anthracnose causes speckle spots followed by defoliation [21]. In 1988, Leite et al. [22] described a new apple leaf spot disease on the Gala and Golden Delicious cultivars in Brazil and demonstrated that it was caused by *G. cingulata* which is the sexual stage of *C. gloeosporioides*. This disease was named *Glomerella* leaf spot (GLS). This report was the first to cite any *Colletotrichum* sp. as the causative agent of leaf spot in the apple orchard. Under favorable conditions, a GLS infestation may result in 75% defoliation by harvest time. It can weaken trees and reducing yield [23, 24]. GLS was first reported in the United States in 1998 as a severe leaf spot on Gala apples [25].

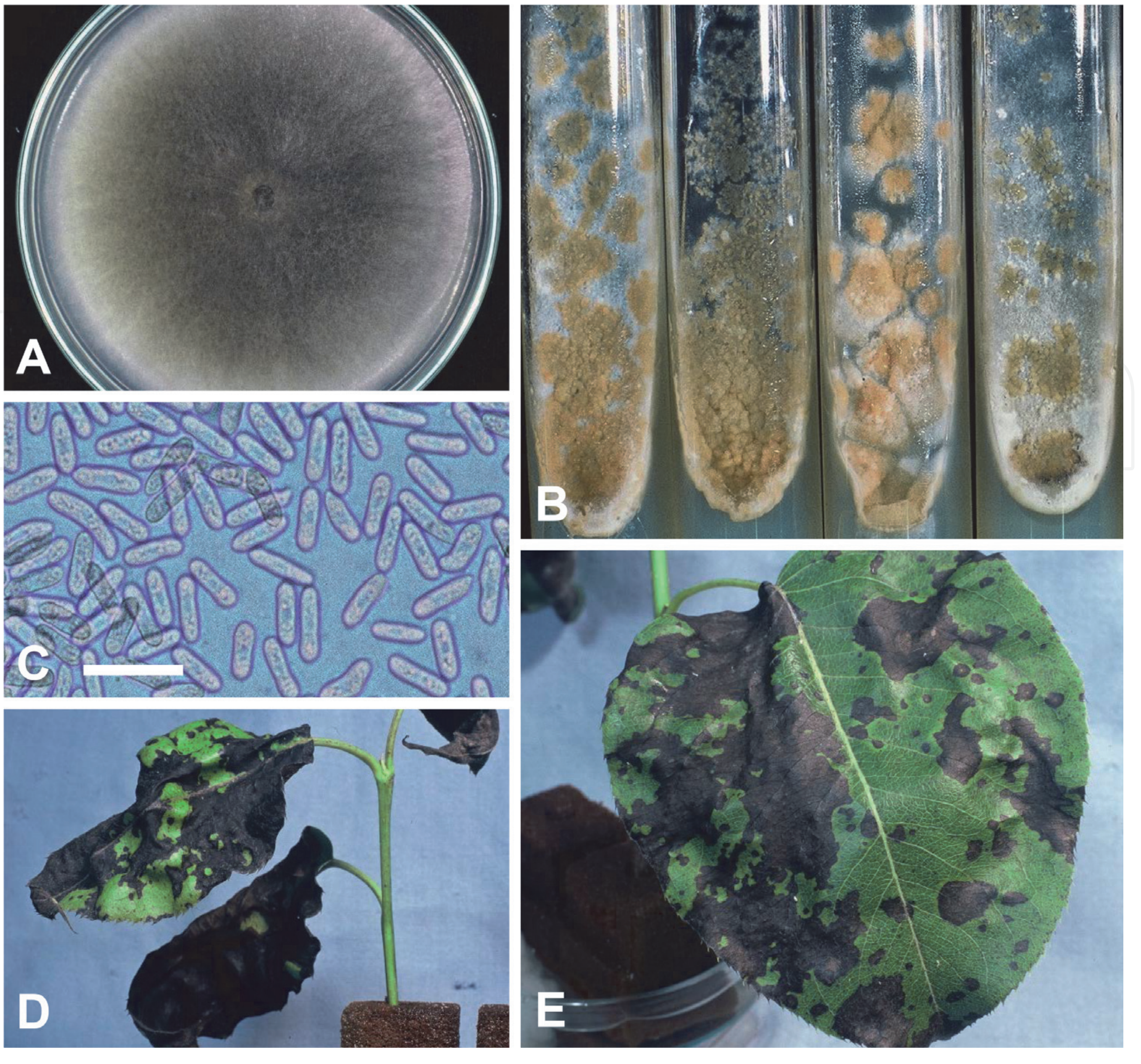

Figure 3.
*Causal pathogen of Japanese pear (*Pyrus pyrifolia *Nakai var.* culta *Nakai) anthracnose and representative symptoms induced by inoculation of conidial suspension under unwounded condition. (A) A 7-day-old colony of strain C-17 grown on potato dextrose agar medium at 25°C, (B) salmon pink spore mass produced on spore-inducing medium (K_2HPO_4 1 g, $MgSO_4$ 0.5 g, peptone 5 g, lactose 10 g, agar 30 g, distilled water 1000 mL), (C) conidia of strain C-17 produced on spore-inducing medium (scale bar = 20 μm), (D) shoots of Japanese pear cv. Housui that showed severe symptoms by inoculation of conidial suspensions of strain C-15 under unwounded condition, (E) black spot lesions of different sizes reproduced by unwounded inoculation of spore suspension.*

Colletotrichum karstii has been reported as a new GLS pathogen [26]. Apple GLS caused by *Glomerella cingulata* was reported in China in 2012 [27].

On the other hand, the compendium makes no reference to foliar anthracnose in pear or Asian pear [21], which occurs on leaves and causes severe defoliation. Since this disease may be unique to Asian and Japanese pear, further investigations of its causal pathogens using molecular diagnostic tools are required.

3. Development of fungicide control technology for JPA (until 2004)

Our aim was to select efficacious fungicides at the Fruit Tree Experiment Station in Saga prefecture [1, 28]. The JPA fungicide spray timing was the same as that for Japanese pear ring rot caused by *Botryosphaeria berengeriana* De Notaris f. sp. piricola (Nose) in Koganezawa and Sakuma. Thus, these diseases had to be addressed simultaneously, and fungicide efficacy on ring rot was also evaluated [2].

3.1 Selection of effective fungicides

3.1.1 Benzimidazoles, benomyl, and thiophanate-methyl

Benzimidazole fungicides, which inhibit β-tubulin assembly during mitosis, were introduced ca. 1970. This group includes thiophanate-methyl, carbendazim, and benomyl. Benomyl (methyl [1-(butylcarbamoyl)benzimidazole-2-yl]carbamate) was registered under the brand name Benlate (50% wettable powder) by DuPont in Japan in 1971. Sumitomo Chemical Co., Ltd. (Tokyo, Japan) acquired the business in 2002. Thiophanate-methyl, dimethyl 4,4′-(o-phenylene) bis(3-thioallophanate), was registered in Japan in 1971 under the brand name Topsin-M (70% wettable powder; Nippon Soda Co., Ltd., Tokyo, Japan).

Initially, benomyl and thiophanate-methyl were considered as broad-spectrum fungicides with low phytotoxicity, and these materials controlled the diseases caused by *Ascomycetes*, *Deuteromycetes*, and *Basidiomycetes*. Thus, benzimidazoles were frequently used on a wide range of crop groups. However, the pathogens rapidly developed field resistance; then, the usage of these fungicides decreased over time. They are still widely used on certain crops as they are broad-spectrum antifungal agents. In Japan, they are often applied to fruit trees.

Benomyl WP and thiophanate-methyl WP have been used since 1975 to prevent Asian pear scab (APS) caused by *Venturia nashicola*. These benzimidazoles were initially highly efficacious [29]. Therefore, their usage increased in frequency. APS fungus resistant to benzimidazoles were first detected in 1980 [30–33]; then the efficacy of benzimidazoles at suppressing APS diminished. In 1985, a demethylation inhibitor (DMI) with significant efficacy against the APS pathogen was introduced [34–37]; then, the use of benzimidazoles against APS was discontinued.

Benzimidazoles are very effective at suppressing ring rot [38] and powdery mildew [39] caused by *Phyllactinia mali* (Duby) U. Braun. Instead of targeting scab disease from April to June, growers applied benzimidazoles three to four times from mid-June until harvest to prevent ring rot and powdery mildew. This time window is also the main JPA infection period. Since benzimidazoles were effective against anthracnose caused by *C. gloeosporioides* sensu lato [11, 40–43], these materials were used often to prevent JPA.

3.1.2 Fungicide screening against the JPA pathogen

We conducted preventive application screening using "Housui" leaves and using fungicides registered for Japanese pears in Japan. Fungicide suspensions were diluted to predetermine concentrations and sprayed onto the leaves on branches excised from the "Housui" tree. The leaves were air-dried and sprayed with a Cgsl spore suspension ($\sim$105 mL^{-1}). The inoculated leaves were maintained in humid conditions at 25°C for 2 days. The lesions on the leaves were counted 7 days after inoculation.

Propyneb WP, dithianon FL, fluazinam FL, organic copper FL, azoxystrobin FL, kresoxim-methyl DF, captan WP, and mancozeb WP had excellent preventive efficacies (**Table 1**). In contrast, the benzimidazoles, benomyl, and thiophanate-methyl which were previously considered effective against anthracnose caused by Cgsl [11] were significantly less efficacious against both strains than the best treatment (**Table 1**), indicating the presence of benzimidazole-resistant strains.

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Generic name	**Trade name in Japan**	**FRAC code**	**Active ingredient (%)**	**Rate applied (mg L^{-1})[1]**	**Control (%)[2]**	
					Strain C-17	**Strain C-25**
Benomyl	Benlate WP	1	50.0	250	0	90.2
Thiophanate-methyl	Topsin-M WP	1	70.0	700	6.8	93.6
Fluazinam	Frowncide SC	29	39.5	198	100	100
Dithianon	Delan FL	M9	42.0	420	98.9	99.1
Propineb	Antracol WG	M3	70.0	1400	100	100
Kresoxim-methyl	Storoby DF	11	50.0	250	100	98.6
Azoxystrobin	Amistar 10 FL	11	10.0	100	99.6	99.8
Oxyquinoline copper	Quinondo FL	—	35.0	350	98.5	96.8
Captan	Orthocide WP 80	M4	80.0	1000	93.8	94.3
Captan/oxyquinoline copper	Oxyrane WP	M4/—	20.0/30.0	400/600	74.1	70.1
Captan/benomyl	Caplate WP	M4/1	60.0/10.0	1000/167	86.8	91.6
Iminoctadine tris(albesilate)	Bellkute WP	M7	40.0	400	53.1	46.4
Mancozeb	Zimandithane WP	M3	80.0	2000	95.1	96.1
Hexaconazole	Anvil FL	3	2.0	20	33.8	28.6
Difenoconazole	Score WG	3	10.0	25	36.3	40.0
Fosetyl	Aliette WP	P7	80.0	1000	7.4	8.1
Mepanipyrim	Frupica FL	9	40.0	200	0	0

[1]*Standards on the use of pesticide in agricultural chemical regulation law of Japan.*
[2]*Control (%) = (1 – average lesion number per leaf with fungicide application/average lesion number per control leaf) × 100.*

Table 1.
Preventive effect of various fungicides for anthracnose on Japanese pear.

3.1.3 Confirming the lack of susceptibility to benzimidazoles among the JPA pathogen strains

Based on the results of the previous study (**Table 1**), we investigated the susceptibility of 122 Cgsl strains to benomyl. The strains were isolated from infected leaves collected in 1999 from nine orchards known to have frequent outbreaks of this disease. Before the experiment, the pathogenicity of these strains was confirmed by inoculation tests. The strains were divided into those with minimum inhibitory concentration (MIC) ≤ 0.39 mg L^{-1} and those with MIC ≥ 1600 mg L^{-1} (**Table 2**). The former were deemed susceptible. The latter were considered highly resistant and were prevalent at all nine orchards investigated (**Table 2**). These highly resistant strains were also highly resistant to thiophanate-methyl, which are very similar to benomyl in the mode of action (**Table 3**). When "Housui" leaves were sprayed with benomyl (250 mg L^{-1}) and then inoculated with the highly

Source orchard	Variety	Number of strains	Number of strains for each MIC (mg L^{-1}) range		
			0.78	25–100	>1600
Minamihata-1	Housui	13	0	0	13
Minamihata-2	Housui	15	4	0	11
Minamihata-3	Housui	14	0	0	14
Minamihata-4	Niitaka	13	0	0	13
Okawa-1	Housui	10	0	0	10
Okawa-2	Housui	10	1	0	9
Okawa-3	Housui	12	0	0	12
Okawa-4	Housui	22	4	0	18
Okawa-5	Niitaka	13	3	0	10
Total		122	12 (9.8)[1]	0 (0.0)	110 (90.2)

[1]*Values in parentheses are the percentage of the total for each category.*

Table 2.
Benomyl sensitivity of C. gloeosporioides *sensu lato, the causal organism of anthracnose in Japanese pear varieties "Housui" and "Niitaka" at Imari district in Saga Prefecture in 1999.*

Strain	Location of isolation[1]	Year of isolation	EC_{50} (mg L^{-1}) values of	
			Benomyl	Thiophanate-methyl
SCG-25	Minamihata town	1999	0.151	0.151
SCG-30	Ohkawa town	1999	0.166	0.206
SCG-64	Ohkawa town	1999	0.155	0.186
SCG-08	Minamihata town	1999	485	2856
SCG-17	Minamihata town	1999	481	2386
SCG-72	Ohkawa town	1999	491	3211

[1]*Minamihata town and Ohkawa town are both in the Imari area of Saga prefecture.*

Table 3.
Effect of benomyl and thiophanate-methyl on the mycelial growth of benzimidazole-sensitive (SCG-25, SCG-30, and SCG-64) strains and highly benzimidazole-resistant (SCG-08, SCG-17, and SCG-72) strains of C. gloeosporioides obtained from lesions of Japanese pear anthracnose.

Strain	Benomyl (250 mg L^{-1}) sprayed trees		Control trees		Control (%)[2]
	Tested leaves	Lesions/leaf	Tested leaves	Lesions/leaf	
SCG-25	27	14.6[a]	26	126.8[a]	88.5[a]
SCG-30	28	6.3[a]	24	98.6[a]	93.6[a]
SCG-17	26	152.5[b]	21	142.3[a]	0[b]
SCG-72	22	96.5[b]	24	106.8[a]	9.6[b]

[1]*The Japanese pear variety "Housui" (2-year-old trees) was sprayed with wettable powder of benomyl and thoroughly dried. Conidial suspensions (approx. 10^5 mL^{-1}) of each strain (benzimidazole-sensitive strains, SCG-25 and SCG-30; highly benzimidazole-resistant strains, SCG-17, SCG-72) were then inoculated. Seven days after inoculation, the development of symptoms was assessed. Values followed by different letter differ significantly in a multiple comparison based on the Tukey–Kramer HSD test ($P < 0.05$).*
[2]*Control (%) = (1 – average lesion number per leaf on the trees with benomyl application/average lesion number per leaf on the control trees) × 100.*

Table 4.
Control efficacy of benomyl against benzimidazole-sensitive (SCG-25 and SCG-30) strains and highly benzimidazole-resistant (SCG-17 and SCG-72) strains of C. gloeosporioides *sensu lato on the leaves of the Japanese pear variety "Housui".*[1]

resistant Cgsl strains, the treated leaves became severely diseased, i.e., benomyl did not suppress JPA (**Table 4**).

Benzimidazole-resistant Cgsl that occurred at a high frequency over a wide range in the Japanese pear-growing areas of Saga prefecture caused benzimidazoles to be no longer effective against JPA. In addition, benzimidazole-resistant Cgsl was also confirmed in Chiba, Oita, and Kochi prefectures. Only highly resistant strains were observed in Chiba prefecture [44], a mixture of highly and moderately resistant strains was detected in Oita prefecture [45], and only moderately resistant strains were confirmed for Kochi prefecture [12].

3.2 Change in detection frequency of highly benzimidazole-resistant strains after discontinuing benzimidazoles

To determine the changes in detection frequency of benzimidazole-resistant strains, Cgsl strains from orchards where benzimidazoles were discontinued were challenged with benomyl in 1999, 2000, 2001, and 2004. The discontinuation of benzimidazole fungicides in each orchard was confirmed from fungicide spray records. The frequency of benzimidazole-resistant strain ranged from 81 to 88% during the study, and there was no indication of a reduction over time (**Figure 4**). Therefore, reintroduction of benzimidazoles to the pear-producing areas of this region was not recommended.

The proportion of benzimidazole-resistant Cgsl strains causing JPA did not decrease even 4 years after discontinuation. Pathogen populations in abscised leaves may be carried over to the following year, and pathogen latently infected with twigs may remain viable for several years [46]. Also, both the resistant and sensitive strains may have similar levels of competitiveness or fitness.

Impacts on the detection frequency of benzimidazole-resistant isolates after the discontinuation were highly variable for other crops and pathogens. The discontinuation of benzimidazole immediately reduced the ratios of highly resistant *Botrytis cinerea* strains causing grape gray mold [47] and *Gloeosporium theae-sinensis* causing tea anthracnose [48]. The ratio of highly resistant *Venturia nashicola* strains causing Japanese pear scab was immediately reduced upon benzimidazole discontinuation; however, the overall ratio of resistant strains did not decline as moderately and weakly resistant strains emerged [49]. As with JPA, the frequency of highly

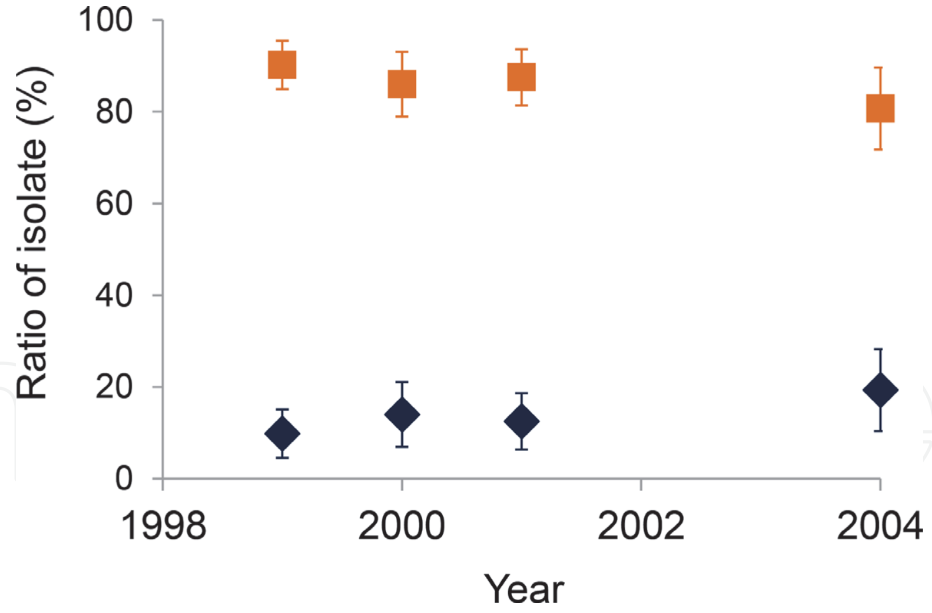

Figure 4.
Change in detection frequency of highly benzimidazole-resistant strains after discontinuing benzimidazoles.

resistant strains did not change for *V. nashicola* [50–52] and *V. inaequalis* which cause pear and apple scab, respectively [53].

3.3 Residual efficacy and rainfastness of fungicides effective against benzimidazole-resistant strains of *Colletotrichum gloeosporioides* sensu lato

3.3.1 Residual efficacy of the sprayed fungicides

To ensure effective pathogen control, it is important to know the length of time fungicidal efficacy persists after product application. Experiments were conducted to determine the period of residual fungicidal activity against JPA. Each fungicide was sprayed onto "Housui" trees in Japanese pear orchards where JPA had never been previously detected. Branches with their leaves intact were excised and brought to the laboratory. A conidial suspension (~105 mL^{-1}) was sprayed onto the leaves. Relative product efficacy was scored based on the number of foliar lesions. Duration of efficacy after product application was also evaluated. Two experiments, where each had different sets of treatments, were conducted in late July and mid-September 2002. In each treatment, 100 leaves from new branches were examined.

For the late July experiment, a mean % disease control (=% suppression of the mean disease incidence relative to the mean disease incidence of the positive control) of >70% was taken as the threshold of satisfactory disease control. The disease control sustainability was measured as days post-application. Dithianon FL and azoxystrobin FL continued to suppress disease onset for 14 days after application (**Table 5**). Satisfactory disease control was observed for fluazinam FL, kresoxim-methyl DF, and captan/benomyl WP until 7 days after application. However, at 14 days after the application, the disease control effect (%) dropped to 69 and 68% for fluazinam FL and captan/benomyl WP, respectively, and 15% for kresoxim-methyl DF. Thus, these fungicides, especially kresoxim-methyl DF, had comparatively shorter disease control durations.

In the mid-September experiment, dithianon FL presented with satisfactory disease control efficacy until 14 days after application as in the previous experiment (**Table 6**). The efficacies of the other fungicides were inferior to that of dithianon FL, and none of the treatment achieved the mean % disease control of >70%. Propineb WG showed no disease control efficacy whatsoever.

Generic name	Trade name in Japan	FRAC code	Active ingredient (%)	Rate applied (mg L^{-1})	Changes of control (%)[1]		
					7 days after	14 days after	21 days after
Azoxystrobin	Amistar 10 FL	11	10.0	100	84	78	45
Kresoxim-methyl	Storobi DF	11	50.0	250	75	15	20
Dithianon	Delan FL	M9	42.0	420	83	90	50
Fluazinam	Frowncide SC	29	39.5	198	77	69	39
Captan/benomyl	Caplate WP	M4/1	60.0/10.0	1000/167	98	68	46

[1]*Control (%) = (1 − mean ratio of diseased leaves of trees with fungicide application/mean ratio of diseased leaves of trees without fungicide application) × 100.*

Table 5.
Residence period of sprayed fungicides against anthracnose on the Japanese pear "Housui" (1).

Generic name	Trade name in Japan	FRAC code	Active ingredient (%)	Rate applied (mg L^{-1})	Changes of control (%)[1]		
					7 days after	14 days after	21 days after
Dithianon	Delan FL	M9	42.0	420	92	83	41
Fluazinam	Frowncide SC	29	39.5	198	59	0	0
Captan/oxyquinoline copper	Oxyrane WP	M4/—	20.0/30.0	400/600	67	57	0
Copper (II) sulfate	IC Bordeaux 48Q	M1	31.2	10,400	69	70	0
Propineb	Antracol WG	M3	70.0	1400	0	0	—

[1]*See* ***Table 5****.*

Table 6.
Residence period of sprayed fungicides against anthracnose on the Japanese pear "Housui" (2).

3.3.2 Rainfastness of the sprayed fungicides

The JPA pathogen propagates and infects during rainfall. The amount of rain determines the degree of attenuation of the fungicide spray on the pear leaves. Thus, the establishment of the rainfastness of various fungicides helps develop an efficient and successful disease control program.

Several fungicide treatments were tested on pot-grown "Housui" trees in 2002. One day after fungicide application, a rainfall treatment of 17 mm h^{-1} and 50 mm d^{-1} was conducted using an artificial rainfall machine (DIK-6000; Daiki Rika Kogyo Co., Ltd., Tokyo, Japan). The leaves were excised from each tree and inoculated with a pathogen conidial suspension (2 × 105 conidia mL^{-1} and 4.0 mL $leaf^{-1}$) before treatment application, at 100, 200, 300, and 400 mm cumulative rain. The efficacy of the fungicide was visually assessed to estimate % disease control.

The level of JPA suppression was high when the leaves received no rainfall, resulting in 100% disease control (=no disease development). As expected, disease control efficacy decreased with increasing cumulative rainfall. For azoxystrobin FL and dithianon FL, the disease control was ≥70% at 200 mm cumulative rainfall after fungicide application (**Figure 5**). Fluazinam FL and kresoxim-methyl DF achieved ≥70% disease control at 100 mm cumulative rainfall, but the disease control efficacy fell to <70% at 200 mm cumulative rainfall. For captan/oxyquinoline-copper WP and captan/benomyl WP, the mean disease control efficacy was 90% and >60% at 100 mm cumulative rainfall but sharply declined to 0 and 23%, respectively, at 200 mm cumulative rainfall (**Figure 5**).

3.3.3 Preventive efficacy of fungicide treatments against JPA in Japanese pear orchards

In the "Housui" orchard, an experiment was conducted over three seasons to determine the efficacy of preventive fungicide application against JPA. Two experiments were conducted in late June 2000. Trees were sprayed at 10- to 14-day intervals. When the cumulative rainfall after the previous application was

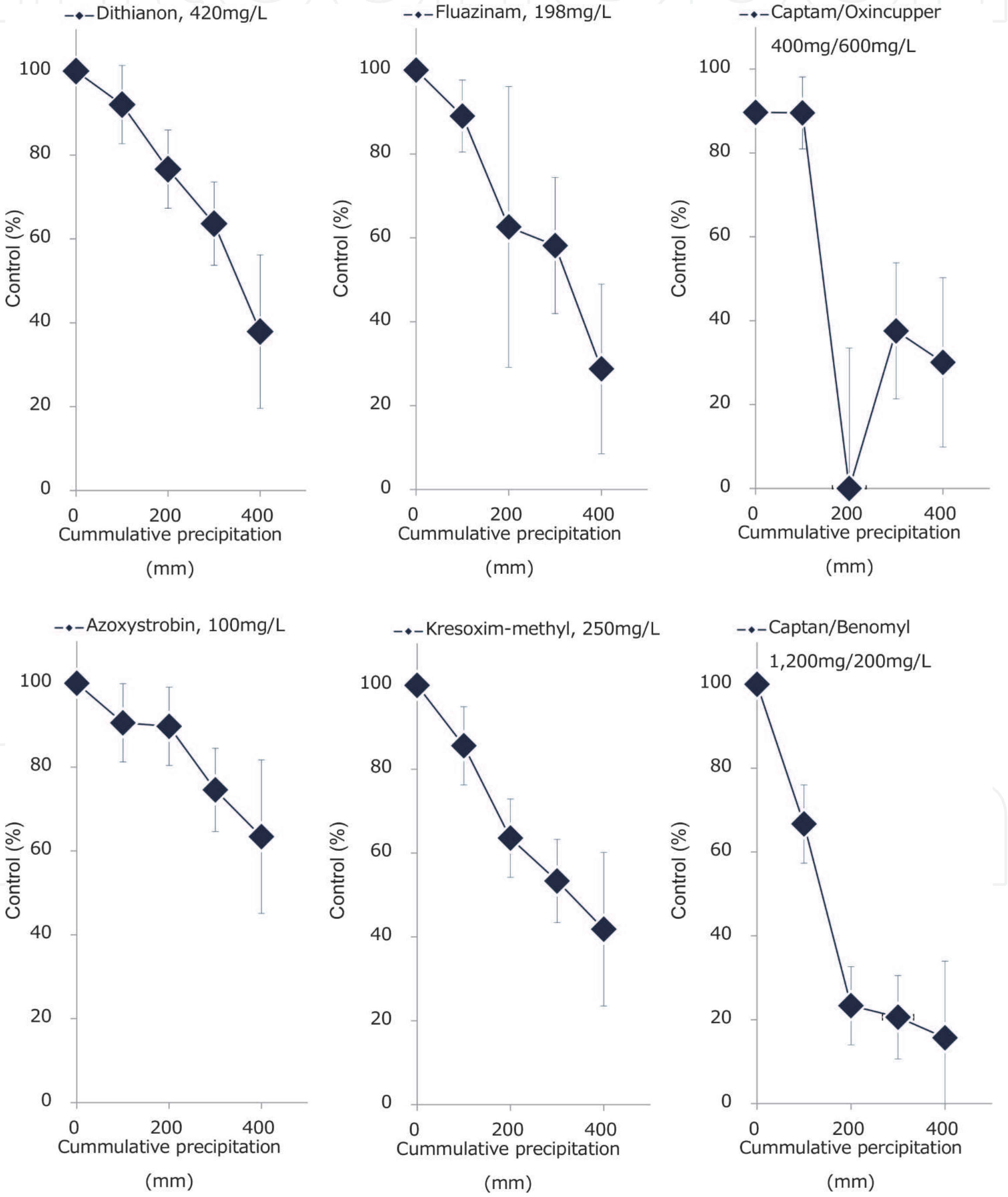

Figure 5.
Reduction of the control effect of various fungicides on Japanese pear anthracnose associated with artificial rainfall after spraying; error bar, 95% confidence interval.

>200 mm, the trees were immediately resprayed to compensate for the product washed off by the rain. Experiments were conducted in mid-June 2001 and mid-May 2002 using a slightly modified spray guideline. The treatments were applied either 20 days after the previous treatment or when the post-application cumulative rainfall was 200 mm. Several heavy rain events increased the cumulative rainfall to >200 mm, but all fungicide treatments were applied before the cumulative rainfall reached 300 mm.

As with the previous experiments, 70% control was set as the efficacy threshold. For all 3 years, preventive azoxystrobin FL and dithianon FL application provided >70% disease control (**Table 7**). Both treatments resulted in consistently high disease control efficacy as they did in the residual efficacy and rainfastness tests (**Tables 5** and **6**).

Kresoxim-methyl DF demonstrated >80% disease control efficacy in one of the residual activity experimental runs in 2000 and 2002, but the results were not consistent among three trials (**Table 7**). In the other trials, the mean % disease control of kresoxim-methyl DF varied from 15 to 75% in the residual efficacy test (**Table 5**), and the mean % disease control efficacy dropped very sharply to below 70% in the rainfastness test at 200 mm cumulative rain fall (**Figure 5**). Thus, the environmental conditions, especially the amount of precipitations, may negatively impact kresoxim-methyl DF to be effective.

Fluazinam showed good levels of disease control (75%) in this experiment (**Table 7**), but it did not perform well with the residual tests (**Tables 5** and **6**), and the mean % disease control dropped at 200 mm cumulative rainfall (**Figure 5**). A trend with propineb was similar where 80% mean disease control was observed in this experiment, but it did not provide any level of control in the residual efficacy test (**Table 6**). We need to investigate more to determine what created these differences.

The lack of disease prevention efficacy for benomyl WP was expected as benzimidazole-resistant strains were detected in this orchard (**Table 7**). The disease prevention efficacy of captan/benomyl WP was ~70% in all 3 years, possibly because of benzimidazole-resistant strains and low rainfastness of captan, which is also shown in the rainfastness test (**Figure 5**). Thus, captan probably needs to be applied with a non-benzimidazole material, and if sprayed with captan alone, it should be applied using a 100 mm cumulative rainfall threshold.

Fungicide application on a 10- to 14-day schedule from the first cover until harvest is the main disease control method that growers use. JPA is very difficult to control after the leaves have been infected with it. Dithianon, fluazinam, strobilurin-quinone outside inhibitor (ST-QoI) fungicides, and captan/benomyl WP provide good disease control when they are applied preventively.

3.3.4 Use of fungicides effective to JPA against ring rot of Japanese pear

We evaluated fungicide efficacy against Japanese pear ring rot because spray application timing was the same as that for JPA [2]. The ST-QoIs azoxystrobin and kresoxim-methyl were highly efficacious against ring rot (**Table 8**). Captan/benomyl also showed high efficacy. In contrast, the efficacy of dithianon against ring rot was highly variable (from 100 to 0% control) during the years it was tested. Fluazinam provided unsatisfactory disease control efficacy against ring rot.

Generic name	Trade name in Japan	FRAC code	Active ingredient (%)	Rate applied (mg L^{-1})	Control (%)[2]			
					In 2000		In 2001	In 2002
					Experiment 1	Experiment 2		
Benomyl	Benlate WP	1	50.0	250	3	—	—	—
Fluazinam	Frowncide SC	29	39.5	198	75	—	85	
Dithianon	Delan FL	M9	42.0	420	82	99	93	95
Propineb	Antracol WG	M3	70.0	1400	80	—	—	—
Kresoxim-methyl	Storobi DF	11	50.0	250	61	79	69	87
Azoxystrobin	Amistar 10 FL	11	10.0	100	81	87	91	88
Oxyquinoline copper	Quinondo FL	—	35.0	350	26		—	—
Captan/benomyl	Caplate WP	M4/1	60.0/10.0	1000/167	65	—	72	67
Hexaconazole	Anvil FL	3	2.0	20	29	—	—	—
			Ratio of diseased leaves in control		67.0%	70.5%	38.3%	63.0%

[1]*Sprays have been done in May to August each year.*
[2]*See* ***Table 5****.*

Table 7.
Control effect of several fungicides against anthracnose on the Japanese pear "Housui".[1]

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Generic name	Trade name in Japan	FRAC code	Active ingredient (%)	Rate applied (mg L^{-1})	Control (%)[2]		
					In 2000	In 2001	In 2002
Benomyl	Benlate WP	1	50.0	250	32	—	—
Fluazinam	Frowncide SC	29	39.5	198	30	37	—
Dithianon	Delan FL	M9	42.0	420	19	0	100
Kresoxim-methyl	Storobi DF	11	50.0	250	39	70	54
Azoxystrobin	Amistar 10 FL	11	10.0	100	47	73	100
Oxyquinoline copper	Quinondo FL	—	35.0	350	0	—	—
Captan/benomyl	Caplate WP	M4/1	60.0/10.0	1000/167	17	69	78
			Ratio of diseased leaves in control		45.0%	40.6%	10.9%

[1]*Sprays have been done in May to August each year.*
[2]*See* ***Table 5****.*

Table 8.
Control effect of several fungicides against ring rot on the Japanese pear "Housui".[1]

3.4 Temporary suspension of the 1999 JPA outbreak

Before the 1999 JPA outbreak, the main disease to control in Japanese pear cultivation was Asian pear scab (APS). Sterol demethylation inhibitor (DMI), belonging to sterol biosynthesis inhibitors (SBIs), was the product most frequently used to control this disease. Iminoctadine tris(albesilate), captan/oxyquinoline copper, and captan were applied for APS a few times. Benzimidazoles were applied three to four times to control ring rot and powdery mildew. However, by 2000, benzimidazoles were no longer recommended in Japanese pear production due to its resistance issue. In their place, local systemic fungicides such as strobilurins (azoxystrobin and kresoxim-methyl) and protective fungicides such as dithianon and fluazinam were applied.

Dithianon FL, fluazinam FL, ST-QoI fungicides (azoxystrobin FL, kresoxim-methyl DF), captan/oxyquinoline copper WP, and captan/benomyl WP were effective against JPA, APS, and ring spot, and all except dithianon were efficacious against powdery mildew. Therefore, these materials were incorporated into the spray calendar with heavy reliance on DMIs, which were popular at that time. As a result, JPA incidence was drastically reduced.

Although Dithianon FL has high JPA control efficacy, it has a 60-day pre-harvest interval (PHI) in Japan. Thus, it cannot be used after mid-June which is a critical JPA control period. The PHI of fluazinam SC was 30 days, so it could be applied until mid-July. Captan/oxyquinoline copper WP has a very short PHI of only 3 days. On the other hand, it leaves visible residues on the fruit and may not be sprayed too soon before harvest.

In contrast, the ST-QoIs (azoxystrobin FL, kresoxim-methyl DF, and pyraclostrobin with boscalid WP in a pre-mix) showed excellent anti-JPA efficacy [28, 44, 54]. These fungicides have a 1-day PHI and can, therefore, be applied up until the day before harvest. Moreover, they leave no visible residues on the fruit. Consequently, the application frequency of ST-QoIs against JPA increased.

4. Emergence of strobilurin (ST)-QoI fungicide-resistant strains and new treatment recommendations after 2011

4.1 ST-QoIs

ST-QoIs or strobilurins were first used in the 1990s and became one of the most important fungicides of the past 25 years. They inhibit ubiquinol oxidation at the quinone outside (Qo) binding site on the cytochrome bc1 complex in the inner mitochondrial membranes of fungi [55]. At the time of introduction, ST-QoIs showed very high efficacy against many different pathogen-crop combinations; however, ST-QoI fungicides are highly prone to inducing resistance in target pathogens that can lead to reduced field efficacy. The ST-QoI resistance risk has been rated high by the Fungicide Resistance Action Committee (FRAC) [56]. ST-QoI-resistant strains have been detected in ~60 fungal and oomycete pathogen species worldwide including powdery and downy mildews, gray mold, *Alternaria* disease, scab, and anthracnose [57]. Currently, disease control strategies that are overly reliant on ST-QoIs are considered undesirable [57]. A major source of ST-QoI resistance is a point mutation in the cytochrome b gene that substitutes alanine for glycine at amino acid position 143. This site may be associated with the pathogen binding affinity of the fungicide [58].

In Japan, ST-QoI resistance has emerged in cucumber powdery mildew (*Podosphaera xanthii*), downy mildew (*Pseudoperonospora cubensis*) [59, 60], eggplant leaf mold (*Mycovellosiella nattrassii*) [61], *Corynespora* cucumber leaf spot (*Corynespora cassicola*) [62], citrus gray mold (*Botrytis cinerea*) [63], European pear black spot (*Alternaria alternata*) [64], *Alternaria* apple blotch (*Alternaria alternata* apple pathotype) [65], grapevine leaf blight (*Pseudocercospora vitis*) [66], strawberry anthracnose (*Colletotrichum gloeosporioides*) [67, 68], tea gray blight (*Pestalotiopsis longiseta*) [69], apple bitter rot (*Colletotrichum gloeosporioides*) [70], rice blast (*Magnaporthe oryzae*) [71], mango anthracnose (*Colletotrichum gloeosporioides*) [72], apple scab (*Venturia inaequalis*) [73], grapevine downy mildew (*Plasmopara viticola*) [74], cucurbits gummy stem blight (*Didymella bryoniae*) [75], chrysanthemum white rust (*Puccinia horiana*) [76], wheat powdery mildew (*Erysiphe* (Blumeria) *graminis f.sp. tritici*), and strawberry powdery mildew (*Sphaerotheca aphanis* var. aphanis) [77].

4.2 Emergence of strains of *Colletotrichum gloeosporioides* sensu lato resistant to ST-QoIs

Over nearly a decade in the Saga and Oita prefectures, ST-QoIs were sprayed three to four times annually between June and early August as countermeasures against JPA and APS. That is, many growers heavily depended on ST-QoIs, especially late in the season because ST-QoIs are phytotoxic to Japanese pear leaves at their early growth stage. In addition, ST-QoIs were also highly efficacious against APS [78, 79].

The alternative material, thiuram FL, has a 30-day PHI; therefore, it cannot be used after mid-July. The other options, such as iminoctadine tris(albesilate)/captan WP, have a relatively shorter PHI (14 days), and captan WP has a 3-day PHI. Captan/oxyquinoline copper WP (3-day PHI), captan WP (3-day PHI), and iminoctadine tris(albesilate)/captan WP (14-day PHI) showed adequate efficacy against JPA [28, 44, 54, 80]. However, the ST-QoIs were preferred over these choices by growers as they were more effective than these; in addition, the common component of these materials, captan, tends to cause stains on the fruit.

As JPA became very prevalent in 2010–2011 in the Oita and Saga prefectures where above-mentioned spraying system. We assessed ST-QoI sensitivity in Cgsl isolates by placing mycelial discs on potato dextrose agar (PDA) containing 100 μg mL^{-1} azoxystrobin and 1000 μg mL^{-1} salicylhydroxamic acid (SHAM). Mycelial elongation was measured 4 days post-inoculation [81]. Isolates from Saga [80] and Oita [45] prefecture grew on the PDA containing azoxystrobin (**Table 9**, **Figure 6**).

To determine the effect of ST-QoI pretreatment on JPA development, conidial suspensions were sprayed on “Housui” leaves previously exposed to azoxystrobin FL. The appearance of JPA lesions caused by the sensitive strain was nearly zero

Source orchard	Number of tested strains	Number of resistant strains[1]
Imari district in Saga prefecture	61	20 (32.8%)[2]
Hita district in Oita prefecture	254	49 (16.2%)

[1]*Number of strains that grew on PDA with 1000 μg mL^{-1} SHAM and 100 μg mL^{-1} azoxystrobin cultured 4 days at 25°C.*
[2]*Values in parentheses are the percentage of the resistant strains.*

Table 9.
Azoxystrobin sensitivity of C. gloeosporioides *sensu lato, the causal organism of anthracnose in Japanese pear varieties “Housui” and “Niitaka” at Imari district in Saga prefecture and Hita district in Oita prefecture both Kyushyu island in 2011.*

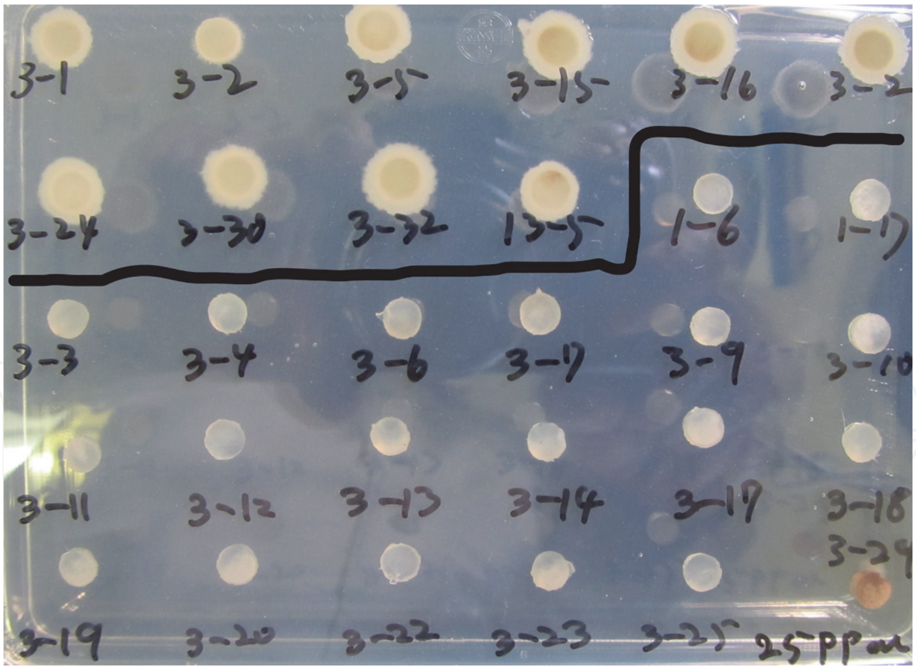

Figure 6.
Effect of azoxystrobin on the mycelial growth of azoxystrobin-resistant and azoxystrobin-sensitive strains of Colletotrichum gloeosporioides *sensu lato, the causal fungus of Japanese pear anthracnose, 4 days after inoculation of mycelial disks (4 mm) at 25°C; medium, potato dextrose agar medium with 25 μg mL^{-1} azoxystrobin and 1000 μg mL^{-1} SHAM; strains above the line, azoxystrobin-resistant strains; strains under the line, azoxystrobin-sensitive strains.*

(99.6% control). In contrast, the two resistant strains induced many lesions, and there was a very low rate of disease control (**Table 10**).

4.3 Effective spraying program in the presence of benzimidazole- and ST-QoI-resistant strains

4.3.1 Use of the adjuvant to reduce the risk of phytotoxicity caused by captan

Products containing captan provide a sufficient level of disease control, but they blemish the fruit to reduce its quality. We investigated the application of spreaders such as Makupika (polyoxyethylene methylpolysiloxane 93.0%; Ishihara Bio-Science Co., Ltd., Tokyo, Japan) and Santokuten 80 (polyoxyethylene dodecyl ether 80.0%; Sumitomo Chemical Co., Ltd., Tokyo, Japan). We also tested the adjuvant

Strain[2]	Azoxystrobin (100 mg L^{-1}) sprayed trees		Control trees		Control (%)[3]
	Tested leaves	Lesions/leaf	Tested leaves	Lesions/leaf	
1–7	5	0.2	5	56.8	99.6
3–1	5	24.8	5	26.8	7.6
3–2	4	5.6	4	16.3	65.5

[1]*The Japanese pear variety "Housui" (2-year-old trees) were sprayed with wettable powder of azoxystrobin and thoroughly dried. Conidial suspensions (approx. 10^5 mL^{-1}) of each strain (azoxystrobin-sensitive strains, 1–7; azoxystrobin-resistant strains, 3–1, 3–2) were then inoculated. Seven days after inoculation, the development of symptoms was assessed.*
[2]*All strains was isolataed at Hita city of Oita prefecture in 2011.*
[3]*Control (%) = (1 – average lesion number per leaf on the trees with azoxystrobin application/average lesion number per leaf on the control trees) × 100.*

Table 10.
Control efficacy of azoxystorobin against azoxystrobin-sensitive (1–7) strains and azoxystrobin-resistant (3–1 and 3–2) strains of C. gloeosporioides *sensu lato on the leaves of the Japanese pear variety "Housui".*[1]

Figure 7.
Fungicide application by air-blast sprayer in the Japanese pear orchard.

squash (sorbitan fatty acid ester 70.0% and polyoxyethylene resin acid ester 5.5%; Maruwa Biochemical Co., Ltd., Tokyo, Japan). These agents render the spray spots inconspicuous by lowering droplet surface tension. All the three agents reduced the visibility of the captan residues on the plant surfaces. There is a concern that the addition of the spreader can decrease the amount of fungicide that attached to the host plant [82, 83]. However, the mixture had nearly the same efficacy levels as captan alone in the field trial [80].

4.3.2 Current recommendation against JPA

By 2014, pear producers had fully recognized the presence of benzimidazole- and ST-QoI-resistant pathogen strains and stopped relying on ST-QoI to manage JPA. The current recommended JPA management protocol for Japanese pear is dithianon FL in early June; thiuram FL, captan/oxyquinoline copper, and iminoctadine tris(albesilate)/captan WP from mid-June to early July; and captan WP with a spreader several times after mid-July. The occurrence of JPA has abated as growers are now comparatively less dependent on ST-QoI fungicides [80].

We also advocate proper spray coverage. For example, we recommend every-row spray over alternate-row spray with an air-blast sprayer (**Figure 7**), because of better fungicide coverage achieved by the former. It has been shown in one of our studies that JPA is more effectively controlled when fungicides are sprayed onto all rows [84]. Moreover, infected and abscised leaves should be promptly removed from orchards to reduce the inoculum pool [85].

5. Potential options for JPA management in the future

Our test results of 1999 and the data obtained at the experiment stations in other prefectures promoted the registration of additional fungicides to control this disease. In 2019, 11 products were registered for use against JPA in Japan (**Table 11**). This step provides a wider selection of fungicides to control or manage this disease.

5.1 Benzylcarbamate (BC)-QoI and pyribencarb

Pyribencarb (methyl{2-chloro-5-[(1E)-1-(6-methyl-2-pyridylmethoxyimino) ethyl]benzyl} carbamate) was formulated by Kumiai Chemical Industry Co., Ltd. and Ihara Chemical Industry Co., Ltd. in Japan. It is a novel benzylcarbamate-type QoI fungicide (BC-QoI) and is active against a wide range of fungal plant pathogens [86]. Pyribencarb is both preventive and curative [87], and its chemical structure

Generic name	Trade name in Japan	FRAC code	Active ingredient (%)	LPHI[2,4]	MNAPS[3,4]	Rate applied (mg L^{-1})[4]	Resistered year in Japan	References
Dithianon	Delan FL	M9	42.0	60	4	420	2003	[28, 44, 54, 80]
Kresoxim-methyl	Storoby DF	11	50.0	1	3	250	2003	[28, 54]
Azoxystrobin	Amistar 10 FL	11	10.0	1	5	100	2006	[28, 54]
Thiuram	Thionoc FL	M3	40.0	30	5	800	2008	[44, 80]
Thiuram	Trenox FL	M3	40.0	30	5	800	2008	[44, 80]
Pyraclostrobin/boscalid	Naria WDG	11/7	6.8/13.6	1	3	34/68	2008	[44]
Captan/oxyquinoline copper	Oxyrane WP	M4/M1	20.0/30.0	3	9	400/600	2009	[44, 54, 80]
Captan	Orthocide WP 80	M4	80.0	3	9	1000	2011	[44, 80]
Iminoctadine tris(albesilate)/captan	Dyepower WP	M7/M4	20.0/45.0	14	5	200/450	2012	[44]
Pyribencarb	Fantasista WDG	11	40.0	1	3	133.3	2013	[44]
Captan/penthiopyrad	Fruitguard WDG	M4/7	70.0/7.5	3	3	700/75	2019	—

[1] *2019 confirmed on September 1, 2019.*
[2] *Legal pre-harvest interval.*
[3] *The maximum number of application per season.*
[4] *Standards on the use of pesticide in agricultural chemical regulation law of Japan.*

Table 11.
Registered fungicides for Japanese pear anthracnose in Japan.[1]

resembles that of ST-QoIs such as kresoxim-methyl and azoxystrobin. However, it has a substitution of the carbonyl moiety on the benzene ring [88]. The binding site of pyribencarb on cytochrome b may be slightly different from that of the ST-QoIs [89].

Pyribencarb more effectively controlled ST-QoI-resistant gray mold isolates than other ST-QoI fungicides [90]. It also had relatively higher efficacy against ST-QoI-resistant *Pestalotiopsis longiseta* which causes tea gray blight [69]. Pyribencarb shows differential cross-resistance patterns to ST-QoI [89].

Since pyribencarb has an excellent effect on JPA [44], it has been recommended to use it in orchards where ST-QoI-resistant strains are present or ST-QoI effects are reduced. However, there have been no reports of the effects of pyribencarb in an orchard where ST-QoI-resistant strains exist. Moreover, the risk of fungal pathogen resistance development of pyribencarb is high [91]. Therefore, it is necessary to take careful approaches to prevent the similar mistake we made with ST-QoIs. The number of pyribencarb application must be limited, and the application should be mixed with another broad-spectrum protective fungicide with a different mode of action.

Pyribencarb may be used less than three times per season on Japanese pear (**Table 11**). The Japan Fungicide Resistance Action Committee (Japan FRAC) guidelines recommend that QoIs be used up to twice annually on Japanese pear [92]. But we believe that it should be used only once between mid-June and early July which is the most critical disease control period of JPA and JPS for proper fungicide resistance management. In addition, pyribencarb must always be co-applied with the protective (multisite) fungicide such as captan, thiuram, iminoctadine tris (albesilate), and iminoctadine tris(albesilate)/captan to reduce the resistant risk. This treatment protocol may enhance disease control efficacy, lower pathogen density, and delay resistant strain development. In the future, comparative field trials would help validate the efficacy of the current treatment recommendations.

5.2 Benzodioxoles and fludioxonil

Fludioxonil is a benzodioxole that affects the signal transduction in the target fungal pathogen. These agents are also known as phenylpyrroles or PP-fungicides. According to the FRAC, the risk of pathogen resistance to this chemical class is low to medium [91]. Fludioxonil had extremely strong efficacy against JPA [93]. As of 2019, however, it has not yet been registered for use on Japanese pear in Japan. Data from field trials are being compiled for fludioxonil registration, and it is hoped that products containing fludioxonil will soon be available so that they may be integrated into our JPA management strategies.

6. Conclusions

Highly efficacious fungicides tend to be used the most. At the same time, the risks of fungicide-resistant fungal pathogen strains against the heavily used fungicide increase with the usage in the field. Fungicides that are prone to inducing pathogen resistance must be used properly by targeting the correct pathogens, applying the agents only at the appropriate times during the season, reducing application frequency, and mixing with other fungicides that are at low risk of inducing pathogen resistance. A mathematical model-based study suggested that the efficacy of high-risk fungicides may be substantially extended if they are mixed with low-risk fungicides [94]. This hypothesis should be validated by field trials, which are costly, time-consuming, and labor-intensive. On the other hand, these field-based data are invaluable in the development of effective measures against fungicide-resistant plant pathogens.

We conceptualized a series of efforts to develop the best plant disease control practice at agricultural sites as an evidence-based control (EBC) [95–103]. The management of plant diseases needs to be developed based on the accumulated evidences, but not anecdotal observations. To gather useful evidence, the data need to be collected from the combination of field, controlled environment, and lab experiments, and then these data must be statistically validated to come up with repeatable and reliable information.

In this chapter, we demonstrated the use of EBC using the development of JPA management strategies against recent outbreaks as an example. JPA outbreak in 1999 and a detection of benzimidazole-resistant Cgsl strains [1, 28] triggered us to investigate alternatives such as fungicides ST-QoI, dithianon, and fluazinam, which were registered for use on Japanese pear [1, 2, 28, 54]. We also established the residual efficacy and rainfastness of these alternative fungicides [54]. We also obtained the evidence of long-term retention of benzimidazole-resistant strains in the field. Based on these results, an effective fungicide spray program without the use of benzimidazoles was established, and JPA was effectively controlled 2 years after the outbreak [1, 28].

However, JPA became conspicuous in 2006 and 2007 in two geographically distant regions, Kyushu (southeast) and Kanto (central). Outbreaks were reported in Oita prefecture in the Kyushu region in 2006 [45] and in Chiba and Kanagawa prefectures in the Kanto region in 2007 [44, 104]. Also a resurgence of JPA was reported around 2011 in Saga prefecture where the 1999 outbreak occurred [80]. Excessive dependence on ST-QoI fungicides induced ST-QoI-resistant Cgsl strains in Oita and Saga prefecture, which contributed to these new outbreaks [45, 80]. In Chiba and Kanagawa prefecture, the occurrence of QoI-resistant strains has not been investigated, but we suspect that the situation is very similar to Oita and Saga prefectures.

In order to increase the number of options to be used in late-season JPA management, we tested the efficacy of adjuvants to reducing visible chemical residues on fruits. Information from these experiments enabled us to determine appropriate and effective combinations of fungicides against JPA without relying on either the benzimidazole or ST-QoI. We intend to keep conducting similar holistic evidence-based approaches to develop effective management strategies for other pathosystems.

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Author details

Nobuya Tashiro[1]*, Youichi Ide[2], Mayumi Noguchi[3], Hisayoshi Watanabe[4] and Mizuho Nita[5]

1 Saga Prefectural Upland Farming Research and Extension Center, Karatsu, Saga, Japan

2 Saga Prefectural Agricultural Research Center, Saga, Japan

3 Saga Prefectural Nishimatsuura Agricultural Extension Center, Imari, Saga, Japan

4 Oita Prefectural Agriculture, Forestry and Fisheries Research Center, Usa, Oita, Japan

5 Alson H. Smith Jr. Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, Winchester, VA, USA

*Address all correspondence to: tashirongreen12@gmail.com

References

[1] Tashiro N, Ide Y, Etho T. Occurrence of benzimidazole tolerant isolate of *Colletotrichum gloeosporioides*, a causal fungus of anthracnose of Japanese pear and its effective fungicides. Kyushu Agricultural Research. 2001;**63**:81 (in Japanese)

[2] Tashiro N, Ide Y. Occurrence of anthracnose of Japanese pear caused by *Colletotrichum gloeosporioides*, and its effective fungicides. Plant Protection. 2003;**57**:111-115 (in Japanese)

[3] Kurosawa R. Anthracnose of Japanese pear (short communication). Syokubutugaku-Zasshi. 1912;**26**:359-360 (in Japanese)

[4] Hara K. Anthracnose on Japanese pear. In: Kazyubyougairon. Nippon Kankitsukai, Tokyo. 1916. pp. 107-110 (in Japanese)

[5] Ikata S. Anthracnose on Leaves in Japanese Pear. Tokyo: Zikkennkazyubyougaihen. Yokendo; 1927. pp. 54-55 (in Japanese)

[6] Ochiai M, Inomata M, Hayashi S. Study on Japanese pear leaf anthracnose like disease (1). Progress of outbreak and isolation of causal organism. Annual Report of the Society of Plant Protection of North Japan. 1976;**27**:81 (in Japanese)

[7] Ochiai M, Hayashi S. Study on Japanese pear leaf anthracnose like disease (2). Pathogenicity of isolated *Colletotrichum* sp. Annual Report of the Society of Plant Protection of North Japan. 1977;**28**:73 (in Japanese)

[8] Ochiai M, Hayashi S. Study on Japanese pear leaf anthracnose like disease (3). Influence of the elapsed days after expand of leaf and the back and forth of the leaves at the inoculation on the disease development. Annual Report of the Society of Plant Protection of North Japan. 1977;**28**:74 (in Japanese)

[9] Ochiai M, Hayashi S. Study on Japanese pear leaf anthracnose like disease (4). Effects of temperature and leaf wetness period on infection and disease incidence. Annual Report of the Society of Plant Protection of North Japan. 1977;**28**:75 (in Japanese)

[10] Ochiai M, Hayashi S. Study on Japanese pear leaf anthracnose like disease (5). Effects of medium and temperature on the growth of pathogen. Annual Report of the Society of Plant Protection of North Japan. 1978;**29**:58 (in Japanese)

[11] Morita Y, Yano K, Matsumoto K, Kotani S, Kurata M. Occurrence and control of anthracnose on leaves of Japanese pear. Bulletin of the Kochi Agricultural Research Center. 1994;**3**: 1-10 (in Japanese)

[12] Yano K, Ishii H, Fukaya M, Kawada Y, Sato T. Anthracnose on Japanese pear caused by intermediately benzimidazole-resistant strains of *Colletotrichum gloeosporioides* (*Glomerella cingulata*). Japanese Journal of Phytopathology. 2004;**70**:314-319 (in Japanese with English summary)

[13] Adaskaveg JE, Hartin RJ. Characterization of *Colletotrichum acutatum* isolates causing anthracnose of almond and peach in California. Phytopathology. 1997;**87**:979-989

[14] Fukaya M. First report of Japanese pear anthracnose disease caused by *Colletotrichum acutatum* and its chemical control. Japanese Journal of Phytopathology. 2004;**70**:184-189 (in Japanese with English summary)

[15] Chung WH, Ishii H, Nishimura K, Fukaya M, Yano K, Kajitani Y. Fungicide sensitivity and phylogenetic relationship of anthracnose fungi isolated from various fruit crops in Japan. Plant Disease. 2006;**90**:506-512

[16] Zhang PF, Zhai LF, Zhang XK, Huang XZ, Hong N, Xu WX, et al. Characterization of *Colletotrichum fructicola*, a new causal agent of leaf black spot disease of sandy pear (*Pyrus pyrifolia*). European Journal of Plant Pathology. 2015;**143**:651-662

[17] Fu M, Crous PW, Bai Q, Zhang PF, Xiang J, Guo YS, et al. *Colletotrichum* species associated with anthracnose of *Pyrus* spp. in China. Persoonia. 2019;**42**: 1-35

[18] Oita Prefecture. Anthracnose of Japanese pear caused by *Colletotrichum fioriniae* (Marcelino & R.G. Shivas & Y.P. Tan). In: Temporary Pest Outbreak Forecast Information. Vol. 3. 2013. pp. 1-2 (in Japanese)

[19] Cho WD, Shin HD. List of Plant Disease in Korea (edition 4). Seoul, Korea Republic: Korean Society of Plant Pathology; 2004. 348 p

[20] Kim WG, Hong SK, Park YS. Occurrence of anthracnose on fruits of Asian pear tree caused by *Colletotrichum acutatum*. Mycrobiology. 2007;**35**:238-240

[21] Sutton TB, Aldwinckle HS, Agnello AM, Walgenbach JF, editors. Compendium of Apple and Pear Diseases and Pests. 2nd ed. St. Paul, MN: APS Publications; 2014

[22] Leite RP Jr, Tsuneta M, Kishino AY. Ocorrência de mancha foliar de Glomerella em macieira no Estado do Paraná. Londrina; Fundação Instituto Agronômico do Paraná. Informe de Pesquisa. 1988;**81**:6 (in Portuguese)

[23] Cerezine PC, Leite RP, Tsuneta M. Efeito de tratamentos químicos no controle da mancha foliar de Glomerella en macieira. No Estado de Paraná. Fitopatologia Brasileira. 1992;**17**:258-267 (in Portuguese)

[24] Sutton TB, Sanhueza RM. Necrotic leaf blotch of Golden delicious-Glomerella leaf spot: A resolution of common names. Plant Disease. 1998;**82**:267-268

[25] González E, Sutton TB. First report of Glomerella leaf spot (*Glomerella cingulata*) of apple in the United States. Plant Disease. 1999;**83**:1074

[26] Velho AC, Stadnik MJ, Casanova L, Mondino P, Alaniz S. First report of *Colletotrichum karstii* causing Glomerella leaf spot on apple in Santa Catarina State, Brazil. Plant Disease. 2014;**98**:157

[27] Wang CX, Zhang ZF, Li BH, Wang HY, Dong XL. First report of Glomerella leaf spot of apple caused by *Glomerella cingulata* in China. Plant Disease. 2012;**96**:912

[28] Tashiro N, Manabe K, Ide Y. Emergence and frequency of highly benzimidazole-resistant *Colletotrichum gloeosporioides*, pathogen of Japanese pear anthracnose, after discontinued use of *benzimidazole*. Journal of General Plant Pathology. 2012;**78**:221-226

[29] Misonou T, Fukatsu R. Effect of benomyl spraying on the infesting of pear scab. Proceedings of the Kanto-Tosan Plant Protection Society. 1970;**17**: 59-60 (in Japanese)

[30] Umemoto S, Nagai Y. Decline of sensitivity of *Venturia nashicola* against benomyl and thiophanate-methyl in Chiba prefecture. Proceedings of the Kanto-Tosan Plant Protection Society. 1976;**23**:56-57 (in Japanese)

[31] Ishii H, Yamaguchi A. Tolerance of *Venturia nashicola* to thiophanate-methyl and benomyl in Japan. Annals of the Phytopathological Society of Japan. 1977;**43**:557-561

[32] Sato S, Fujikawa T, Tomiku T, Ando S, Goto D, Mino T. Occurrence of benomyl tolerant *Venturia nashicola* causing Japanese pear scab. Proceedings of Association of Plant Protection Kyushu. 1978;**24**:74-76 (in Japanese)

[33] Ishizaki H, Kohno M, Tsuchida M, Umino M, Hazumi Y, Katoh S, et al. Yearly fluctuation in the occurrence of thiophanatemethyl-resistant strains of the pear scab fungus in pear orchards at Karasu town, Mie Prefecture. Japanese Journal of Phytopathology. 1983;**49**: 347-351 (in Japanese with English summary)

[34] Hashimoto S, Sano S, Murakami A, Mizuno M, Nishikawa H, Yasuda Y. Fungitoxic properties of triflumizole. Annals of the Phytopathological Society of Japan. 1986;**52**:599-609

[35] Umemoto S, Murata A. Effects of the different spraying time of some fungicides on Japanese pear scab. Proceedings of the Kanto-Tosan Plant Protection Society. 1988;**35**:99-102 (in Japanese)

[36] Tomita Y, Ogawara S, Nagatsuka H. Control of pear scab by sterol demethylation inhibitors (DMIs). Annual Report of the Kanto-Tosan Plant Protection Society. 2003;**50**:75-77 (in Japanese)

[37] Umemoto S, Kaneko Y, Kameda K, Yamamoto A, Suzuki J, Fukuda H, et al. Fungicidal effect and effective duration period of sterol demethylation inhibitor (DMI) fungicides in Japanese pear scab control. Annual Report of the Kanto-Tosan Plant Protection Society. 2012;**59**: 115-118 (in Japanese with English summary)

[38] Nitta H, Ogasawara S. Chemical control of Physalospora canker to Japanese pear's current shoots. Annual Report of the Kansai Plant Protection Society. 1997;**39**:57-58 (in Japanese)

[39] Imamura S, Saito Y. Application timing and effects of some fungicides against powdery mildew of pear. Annual Report of the Kanto-Tosan Plant Protection Society. 1979;**26**:73 (in Japanese)

[40] Ohnuma T, Hirasawa H, Tanaka T, Shibahashi T. Studies on the ecology and control of anthracnose of cherry caused by *Glomerella cingulate* Spaulding et Schrenk. Bulletin of the Yamagata Prefectural Horticultural Experiment Station. 1986;**5**:9-27 (in Japanese with English summary)

[41] Adaskaveg JE, Forster H. Occurrence and management of anthracnose epidemics caused by *Colletotrichum* species on tree fruit crops in California. In: Prusky D, Freeman S, Dickman MB, editors. Colletotrichum: Host Specificity, Pathology, and Host-Pathogen Interaction. St. Paul: APS Press; 2000. pp. 317-336

[42] Timmer LW, Brown GE. Biology and control of anthracnose disease of citrus. In: Prusky D, Freeman S, Dickman MB, editors. Colletotrichum: Host Specifity, Pathology, and Host-Pathogen Interaction. St. Paul: APS Press; 2000. pp. 300-316

[43] Om P, Misra AK, Pandey BK. Anthracnose disease of tropical and subtropical fruits. In: Agnihotri VP, Prakash O, Kishun R, Misa AK, editors. Disease Scenario in Crop Plants, Vol. 1. Fruits and Vegetables. Pitampura, New Delhi: International Books and Periodicals Supply Service; 1996. pp. 1-27

[44] Kaneko Y, Suzuki T, Takeuchi T. Occurrence and control of anthracnose of Japanese pear caused by *Glomellera cingulate*. CAFRC Research Bulletin. 2010;**2**:7-16 (in Japanese with English summary)

[45] Watanabe H. Strobilurin & benzimidazole resistant in *Colletotrichum gloeosporioides*, the anthracnose fungus of Japanese pear in Oita prefecture. Bulletin of Oita Prefectural Agriculture, Forestry and Fisheries Research Center. 2013;**3**: 23-30 (in Japanese)

[46] Kaneko Y, Umemoto S, Takeuchi T. Investigation of the primary inoculum source for anthracnose caused by *Glomerella cingulata* on Japanese pear.

Japanese Journal of Phytopathology. 2010;**76**:282-285 (in Japanese with English summary)

[47] Fukaya M, Katoh S, Satoh H. Studies on the tolerance of grape gray mold (*Botrytis cinerea* Persoon) 1. Tolerance of grape gray mold (*Botrytis cinerea*) for thiophanate-methyl and some of its characteristics. Bulletin of Akita Fruit-Tree Experiment Station. 1979;**11**:33-38 (in Japanese with English summary)

[48] Nonaka T. Tolerance of tea anthracnose fungus, *Gloeosporium theae-sinensis*, to thiophanate-methyl and benomyl fungicides. (4) Fluctuation in the occurrence of resistant fungi under restricted use of fungicides. Proceedings of Association of Plant Protection Kyushu. 1984;**30**:73-76 (in Japanese)

[49] Ishii H, Udagawa H, Yanase H, Yamaguchi A. Resistance of *Venturia nashicola* to thiophanate-methyl and benomyl: Build-up and decline of resistance in the field. Plant Pathology. 1985;**34**:363-368

[50] Tomikawa A, Nagae S. Studies on resistance of the pear scab fungus (*Venturia nashicola*) to thiophanate-methyl (1). Pear scab fungus in Mie Pref. and yearly fluctuation in the occurrence of thiophanate-methyl resistant-strains of the pear scab fungus in pear orchards. Bulletin of Mie Agricultural Research Center. 1984;**12**: 21-28 (in Japanese)

[51] Umemoto S, Murata A. Change of benzimidazole-resistant populations of *Venturia nashicola* (Japanese pear scab fungus) in pear orchards. Bulletin of Chiba Agricultural Experiment Station. 1988;**29**:137-147 (in Japanese with English summary)

[52] Osada S. Yearly fluctuation in the occurrence of benomyl-resistant strain of Japanese pear scab fungus in Miyagi Prefecture. Annual Report of the Society of Plant Protection of North Japan. 1990;**41**:86-88 (in Japanese with English summary)

[53] Schwabe WTS. Resistance of the apple scab fungus (*Venturia inaequalis*) to benzimidazole fungicides. Deciduous Fruit Grower. 1979;**29**:418-422

[54] Ide Y, Tashiro N. Evaluation of fungicides about residue, rainfastness and efficacy of disease inhibition for the purpose of efficient control to the anthracnose with *Colletotrichum gloeosporioides* on Japanese pear. Japanese Journal of Phytopathology. 2004;**70**:1-6 (in Japanese with English summary)

[55] Ishii H. QoI fungicide resistance: Current status and the problems associated with DNA-based monitoring. In: Gisi U, Chet I, Gullino ML, editors. Recent Developments in Management of Plant Diseases, Plant Pathology in the 21st. Dordrecht, Heidelberg: Springer Science + Business Media B.V; 2010. pp. 37-45

[56] Brent KJ, Hollomon DW. QoIs (quinone outside inhibitors). In: Fungicide Resistance in Crop Pathogens: How Can it be Managed?. FRAC Monograph No. 1. 2nd revised ed. Brussels: The Fungicide Resistance Action Committee; 2007. pp. 42-43

[57] Hirooka T, Ishii H. Chemical control of plant diseases. Journal of General Plant Pathology. 2013;**79**:390-401

[58] Knight SC, Anthony VM, Brady AM, Greenland AJ, Heaney SP, Murray DC, et al. Rationale and perspectives on the development of fungicides. Annual Review of Phytopathology. 1997;**35**:349-372

[59] Ogasawara K, Enoyoshi T, Miyahara T, Kojiguchi, Kadota G, Takamatsu S, et al. Cucurbit powdery mildew resistant to strobilurins. Japanese Journal of Phytopathology. 1999;**65**:655 (Japanese abstract)

[60] Ishii H, Fraaije BA, Sugiyama T, Noguchi K, Nishimura K, Takeda T, et al. Occurrence and molecular characterization of strobilurin resistance in cucumber powdery mildew and downy mildew. Phytopathology. 2001; **91**:1166-1171

[61] Yano K, Kawada Y. Occurrence of strobilurin-resistant strains of *Mycovellosiella nattrassii*, causal fungus of leaf mold of eggplants. Japanese Journal of Phytopathology. 2003;**69**: 220-223 (in Japanese with English summary)

[62] Fuji M, Yamaguchi J, Furuta A, So K. Sensitivity of *Corynespora* leaf spot on cucumber fungus isolated from Saga Prefecture to strobilurin fungicides and methods for testing the sensitivity. Japanese Journal of Phytopathology. 2003;**69**:299-300 (Japanese abstract)

[63] Kansako M, Yoneda Y, Shimadu K, Ishii H. Occurrence of strobilurin-resistant *Botrytis cinerea*, pathogen of citrus gray mold. Japanese Journal of Phytopathology. 2005;**71**:249 (Japanese abstract)

[64] Tanahashi M, Nakano T, Ishii H, Kodama M, Otani H. Possible occurrence of strobilurin resistant strains in *Alternaria alternata* causing black spot of European pear. Japanese Journal of Phytopathology. 2006;**72**:275 (Japanese abstract)

[65] Tsushima Y, Yukita K, Fukushi Y, Akahira T. Resistance to strobilurin fungicides of *Alternaria alternata* apple pathotype and simplified test method. Japanese Journal of Phytopathology. 2007;**73**:51-52 (Japanese abstract)

[66] Koya N, Inoue K, Kawaguchi A. Occurrence of strobilurin resistant isolates of *Pseudocercospora vitis*, grapevine leaf blight fungus. Japanese Journal of Phytopathology. 2008;**74**: 73-74 (Japanese abstract)

[67] Inada M, Ishii H, Chung W-H, Chung WH, Yamada T, Yamaguchi J, et al. Occurrence of strobilurin-resistant strains of *Colletotrichum gloeosporioides* (*Glomerella cingulata*), the causal fungus of strawberry anthracnose. Japanese Journal of Phytopathology. 2008;**74**: 114-117 (in Japanese with English summary)

[68] Hirayama Y, Kawamoto Y, Matsutani S, Nishizaki M, Okayama K. Occurrence of fungicides resistant isolates of *Glomerella cingulata* causing the strawberry anthracnose in Nara Prefecture. Annual Report Kansai Plant Protection. 2008;**50**:93-94 (in Japanese)

[69] Tomihama T, Nonaka T, Omatsu N, Nishi Y. Occurrence of QoI resistance in *Pestalotiopsis longiseta*, the pathogen causing gray blight disease in tea plants. Kyushu Plant Protection Research. 2009;**55**:83-88 (in Japanese with English summary)

[70] Akahira T, Hanaoka T. Occurrence of trifloxystrobin resistant strains of *Colletotrichum gloeosporioides* (*Glomerella cingulata*), the causal fungus of apple bitter rot in Aomori Prefecture. Japanese Journal of Phytopathology. 2013;**79**:197-198 (Japanese abstract)

[71] Miyagawa N, Fuji M. Occuurrence of QoI-fungicide-resistant strains of *Magnaporthe oryzae* on rice and fungicidal effective. In: Abstracts of the 23rd Symposium of PSJ Research Committee on Fungicide Resistance, Gifu, Japan. 2013. pp. 25-35 (in Japanese with English summary)

[72] Takushi T, Kadekaru K, Arasaki C, Taba S. Occurrence of strobilurin-resistant strains of *Colletotrichum gloeosporioides*, the causal fungus of mango anthracnose. Japanese Journal of Phytopathology. 2014;**80**:119-123 (in Japanese with English summary)

[73] Hirayama K, Akahira T, Hanaoka T. QoI-resistant strains of *Venturia*

inaequalis causing apple scab in Aomori Prefecture, Japan. Annual Report of the Society of Plant Protection of North Japan. 2017;**68**:115-119 (in Japanese with English summary)

[74] Furuya S, Mochizuki M, Saito S, Kobayashi H, Takayanagi T, Suzuki S. Monitoring of QoI fungicide resistance in *Plasmopara viticola* populations in Japan. Pest Management Science. 2010; **66**:1268-1272

[75] Orihara N, Uekusa H, Miyakawa K, Okamoto M, Kobatashi N. Occurrence of QoI-resistant strains of *Didymella bryoniae*, causal fungus of gummy stem blight of cucurbits in Kanagawa prefecture. Annual Report of the Kanto-Tosan Plant Protection Society. 2013;**60**: 31-33 (in Japanese)

[76] Matsuura S. Current status of QoI (strobilurin) fungicides sensitivity in isolates of *Puccinia horiana*, the causal agent of chrysanthemum white rust, occur in western Japan. Plant Protection. 2019;**73**:370-373 (in Japanese)

[77] Ishii H. Current status of QoI fungicide resistance. Plant Protecton. 2015;**69**:469-474 (in Japanese)

[78] Ogata T, Kanno E. Application period and selection of effective chemicals for the Japanese pear scab control in autumn. Annual Report of the Society of Plant Protection of North Japan. 2000;**51**:141-143 (in Japanese)

[79] Tomita Y, Ogawara T, Miyamoto T. Fungicidal control of Japanese pear scab caused by *Venturia nashicola* in Ibaraki Prefecture. Plant Protection. 2011;**65**: 131-133 (in Japanese)

[80] Noguchi M. Occurrence of QoI-resistance to *Glomerella cingulate* on Japanese pear and countermeasures in Saga Prefecture. Plant Protection. 2015; **69**:494-497 (in Japanese)

[81] Watanebe H. Methods for detecting QoI fungicide resistance in *Colletotrichum gloeosporioides* (Japanese pear anthracnose). Plant Protection. 2017;**71**:327-330 (in Japanese)

[82] Tashiro N, Sadamatsu M. The effect of additional wetting spreaders on the effect of fungicides for control of Japanese pear black spot caused by *Alternaria alternata* Japanese pear pathotype. Bulletin of Saga Prefectural Fruit Tree Experiment Station. 1996;**13**:104-113 (in Japanese with English summary)

[83] Tashiro N. The effective use of the spreading agent in the disease control of fruit trees. Plant Protection. 2009;**63**: 212-217 (in Japanese)

[84] Ide Y, Tashiro N. Effect of row-passage styles by speed sprayer on the efficacy against the scab by *Venturia nashicola* and anthracnose by *Colletotrichum gloeosporioides* and chemical adhesion on Japanese pear leaves. Japanese Journal of Phytopathology. 2007;**73**:289-294 (in Japanese with English summary)

[85] Kaneko Y, Fukuta H. Use of 'Makupika' spreader for the purpose of reducing fruit stains with captan wettable powder just before the pear harvest period in Chiba Prefecture. CAFRC. Research Bulletin. 2010;**9**:49-55 (in Japanese)

[86] Ozaki M, Fukumoto S, Tamai R, Yonekura N, Ikegaya K, Kawashima T, et al. Kumiai Chemical Industry Co, Ltd. and Ihara Chemical Industry Co. Ltd.; 2001. WO 01/10825 A1

[87] Takagaki M, Kawata M, Fukumoto S, Miura I. Efficacy and in vitro activity of new fungicide KUF-1204 on *Botrytis cinerea*. Japanese Journal of Phytopathology. 2005;**71**:256 (Japanese abstract)

[88] Kataoka S, Takagaki M, Kaku K, Shimizu T. Mechanism of action and

selectivity of a novel fungicide, pyribencarb. Journal of Pesticide Science. 2010;**35**:99-106

[89] Ishii H. Fungicide research in Japan—An overview. In: Dehne HW, Deising HB, Gisi U, Kuck KH, Russell PE, Lyr H, editors. Modern Fungicides and Antifungal Compounds V. 15th International Reinhardsbrunn Symposium May 06–10, 2007, Friedrichroda, Germany. DPG Selbstverlag, Braunschweig. 2008. pp. 11-17

[90] Takagaki M, Kataoka S, Fukumoto S, Ishii H, Yamaguchi J, Inada M, et al. The efficacy of the novel fungicide pyribencarb to the several QoI resistant fungal strains. Japanese Journal of Phytopathology. 2006;**72**:274-275 (Japanese abstract)

[91] Fungicide Resistance Action Committee. FRAC Code List 2019: Fungal control agents sorted by cross resistance pattern and mode of action (including FRAC Code numbering). Available at: https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2019.pdf?sfvrsn=98ff4b9a_2 [Accessed: 14 October 2019]

[92] Japan Fungicide Resistance Action Committee. Guide line of QoIs in Japan. Available at: https://www.jcpa.or.jp/labo/jfrac/guidelines.html [Accessed: 14 October 2019]

[93] Kaneko Y. Screening of substitutes for quinone outside inhibitor fungicides for control of Japanese pear anthracnose caused by *Glomerella cingulata* and their effects on several Japanese pear diseases. CAFRC Research Bulletin. 2016;**8**:1-7 (in Japanese with English summary)

[94] Hobbelen PHF, Paveley ND, van den Bosch F. Delaying selection for fungicide insensitivity by mixing fungicides at a low and high risk of resistance development: S modeling analysis. Phytopathology. 2011;**101**: 1224-1233

[95] Tashiro N. New concept of disease and pest management: Construction of a control system with less frequent spray by EBC (evidence-based control). In: Proceedings of the 9th Forum of MAFF on Disease and Pest Management for Agricultural Product. Tokyo, Japan. 2003. pp. 18-27 (in Japanese)

[96] Tashiro N. Evidence-based control: The new concept of pest management. Journal of Evidence-Based Control. 2011;**7**:26-29 (in Japanese)

[97] Tashiro N. Evidence-based control: The new concept of pest management. Plant Protection. 2005;**59**:69-73 (in Japanese)

[98] Tashiro N. EBC (evidence-based control) is necessary for development of plant protection in the future. Annual Report of the Society of Plant Protection of North Japan. 2007;**58**:1-15 (in Japanese)

[99] Kawaguchi A. Concept for study of plant disease control at research committee for the EBC (evidence-based control), on the 7th workshop. Journal of Evidence-based Control. 2011;**7**:26-29 (in Japanese with English summary)

[100] Kawaguchi A. Concept and practice of EBC (evidence-based control) for on-farm research. Plant Protection. 2012;**66**:450-455 (in Japanese)

[101] Ide Y. Evidence of the chemical control to the disease on fruit trees. Plant Protection. 2012;**66**:456-459 (in Japanese)

[102] Tashiro N. Study on the improvement of fruit tree disease management using evidence-based control. Journal of General Plant Pathology. 2015;**81**:470-475

[103] Tashiro N, Yamaguchi S, Nakashima K, Syouji K, Matsuo Y, Yamaguchi M. Evidence-based control (EBC) to greenhouse mandarin sooty mold caused by *Cladosporium cladosporioides*. Japanese Journal of Phytopathology. 2016;**82**:79 (Japanese abstract)

[104] Kanagawa Prefecture. Anthracnose of Japanese pear (in Japanese). In: Temporary Pest Outbreak Forecast Information. Vol. 3. 2007. pp. 1-2 (in Japanese)