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.
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 (K₂HPO₄ 1 g, MgSO₄ 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
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].
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. piriicola (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 (~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.
| Generic name | Trade name in Japan | FRAC code | Active ingredient (%) | Rate applied (mg L\(^{-1}\))\(^1\) | Control (%)\(^2\) |
|--------------|---------------------|-----------|-----------------------|--------------------------------------|------------------|
| Benomyl      | Benlate WP          | 1         | 50.0                  | 250                                  | Strain C-17: 0, Strain C-25: 90.2 |
| Thiophanate-methyl | Tospin-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.
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DOI: http://dx.doi.org/10.5772/intechopen.90018

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”.

| 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\(^{b}\) |

1The 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).

2Control (%) = (1 − average lesion number per leaf on the trees with benomyl application/average lesion number per leaf on the control trees) × 100.

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
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.
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\(\times\)10\(^5\) 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 \(\geq 70\%\) at 200 mm cumulative rainfall after fungicide application (Figure 5). Fluazinam FL and kresoxim-methyl DF achieved \(\geq 70\%\) disease control at 100 mm cumulative rainfall, but the disease control

| 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           |
| Captain/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) \times 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 (%)\(^1\) |
|-----------------------------------|---------------------|-----------|-----------------------|-----------------------------|----------------------------|
| Dithianon                         | Delan FL            | M9        | 42.0                  | 420                         | 92           | 83           | 41           |
| Fluazinam                         | Frowncide SC        | 29        | 39.5                  | 198                         | 59           | 0            | 0            |
| Captain/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            | —            |

\(^{2}\)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

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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 \(\geq 70\%\) at 200 mm cumulative rainfall after fungicide application (Figure 5). Fluazinam FL and kresoxim-methyl DF achieved \(\geq 70\%\) 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
>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 rainfall (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-Qols 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 (%)<sup>2</sup> |
|---------------------|---------------------|-----------|-----------------------|-----------------------|-------------------------|
|                     |                     |           |                       |                       | 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     | Storbi 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             | 100/167               | 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% |

<sup>1</sup>Sprays have been done in May to August each year.
<sup>2</sup>See Table 5.

Table 7.
Control effect of several fungicides against anthracnose on the Japanese pear “Housui”.<sup>3</sup>
### Table 8.
Control effect of several fungicides against ring rot on the Japanese pear “Housui”.

| Generic name               | Trade name in Japan | FRAC code | Active ingredient (%) | Rate applied (mg L⁻¹) | Control (%)² |
|----------------------------|---------------------|-----------|-----------------------|-----------------------|--------------|
| Benomyl                    | Benlate WP          | 1         | 50.0                  | 250                   | 32           |
| Fluazinam                  | Frowncide SC        | 29        | 39.5                  | 198                   | 30           |
| Dithianon                  | Delan FL            | M9        | 42.0                  | 420                   | 19           |
| Kresoxim-methyl            | Storobi DF          | 11        | 50.0                  | 250                   | 39           |
| Azoxystrobin               | Amistar 10 FL       | 11        | 10.0                  | 100                   | 47           |
| Oxyquinoline copper        | Quinondo FL         | —         | 35.0                  | 350                   | 0            |
| Captan/benomyl             | Caplate WP          | M4/1      | 60.0/10.0             | 1000/167              | 17           |

|                           | In 2000 | In 2001 | In 2002 |
|---------------------------|---------|---------|---------|
| Ratio of diseased leaves in control | 45.0%   | 40.6%   | 10.9%   |

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

Emergence of Benzimidazole- and Strobilurin-Quinone Outside Inhibitor-Resistant Strains... DOI: http://dx.doi.org/10.5772/intechopen.90018
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 (azoxyystrobin and kresoxim-methyl) and protective fungicides such as dithianon and fluazinam were applied.

Dithianon FL, fluazinam FL, ST-QoI fungicides (azoxyystrobin FL, kresoxim-methyl DF), captain/oxyquinoline copper WP, and captain/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. Captain/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 (azoxyystrobin 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 (Pestalotiopsis n. sp.) [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-Qol 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 strains¹ |
|---------------------------------|--------------------------|----------------------------|
| Imari district in Saga prefecture | 61                      | 20 (32.8%)²                |
| Hita district in Oita prefecture | 254                     | 49 (16.2%)                 |

¹Number of strains that grew on PDA with 1000 μg mL⁻¹ SHAM and 100 μg mL⁻¹ azoxystrobin cultured 4 days at 25°C.

²Values 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 “Nitaka” at Imari district in Saga prefecture and Hita district in Oita prefecture both Kyushu island in 2011.
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

| Strain2 | 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         |

1The 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.

2All strains was isolated at Hita city of Oita prefecture in 2011.

3Control (%) = (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 azoxystrobin 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
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<sup>2,4</sup> | MNAPS<sup>3,4</sup> | Rate applied (mg L<sup>-1</sup>)<sup>4</sup> | 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]   |
| Thiram                               | Thionoc FL          | M3        | 40.0                  | 30                  | 5                   | 800                                           | 2008                   | [44, 80]   |
| Thiuram                              | Trenox FL           | M3        | 40.0                  | 30                  | 5                   | 800                                           | 2008                   | [44, 80]   |
| Pyraclostrobin/bosalid               | 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                   | —          |

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

**Table 11.**  
Registered fungicides for Japanese pear anthracnose in Japan.<sup>1</sup>
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-Qol-resistant gray mold isolates than other ST-Qol fungicides [90]. It also had relatively higher efficacy against ST-Qol-resistant *Pestalotiopsis longiseta* which causes tea gray blight [69]. Pyribencarb shows differential cross-resistance patterns to ST-Qol [89].

Since pyribencarb has an excellent effect on JPA [44], it has been recommended to use it in orchards where ST-Qol-resistant strains are present or ST-Qol effects are reduced. However, there have been no reports of the effects of pyribencarb in an orchard where ST-Qol-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.

Acknowledgements

We appreciate the collaboration and information exchange with Dr. Yohei Kaneko of the CAFRC. In addition, we sincerely thank Dr. Kayo Manabe of Nippon Steel Eco-tech Corporation for assisting in the collection of references and Ms. Noriko Orihara and Mr. Makoto Suzuki of the Kanagawa Agricultural Technology Center for providing photos of JPA outbreak. Moreover, for implementation of the study, we sincerely thank the staff members of the Plant Protection Laboratory of Saga Prefectural Fruit Tree Experiment Station including Ms. Hisako Fukumoto, Setsumi Morinaga, and Hatsumi Nakayama and students of Saga Prefectural Agricultural College Fruit Tree Branch School.
Emergence of Benzimidazole- and Strobilurin-Quinone Outside Inhibitor-Resistant Strains...
DOI: http://dx.doi.org/10.5772/intechopen.90018

Author details

Nobuya Tashiro¹*, Youichi Ide², Mayumi Noguchi³, Hisayoshi Watanabe⁴ and Mizuho Nita⁵

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

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