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Fungicides Application against Fusarium Head Blight in Wheat and Barley for Ensuring Food Safety

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1. Introduction

Fusarium head blight (FHB), or scab, caused by several *Fusarium* species, especially *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch], is a widespread and destructive disease of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and other small-grain cereals (Liddell 2003; Parry et al. 1995; Pirgozilev et al. 2003). These pathogens infect spikes and reduce grain yield and quality. Moreover, *Fusarium* species that cause FHB produce trichothecene mycotoxins such as deoxynivalenol (DON) and nivalenol (NIV), which are toxic to humans and other animals. The Joint WHO/FAO Expert Committee on Food Additives evaluated the risk of DON and set a provisional maximum tolerable daily intake of DON as 1 µg/kg body weight in 2001. Accordingly, the Japanese government determined provisional guidelines for DON content in unpolished wheat grains as 1.1 mg kg⁻¹ in 2002. Many other countries have also established maximum allowed levels for DON in cereals and cereal products.

There is currently no robust single control measure by which to manage either FHB or mycotoxin contamination in barley and wheat. Fungicide application is one measure available to reduce the risk; however, results have not been highly effective or consistent (Horsley et al. 2006; Jones 2000; McMullen et al. 1997; Mesterhazy 2003). To obtain increased chemical control of FHB, the timing of fungicide application is an important factor, as well as fungicide selection, application rate, and good coverage of the spike (Mesterhazy 2003).

This chapter introduces FHB disease and its related mycotoxins, and summarizes our research on the chemical control of mycotoxin contamination.

2. Fusarium head blight of cereals

Symptoms caused by the fungus in wheat include premature bleaching of the spikelets or entire spike (Fig. 1a). These white heads are very conspicuous in a green field. Frequently, only part of the head is affected by FHB. These partly white and partly green heads are diagnostic. Additional indications of FHB infection are pink to salmon-orange spore masses of the fungus often seen on the infected spikelet and glumes during prolonged wet weather (Fig. 1a). Bleached spikelets usually are sterile or contain shriveled and/or discolored seeds

(Fig.1c). These kernels are sometimes called "tombstones" because of their chalky, lifeless appearance. Other Fusarium-infected kernels may be more normal in size, if infection occurred late in kernel development. In barley, FHB infections are not always readily apparent in the field. Infected spikelets may show a browning or water-soaked appearance (Fig. 2a). Infected barley kernels show a brown discoloration similar to that caused by other kernel blighting organisms (Fig. 2c).
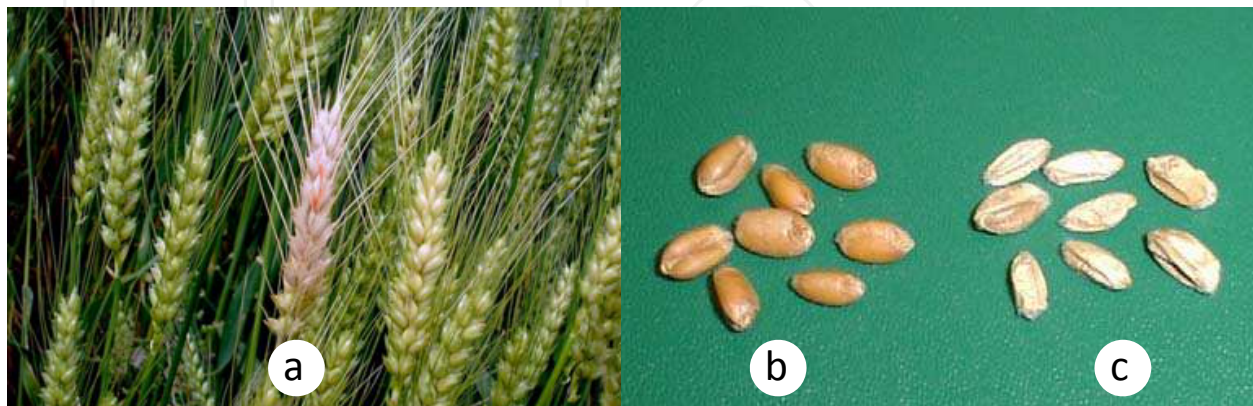

Fig. 1. Symptom of Fusarium head blight (a), healthy seeds (b) and diseased seeds (c) in wheat.

Fig. 2. Symptom of Fusarium head blight (a), healthy seeds (b) and diseased seeds (c) in barley.

3. Toxicity of mycotoxins

Trichothecene mycotoxins produced by FHB pathogens, especially DON and NIV (Fig. 3) possess common biochemical and cellular toxicities. These have a strong inhibitory effect on protein synthesis by binding to the ribosomes, an inhibitory effect on RNA and DNA synthesis, and toxic effects on cell membranes (Sugita-Konishi & Kumagai, 2005). Furthermore, their capacity to inhibit protein synthesis may induce apoptosis in thymus, lymphatic, and hematopoietic tissue via mitogen-activated protein kinase. Crops contaminated with trichothecene may result in serious food poisoning accompanied by nausea, vomiting, and diarrhea. Immunotoxicity, which is a chronic effect of trichothecene mycotoxins, decreases host resistance. Selective upregulation of serum IgA caused by dietary exposure to DON or NIV induces IgA nephropathy. Its cancer-promoting effects seem to be responsible for the immunotoxicity.
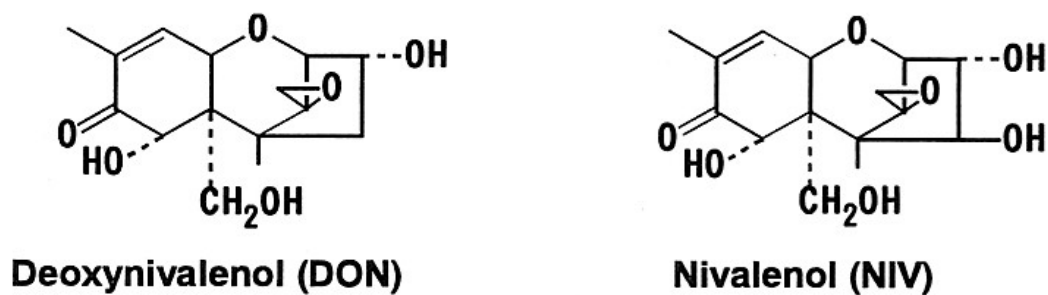
Fig. 3. Chemical structure of deoxynivalenol (DON) and nivalenol (NIV)

The toxicity of mycotoxins can be compared to that of agricultural fungicides for FHB control using acceptable daily intake (ADI) and provisional tolerable dietary intake (PTDI); both are estimates of the amount of a substance in a food or crop that can be ingested daily over a lifetime without appreciable health risk. The ADI of four main fungicides for FHB control are much higher than the PTDI of DON or NIV, indicating that the risk of mycotoxins are much higher than that of fungicides. Therefore, appropriate application of fungicides for FHB control is better for ensuring food safety. This is good example for explanation of benefit of fungicides.

Fungicide	Acceptable daily intake (µg/kg bw/day)	Maximum residue limit in wheat (mg/kg)
Thiophanate-methyl	120 [a]	0.6 [a]
Tebuconazole	29 [a]	0.5 [a]
Propiconazole	18 [a]	1.0 [a]
Metconazole	40 [a]	0.2 [a]
Mycotoxin	**Provisional tolerable dietary intake** (µg/kg bw/day)	**Provisional standard in wheat** (mg/kg)
Deoxynivalenol	1.0 [b]	1.1 [a]
Nivalenol	0.7 [c]	N.D.

Table 1. Comparison of toxicity between Fusarium head blight (FHB) mycotoxins and fungicides for FHB control, as a) determined by the Japanese government, b) evaluated by Joint FAO/WHO Expert Committee on Food Additives, and c) evaluated by European Food Safety Authority.

4. Screening of fungicides for the reduction of mycotoxins

Since the provisional standard of 1.1 ppm for DON in wheat was set by the Japanese government in 2002, the endpoint of our research had to be changed from disease severity to mycotoxin contamination. Therefore, a re-evaluation of registered fungicides and screening of new candidates to control mycotoxin contamination became mandatory. We tested 24 fungicides with different modes of action. Three experiments were conducted for 2 years (Nakajima, 2004).

In a paddy field, we sprayed fungicides 2 days before flowering and 5 days after flowering. Inoculations of *F. graminearum* were conducted at flowering and 7 days after flowering. In 2002, we used DON producer, and in 2003, we used a mixture of DON and an NIV
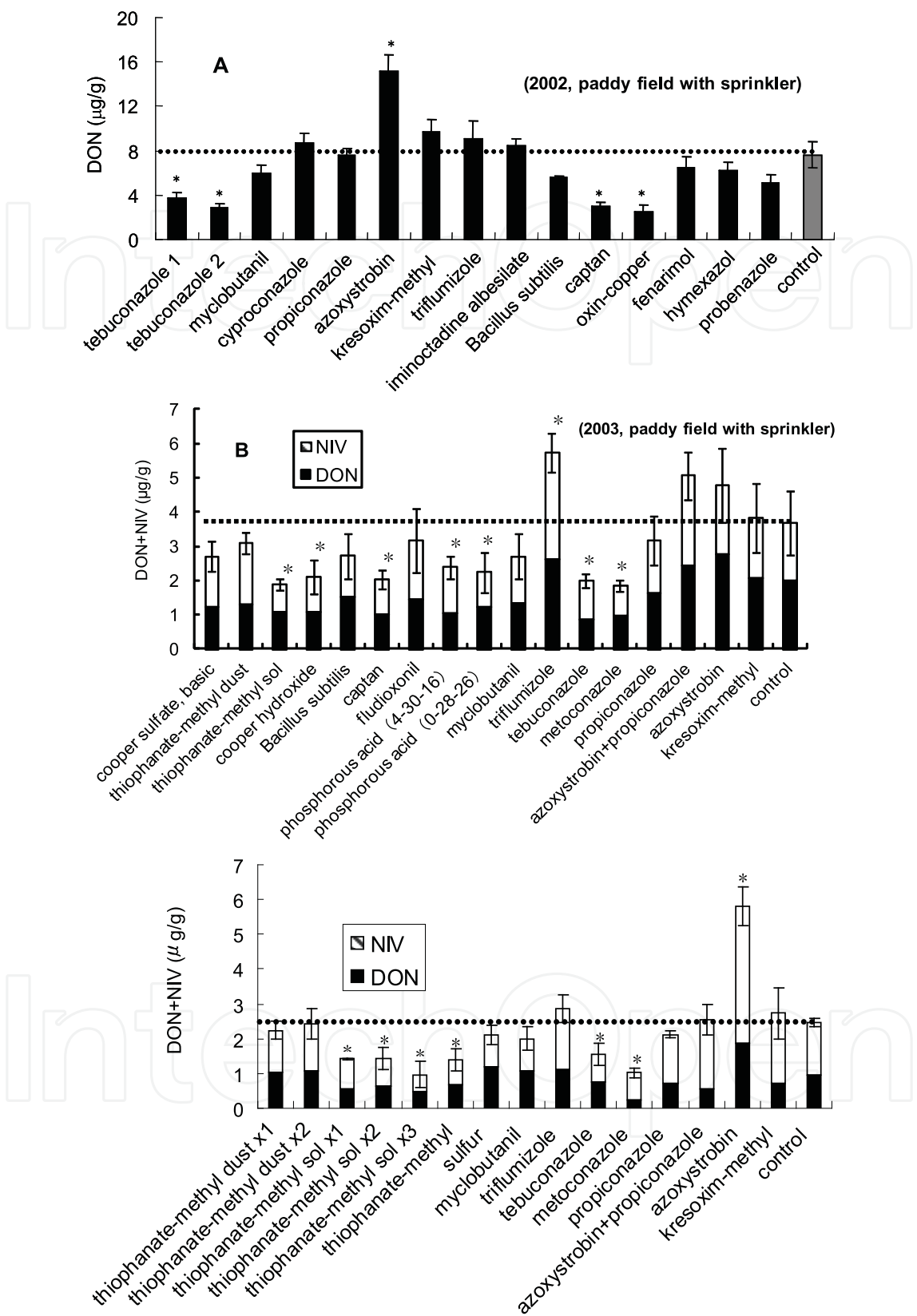
Fig. 4. Effects of fungicides on the level of deoxynivalenol (DON) and nivalenol (NIV) (Nakajima, 2004). * significantly different from control plot at p < 0.05. Bars indicate standard error.

producer. A sprinkler was used to promote disease development. In addition, an upland field experiment was performed in 2003. Corn grain inoculum of a mixture of DON and an NIV producer were used under natural rainfall conditions.

Most of the fungicides controlled FHB disease severity. In particular, tebuconazole, captan, and oxin-copper were highly effective. Azoxystrobin was not so effective but its efficacy was about 40%. In general, the fungicides were less efficacious against DON than against disease severity. However, tebuconazole, captan, and oxin-copper decreased the DON level significantly compared to the control plot (Fig. 4-A). In contrast, azoxystrobin increased the DON level significantly. Other fungicides did not affect the DON level.

In the paddy field, most fungicides except trifulumizole were highly effective in 2003. The reason for the failure of trifulumizole was unknown. Control of DON + NIV was more difficult than control of disease severity. In 2003, two applications were insufficient to decrease mycotoxin levels. Thiophanate-methyl sol, cooper hydroxide, captan, and two-kinds of phosphorous acid, tebuconazole and metoconazole decreased significantly DON+NIV level than control plot (Fig.4-B). Trifulumizole was not effective for disease or toxin control. Azoxystrobin and a mixture of azoxystrobin and propiconazole were effective for disease control but not as effective against mycotoxins.

We inoculated a corn grain inoculum with a DON + NIV mixture in 2003 in an upland field to simulate a natural infection. In this case, most of the fungicides were highly effective. Two applications was enough to control disease severity. However, control of DON + NIV was more difficult. The efficacy of toxin control was lower than that in the paddy field in which spore inoculation was performed (Fig. 4-C). Corn inoculum probably supplies conidiospores continually during the maturation period. Therefore, a nonvisible infection might increase mycotoxin levels. Thiophanate-methyl sol significantly decreased the DON + NIV level compared to thiophanate-methyl dust. Tebuconazole and metoconazole decreased the DON+NIV level significantly compared to the control plot. In contrast, azoxystrobin increased the DON + NIV level, especially the NIV level, significantly. In this case, a mixture of propiconazole and azoxystrobin did not increase or decrease mycotoxin levels. Interestingly, the mode of action of kresoxim-methyl was similar to that of azoxystrobin but its effect on mycotoxin levels seemed to be different.

Several studies have reported that some fungicides stimulate DON accumulation in wheat grains infected by FHB fungi (Magan et al., 2002; Simpson et al., 2001). Simpson et al. (2001) suggested that eliminating the competition between DON-producing *Fusarium* species and *M. nivale* by selectively controlling *M. nivale* increases the proportion of DON producers in the host plant. In our tests, however, *M. nivale* was not responsible for FHB. Ramirez et al. (2004) also reported a direct effect of fungicides on DON production by *F. graminearum* when cultured in vitro with viable wheat grains. Five fungicides stimulated DON production under certain water activities, temperatures, fungicide concentrations, and durations of incubation (Ramirez et al., 2004). Microarray analysis revealed that trichothecene biosynthesis genes were highly expressed in *F. culmorum* grown under optimal conditions and under mild temperature and water stress (Schmidt-Heydt et al., 2008). Thus, suboptimal growth conditions generated by a particular fungicide application and environmental stress enhanced DON production by *Fusarium* species.

Therefore, to screen fungicides, we must consider the effects they have on DON or NIV levels. These results suggest that a new fungicide evaluation system based on efficacy for mycotoxin contamination should be introduced.

5. Critical control point for the control of the mycotoxins in wheat

In our field evaluation of fungicides, the efficacy for DON and NIV control was consistently lower than the efficacy for FHB severity (Nakajima, 2004). Based on this finding, we hypothesized that the critical control point of DON and NIV might be different than that of FHB severity. The general recommendation for fungicide application timing is the beginning of flowering, because plants are most susceptible to infection at this stage. An additional application at 7–10 days after the first application is recommended in Japan. The frequency of fungicide application is also crucial for mycotoxin reduction.
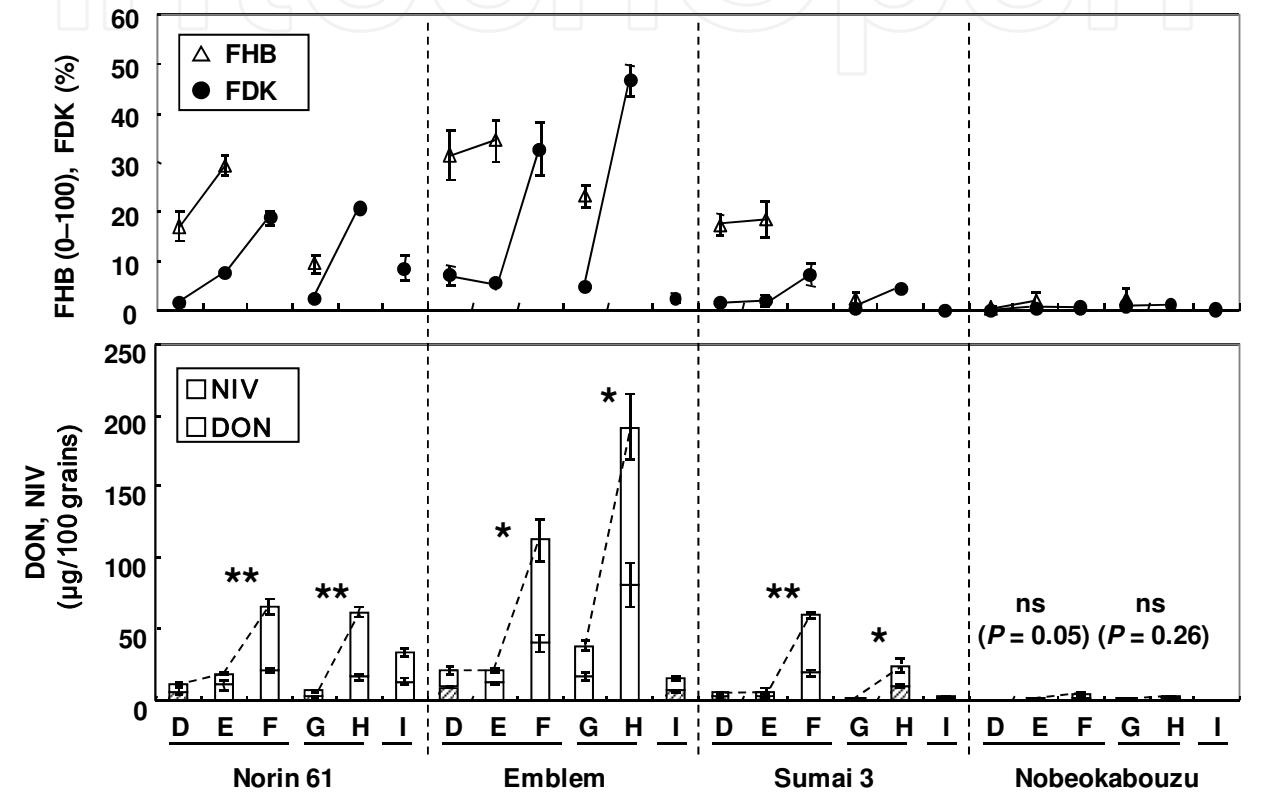

Fig. 5. Deoxynivalenol (DON) and nivalenol (NIV) concentrations in grains, Fusarium head blight (FHB) severity, and the percentage of Fusarium-damaged kernels (FDK) during host development after different timings of inoculation for the four cultivars in the greenhouse experiments in 2004 (Yoshida & Nakajima, 2010). In treatments (Trts) D, E, and F, plants were inoculated at anthesis and sampled 10 days after anthesis (DAA), 20 DAA, and maturity (38 to 40 DAA), respectively. In Trts G and H, plants were inoculated at 10 DAA and sampled at 20 DAA and at maturity, respectively. In Trt I, plants were inoculated at 20 DAA and sampled at maturity.

To develop effective timing for controlling FHB in wheat, particularly for reducing the risk of toxin contamination, it is necessary to elucidate the manner in which toxin accumulation occurs in wheat grain.

Four cultivars were tested in a greenhouse environment (Yoshida & Nakajima, 2010), where the plants were spray-inoculated at three different stages with a mixture of *F. graminearum* DON and NIV chemotypes. To determine the actual manner in which the toxin increases in the developing grain, the toxin content of grains after inoculation of each cultivar was
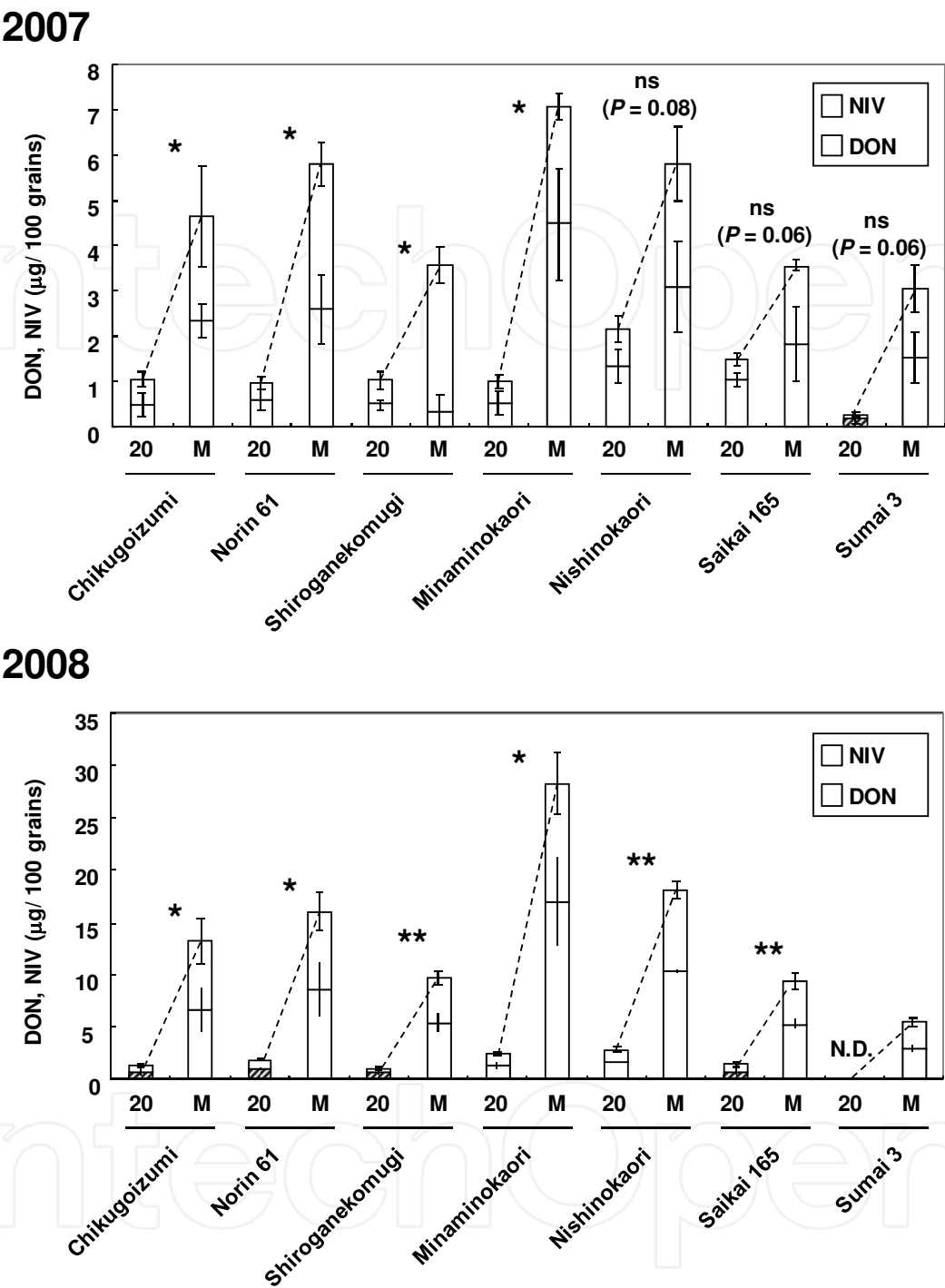
2007

2008

Fig. 6. Deoxynivalenol (DON) and nivalenol (NIV) concentration in grains at two developmental stages for the seven cultivars in the field experiments in 2007 and 2008 (Yoshida & Nakajima, 2010). Signs below the graph (20 and M) indicate the timing when the samples were harvested, 20 days after anthesis (DAA) and maturity (40 DAA), respectively. Each data point represents the mean of three replications. Bars represent standard errors. N.D., not detected; * and ** indicate $P < 0.05$ and 0.01, respectively, for differences in toxin (DON + NIV) concentration in grains (micrograms per 100 grains) between the two harvests (i.e., 20 DAA and at maturity) (one-tailed t-test); ns, difference not significant.

measured on a grain number basis (µg/100 grains) instead of a grain weight basis (e.g., µg g^{-1}, ppm, etc.), considering the influence of grain-weight increase during development. The results indicated that high levels of DON and NIV were produced beyond 20 days after anthesis (DAA), even following early infection (Fig. 5). The results of field experiments performed on seven cultivars, in which the inoculation was conducted using colonized maize kernel inoculum, were consistent with the greenhouse results (Fig. 6). Furthermore, in the greenhouse experiments, late infection, at least as late as 20 DAA, caused grain contamination with these toxins, even without clear disease symptoms on the spike (Fig. 5).

Strategies for controlling FHB and toxin contamination have been developed, mostly focusing on infection during the host flowering stage. However, our results indicate the importance of the late stages of grain development for toxin contamination in addition to the early stage, showing that the amount of DON and NIV largely increases after 20 DAA (late milk stage), even with infection at earlier stages, and that infection at late stages, at least as late as 20 DAA, can cause non-negligible levels of contamination, even without clear FHB symptoms. Thus, control strategies should be established covering the late stage as well as the time around the flowering stage to effectively reduce the risk of DON and NIV contamination.

Application of fungicides or other control agents at the late stage may be an effective measure for reducing the final level of toxin accumulation. Agents used for such 'late control' are expected to possess effects not only to prevent primary infection at late stages but also to prevent toxin production in late stages by previous fungal infection. Studies to test the effectiveness of such 'late control' for reducing toxin contamination are needed. The use of resistant cultivars with a lower risk of toxin contamination throughout the developmental period is also important. To properly assess the total risk of toxin contamination in wheat cultivars, inoculation tests that cover the late as well as the early stages are required. Among the tested cultivars in this study, 'Nobeokabouzu', which is a FHB-resistant cultivar widely used in FHB-resistance breeding, as well as 'Sumai 3' (Rudd et al., 2001; Shi et al., 2008) showed the highest resistance to mycotoxin accumulation for all timings of inoculation (Fig. 5). 'Nobeokabouzu' seems to possess consistent resistance to mycotoxin accumulation as well as to FHB during grain development. Such a cultivar may be a useful resistance source, especially for introducing resistance to mycotoxin accumulation during all grain development stages.

6. Extrusion of the spent anthers in the cleistogamous barley cultivar

Anthesis is considered the optimal growth stage for fungicide application to control FHB in wheat (Mesterhazy 2003; Paul et al. 2007). This is reasonable because wheat is most susceptible to FHB during anthesis (Atanasoff, 1920; Parry et al., 1995; Pugh et al., 1933; Sutton 1982), at which time anthers extrude from the florets. An initial *Fusarium* infection commonly occurs on extruded anthers (Pugh et al. 1933). In most studies on the chemical control of FHB in barley, fungicides were applied at or near anthesis (Havlova et al. 2006; Ioos et al. 2005; Jones 2000; Vanova et al. 2007) similar to the case of wheat, or at full head emergence (Havlova et al. 2006; Jones 2000), which may or may not be concurrent with the time of anthesis. Although barley usually undergoes anthesis after heading similar to wheat, in some barley-growing regions anthesis occurs while the spike is still enclosed within the flag leaf sheath (McCallum and Tekauz 2002; Steffenson 2003). In such cases, the time of full head emergence should be after anthesis is completed. In Japan, where anthesis in barley

usually occurs at or a few days after full heading, fungicide application for FHB has been performed around anthesis regardless of cultivar. However, the influence of fungicide application timing on reducing FHB and mycotoxin accumulation in barley has not been investigated to date.

While wheat is generally chasmogamous (open-flowering type) and extrudes anthers at anthesis, barley has two flowering types: chasmogamous and cleistogamous (closed-flowering type). In cleistogamous cultivars, the florets do not open and anthers are not extruded at anthesis (Fig.7). However, the anthers may be pushed out from the tips of florets by the developing grain several days after anthesis (Fig.7). In Japan, this phenomenon is observed commonly in cleistogamous cultivars. Thus, it can be said that the timing of anther extrusion differs with flowering type in barley.
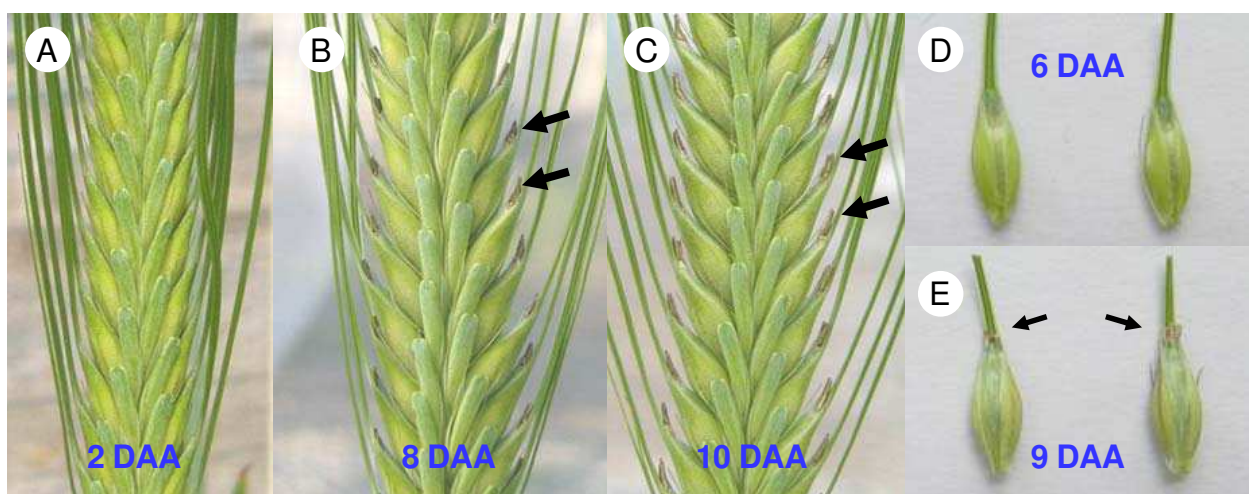

Fig. 7. Extrusion of the spent anthers in the cleistogamous barley cultivar 'Nishinochikara' (Yoshida et al., 2007). A–C, Spikes (A) 2 days after anthesis (DAA), (B) 8 DAA, and (C) 10 DAA. D and E, Appearance of the ventral side of florets (D) 6 DAA and (E) 9 DAA. Anthers were still contained within the closed florets at 6 DAA; most anthers were partially extruded at 8 DAA; most anthers were completely extruded at 10 DAA. Arrows indicate the extruded spent anthers.

In Japanese barley cultivars, there is a strong association between row type and flowering type; most two-rowed cultivars are cleistogamous, whereas most six-rowed cultivars are chasmogamous. In most cases, the former are relatively resistant and the latter are susceptible to FHB (Yoshida et al., 2005), consistent with the general observation that two-rowed types are more resistant than six-rowed types (Choo et al., 2004; Heta & Hiura, 1963; McCallum, et al., 2004; Steffenson, 2003; Takeda & Heta, 1989 & Zhou et al., 1991). Cleistogamy contributes to the resistance of barley cultivars to FHB, at least for infection that occurs at anthesis (Yoshida et al., 2005). Even in the two-rowed cleistogamous cultivars with their relative resistance, however, high levels of DON and NIV contamination can occur (Yoshida et al., 2007).

In the cleistogamous cultivars, anthers did not protrude at anthesis; however, as the grain developed after pollination, the spent anthers were extruded from the tip of the floret between the tip of the palea and lemma (Fig. 7). Spent anther extrusion began around 7 DAA and was completed by 10 DAA.
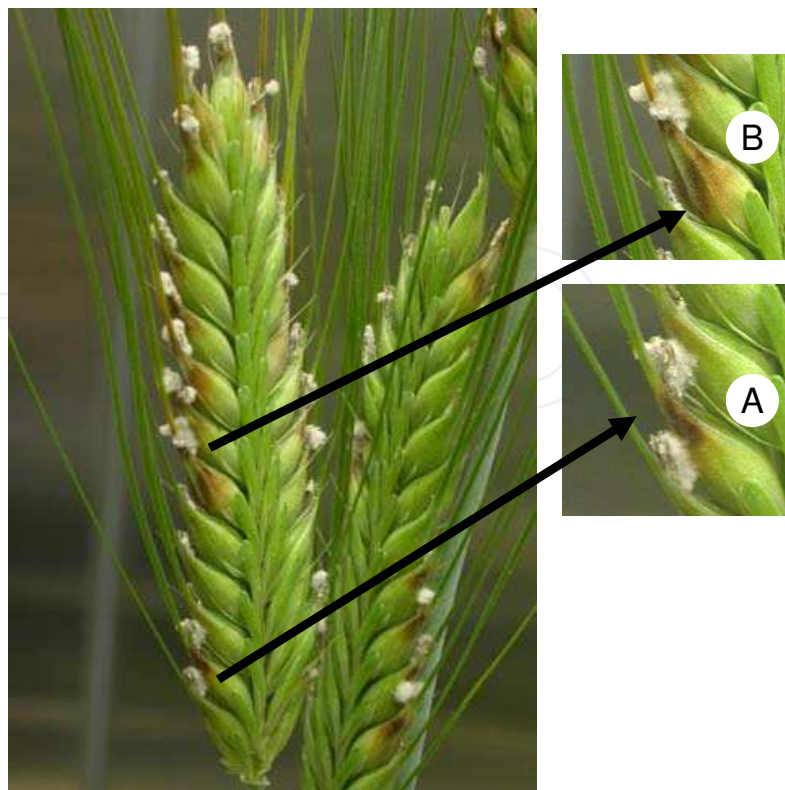
Fig. 8. Initial symptoms of Fusarium head blight (FHB) appeared in two-rowed closed-flowering cultivars inoculated at the timing of spent anther extrusion. (A) Fungal colonization of the spent anthers and initial symptoms appeared around the tips of florets. (B) The discoloration progressed toward the bases of the florets.

7. Effect of infection timing on Fusarium head blight and mycotoxin accumulation in barley

Thirteen barley cultivars (nine two-rowed and four six-rowed cultivars shown in Fig. 9) were evaluated for FHB resistance at anthesis and 10 DAA. Among the cultivars, all six-rowed cultivars are chasmogamous and the two-rowed varieties are cleistogamous, except for the chasmogamous two-rowed cultivar Satsuki Nijo. The cultivars were planted in pots (four plants per pot) and grown in a greenhouse. Japanese isolates of *F. graminearum* Schwabe (DON chemotype) was used as the inoculum. The spikes were spray inoculated at the respective stages and were nursed overnight in a greenhouse at 18–25°C and 95–100% humidity. After inoculation, the plants were placed in the greenhouse (18–25°C) equipped with a sprinkler system that intermittently produces a fine mist to keep the spikes wet during the test. FHB severity was assessed 2 weeks after inoculation on a scale of 0 to 100 (0, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100), according to a visual rating of the percentage of infected florets.

The results showed that infection timing differentially influenced FHB severity among cultivars (Fig. 9). The two-rowed, cleistogamous cultivars were severely diseased by 10 DAA inoculation, whereas they showed good resistance to infection at anthesis. The drastic decline in resistance during the period seemed to be related to the extrusion of old anthers, which occurred around 10 DAA in the cleistogamous cultivars.
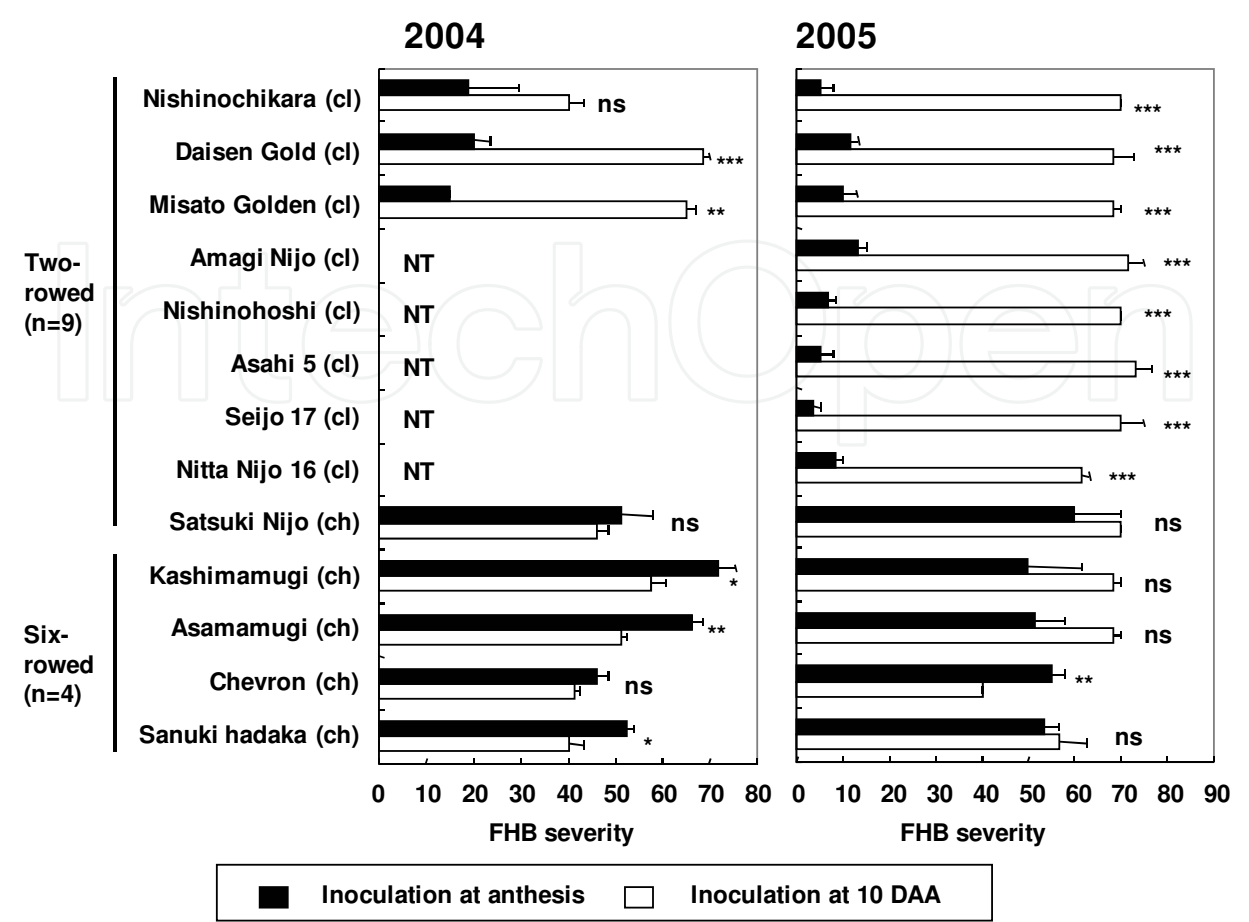
Fig. 9. Fusarium head blight (FHB) severity (0–100 scale) for 13 cultivars inoculated with *Fusarium graminearum* at anthesis and 10 days after anthesis (DAA) in 2004 and 2005 (Yoshida et. al, 2007). Bars represent standard errors. *P < 0.05, **P < 0.01, *** P < 0.001 for differences between the two inoculation times (t-test). Ns, the difference was not significant; NT, not tested; cl, cleistogamous cultivar; ch, chasmogamous cultivar.

Five barley cultivars (three two-rowed and two six-rowed cultivars shown in Fig. 10) were tested. The cultivars were planted in pots (five to six plants per pot) and at the heading stage, late tillers of each plant were removed, thereby allowing 7–12 spikes that appeared to be at the same stage to grow per pot. A mixture of two Japanese isolates of *F. graminearum* Schwabe differing in trichothecene chemotype (DON chemotype and NIV chemotype) was used as the inoculum. Spray inoculation was conducted at anthesis (= 0 DAA), 10 DAA, and 20 DAA. The inoculated plants were kept wet for 6 days after inoculation in a greenhouse equipped with a sprinkler system. Then the plants were returned to the greenhouse in which they were grown. FHB severity was visually assessed 20 DAA. Sampling was conducted at maturity (33 to 35 DAA), and the grain samples were analyzed for DON and NIV content using ELISA (Yoshizawa et al. 2004).

In this experiment, the two-rowed, cleistogamous cultivars accumulated much more mycotoxins when inoculated 10 DAA or 20 DAA than when inoculated at anthesis, whereas the six-rowed, chasmogamous cultivars accumulated mycotoxins the most when inoculated at anthesis (Fig. 10). Based on these results, we concluded that the most critical timing of infection for FHB and mycotoxin accumulation in barley differs with cultivar, probably associated with flowering type. This was probably because the extruded spent anthers

provide an initial base for the colonization of *F. graminearum* in cleistogamous cultivars (Fig. 8). These results suggest that the optimal timing of chemical control may depend on cultivar, although fungicide application to control FHB has been performed uniformly around anthesis, which is several days after anthesis in Japan.
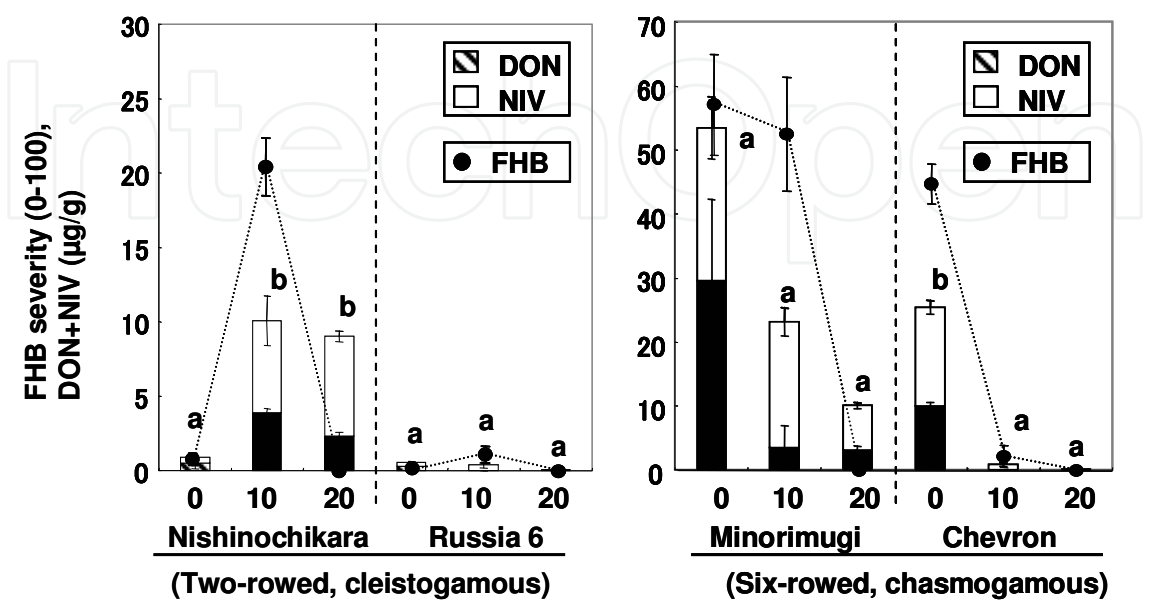

Fig. 10. Fusarium head blight (FHB) severity caused 20 days after anthesis (DAA) and mycotoxin concentration in five barley cultivars and different inoculation times in 2004 (Yoshida et. al 2007). Numbers below the graph indicate the timing of inoculation in DAA. Each point represents the mean of three replicates; bars represent standard errors. Within cultivars, mycotoxin concentrations (the total of deoxynivalenol [DON] plus nivalenol [NIV]) with the same letter were not significantly different at P = 0.05 (Tukey-Kramer multiple comparison).

8. Effect of the timing of fungicide application in barley

The two-row cleistogamous cultivar 'Nishinochikara' was used for the field trials in 2005 and 2006. The timings of fungicide application (see Table 2) were represented by codes from I to VI, which were arranged by the authors. A wettable powder of thiophanate-methyl (1050 g ai/ha) was used as the fungicide treatment. Treatments (different timings of fungicide application and no-fungicide control) were assigned to 4-m single-row plots that were arranged at 0.5-m intervals in a randomized complete block design in the field consisted of 12 50-m-long rows spaced 0.8 m apart. Adjacent rows were left untreated as border rows. Inoculation of *F. graminearum* was performed using colonized maize kernels inoculum (Champbell and Lipps 1998, Dill-Macky 2003), which generate ascospores over a period of testing season in the field. Mist irrigation for promoting ascospores production and the fungal infection was performed in the field. Disease assessments were done at 22 and 24 DAA in 2005 and 2006, respectively. After the harvest, the percentage of discolored kernels (DK), which showed tan to dark brown discoloration on more than 5 % of the grain surface, thousand kernel weight (TKW, g), DON and NIV content were also measured for each grain sample.

Code	Plant growth stage description	ZGS[e]	2005		2006	
			DAA	Date	DAA	Date
I	Before anthesis	56–57	–3	15-Apr	–2	16-Apr
II	Anthesis	(59,) 64–65	0	18-Apr	0	18-Apr
III-1	4–5 DAA[a]	71	4	22-Apr	5	23-Apr
III-2	9 DAA, before spent anther extrusion		9	27-Apr	9	27-Apr
IV-1	Beginning of spent anther extrusion[b]	73	11	29-Apr	12	30-Apr
IV-2	Spent anther extrusion half-way[c]		–	–	14	2-May
IV-3	Spent anther extrusion complete[d]	75	15	3-May	16	4-May
V	Late milk	77	21	9-May	21	9-May
VI	Soft dough	85	29	17-May	30	18-May

Table 2. Timing of fungicide application for closed-flowering barley (cv. 'Nishinochikara') grown in the test field in Koshi Japan in 2005 and 2006.
[a] Days after anthesis.
[b] Spent anther tips emerged in 50% of spikes.
[c] Spent anthers fully extruded in 50% of spikes.
[d] Spent anthers fully extruded in > 90% of spikes.
[e] Zadoks growth stage (ZGS) corresponded to each timing. ZGS 56–57: 3/4 of the inflorescence emerged in > 50% of spikes; ZGS 59: emergence of inflorescence completed; ZGS 64–65: anthesis half-way in > 50% of spikes; ZGS 71: caryopsis water ripe; ZGS 73: early milk; ZGS 75: medium milk; ZGS 77: late milk; ZGS 85: soft dough.

The disease level of the no-fungicide control plots was moderately severe in 2005 (FHB incidence: 98.0%, FHB severity: 13.7), whereas it was slight in 2006 (FHB incidence: 59.5%, FHB severity: 3.5). The mycotoxin content in grains from the control plots was also higher in 2005 (DON + NIV: 25.0 µg/g) than in 2006 (DON + NIV: 6.1 µg/g). The DK percentage, however, was higher in 2006 (11.1%) than in 2005 (8.7%). In both years, the effect of fungicide timing was significant (ANOVA; $P < 0.05$) for all disease and post-harvest parameters evaluated except TKW ($P = 0.10$ in 2005 and $P = 0.16$ in 2006). To assess the effects of fungicide application timing, we adopted the inoculation method using colonized maize kernels and sprinkler irrigation. It would be difficult to assess the effects of fungicide application timing with experiments using spray inoculation or under natural infection conditions, because with such methods, the timing of spray inoculation or natural precipitation would affect the results. The inoculation method used in this study, which provides periodic moist conditions and generates *F. graminearum* spores throughout the testing season, was most appropriate for the purposes of this study.

In both test years, fungicide application around the beginning of spent anther extrusion resulted in the best efficacy for controlling FHB and mycotoxin content in a cleistogamous barley cultivar (Fig. 10). In addition, it is noteworthy that fungicide application at later stages (21 and 29–30 DAA, ZGS 77 and 85, respectively) resulted in significantly lower DON and NIV content compared to the no-fungicide control, although it did not significantly affect disease levels. These results suggest that the optimal time for chemical control of FHB and mycotoxin contamination in cleistogamous barley is around the beginning of spent anther extrusion, rather than at anthesis. In addition, fungicide application as late as 30 DAA may also be effective for controlling mycotoxin accumulation in grain.

Although the effectiveness of late-stage fungicide application for controlling mycotoxins without controlling disease was indicated, such late application can lead to residues in the harvested grain. Thus, for commercial barley production, the use of some fungicides at late stages may be restricted. Nevertheless, our results demonstrate the potential of the late timing of treatment for controlling mycotoxin contamination in barley.

The type of fungicide may affect the results of application. We used Topsin M (thiophanate-methyl, a benzimidazole), which is a broad-spectrum systemic fungicide with both preventive and curative properties. Thiophanate-methyl is converted to methyl benzimidazole carbamate (carbendazim), and this compound interferes with nuclear division in sensitive fungi. It is likely that the systemic and/or curative properties of Topsin M are critical to effectively control mycotoxin accumulation in grain, especially when
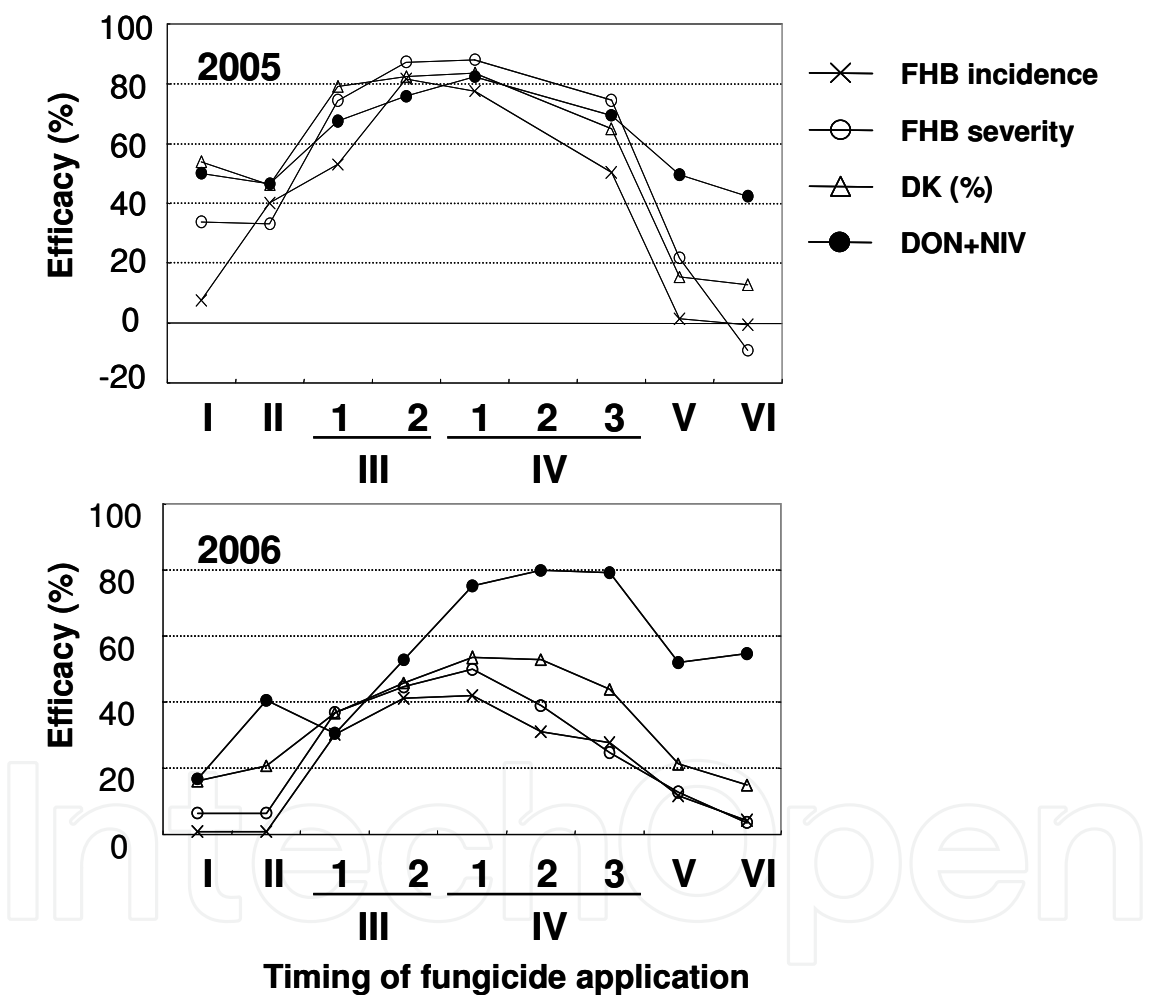

Fig. 11. Control efficacy for *Fusarium* head blight (FHB) incidence, FHB severity, percentage of discolored kernels (DK), and concentration of deoxynivalenol (DON) plus nivalenol (NIV) at various times of thiophanate-methyl fungicide application in each study year (Yoshida et al., 2008). The timing of fungicide application is indicated in the stage codes described in Table 1 (i.e., I: before anthesis; II: anthesis; III-1: 4–5 days after anthesis [DAA]; III-2: 9 DAA; before spent anther extrusion; IV-1: beginning of spent anther extrusion; IV-2: spent anther extrusion half-way; IV-3: spent anther extrusion complete; V: late milk; VI: soft dough).

applied at later stages. Further studies are needed to evaluate the efficacy of other fungicides with different properties or different modes of action in applications at the beginning of spent anther extrusion and at later stages in cleistogamous barley.

In conclusion, spent anther extrusion has not been given adequate attention thus far and is poorly documented worldwide. Not only the timing, but also the degree of extrusion may differ among cultivars or environments. It would be helpful, not only in Japan but also in other barley-growing regions, to observe spent anther extrusion in cleistogamous barley cultivars to understand the cultivar's response to FHB and to determine the timing of chemical control against FHB and mycotoxin contamination. Figure 12 shows the information transfer sheet for fungicide application timing by regional extension center. Our findings have already been confirmed by field trials by some prefectural researchers and have been distributed to farmers through the extension center.
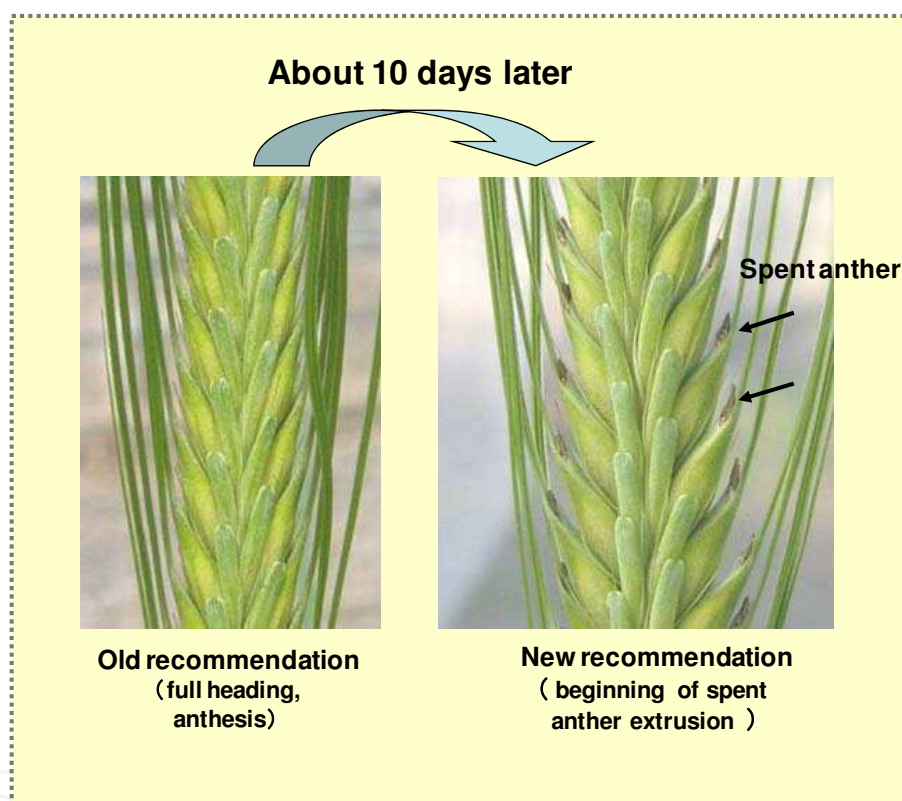
Fig. 12. Information transfer sheet prepared by the regional extension center for fungicide application timing to control FHB in closed-flowering barley.

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Fungicides

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Plant and plant products are affected by a large number of plant pathogens among which fungal pathogens. These diseases play a major role in the current deficit of food supply worldwide. Various control strategies were developed to reduce the negative effects of diseases on food, fiber, and forest crops products. For the past fifty years fungicides have played a major role in the increased productivity of several crops in most parts of the world. Although fungicide treatments are a key component of disease management, the emergence of resistance, their introduction into the environment and their toxic effect on human, animal, non-target microorganisms and beneficial organisms has become an important factor in limiting the durability of fungicide effectiveness and usefulness. This book contains 25 chapters on various aspects of fungicide science from efficacy to resistance, toxicology and development of new fungicides that provides a comprehensive and authoritative account for the role of fungicides in modern agriculture.

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