Chemical control of *Fusarium* head blight and mycotoxin contamination in barley and wheat based on mycotoxin accumulation during grain development

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Summary

Fusarium head blight (FHB) is a widespread, destructive disease of wheat and barley that is primarily caused by *Fusarium graminearum* Schwabe. *F. graminearum* produces mycotoxins such as deoxynivalenol (DON) and nivalenol (NIV) in infected grain. Fungicide application is the most practical way to control FHB, but it is not consistently effective. To obtain better chemical control, the timing of application is important. In both wheat and barley, FHB fungicides have been usually applied at or near anthesis (flowering stage) regardless of the cultivar because this stage in wheat is most susceptible. However, we found that in barley the most critical time for *F. graminearum* infection and mycotoxin accumulation differs among cultivars. Whereas chasmogamous (open-flowering) cultivars were most susceptible at anthesis, cleistogamous (closed-flowering) cultivars showed good resistance at anthesis, but were relatively susceptible after 'spent' anther extrusion (SAE). As expected from these observations, field experiments using thiophanate-methyl fungicide indicated that the optimal timing for chemical control in cleistogamous barley is around the beginning of SAE, rather than at anthesis. For wheat, the manner in which DON and NIV accumulate in grain infected with *F. graminearum* and the influence of the time of infection on mycotoxin accumulation were investigated. High levels of DON and NIV were produced in grain beyond 20 days after anthesis. This indicates the importance of the late stage of grain development in mycotoxin contamination in wheat, suggesting that control strategies that cover both the early and late stages of grain development should be developed.

Key words : barley, chemical control, deoxynivalenol, *Fusarium* head blight, nivalenol, trichotecene, wheat

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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 cereals¹⁻³⁾. These pathogens infect spikes and reduce grain yield and quality. Moreover, *Fusarium* species that cause FHB

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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 (JECFA) evaluated the risk of DON and set a provisional tolerable daily intake (PTDI) 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 in 2002. Many other countries have also established maximum allowed levels for DON in cereals and cereal products. More recently, in 2010, the Food Safety Commission in Japan set tolerable daily intake (TDI) of DON and NIV as 1 and 0.4 µg/kg body weight, respectively. In Japan, natural contamination of wheat and barley with both DON and NIV is common, therefore, control of both DON and NIV contamination is essential for wheat and barley production.

There is currently no robust single control measure by which to manage either FHB or mycotoxin contamination in wheat and barley. Fungicide application is one measure available to reduce the risk; however, results have not been highly effective or consistent⁽⁴⁻⁷⁾. To obtain increased chemical control of FHB, the timing of fungicide application would be an important factor, as well as fungicide selection, application rate, and good coverage of the spike⁷⁾.

This article introduces our recent research mainly aimed to determine the timing of fungicide application for effective reduction of mycotoxin (DON and NIV) contamination in wheat and barley⁸⁻¹⁰⁾.

Study on barley

In both wheat and barley, FHB fungicides have been usually applied at or near anthesis (flowering stage) regardless of the cultivar. This is reasonable for wheat because wheat is most susceptible to FHB during anthesis^{2, 11-13)}, at which time anthers extrude from the florets. An initial *Fusarium* infection commonly occurs on extruded anthers¹²⁾. 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. 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 FHB¹⁴⁾, consistent with the general observation that two-rowed types are more resistant than six-rowed types¹⁵⁻²⁰⁾. Cleistogamy contributes to the resistance of barley cultivars to FHB, at least for infection that occurs at anthesis¹⁴⁾. Even in the two-rowed cleistogamous cultivars with their relative resistance, however, high levels of DON and NIV contamination can occur⁸⁾.

In cleistogamous cultivars, the florets do not open and anthers are not extruded at anthesis, however, the 'spent' anthers may be pushed out from the tips of florets by the developing grain several days after anthesis²¹⁾. In Japan, this phenomenon is observed commonly in cleistogamous cultivars (Fig. 1). Such 'spent' anther extrusion (SAE) generally occurs around 10 days after anthesis (DAA). Thus, it can be said that the timing of anther extrusion differs with flowering type in barley.

With greenhouse experiments, we found that the most critical time for *F. graminearum* infection and mycotoxin accumulation in barley differs between open- and closed-flowering types. Whereas chasmogamous cultivars were found to be most susceptible at anthesis, cleistogamous cultivars were considerably resistant against FHB at anthesis, but became susceptible shortly after SAE (i.e., 10 DAA in the greenhouse experiments)⁸⁾ (Figs. 2 and 3). This was probably because the extruded spent anthers provide an initial base for the

Fig. 1. Spent anther extrusion (SAE) in the cleistogamous barley cultivar 'Nishinochikara' in a greenhouse environment⁸⁾. 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.

Fig. 2. Fusarium head blight (FHB) severity (0-100 scale) for 13 barley cultivars inoculated with *Fusarium graminearum* at anthesis and 10 days after anthesis (DAA) in the greenhouse experiments in 2004 and 2005⁸⁾. 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.

colonization of *F. graminearum* in cleistogamous cultivars.

From these results, it seemed likely that the optimal timing for chemical control of FHB and mycotoxin contamination in barley would differ with flowering type. For cleistogamous cultivars, we expected that the efficacy of fungicide application would be improved by changing the application timing from anthesis to around SAE. Therefore, we evaluated the effect of the timing of fungicide application on FHB and mycotoxin accumulation in cleistogamous barley.

The two-row cleistogamous cultivar 'Nishinochikara' was tested in the field trials in 2005 and 2006. The timings of fungicide application (see Table 1) were represented by codes from I to VI, which were arranged

Fig. 3. Fusarium head blight (FHB) severity caused 20 days after anthesis (DAA) and mycotoxin concentration at maturity in four barley cultivars and different inoculation times in the greenhouse experiment in 2004⁸⁾. 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).

Table 1. Timing of fungicide application to closed-flowering barley (cv. 'Nishinochikara') grown in the test field in Koshi Japan in 2005 and 2006.

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

^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)²²⁾ 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.

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^{23, 24)}, 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. At physiological maturity (38 and 41 DAA in 2005 and 2006, respectively), all spikes within 2 m of the middle of each single-row-plot were harvested by hand. The spikes were dried at 70 °C for 24 h, and were threshed with a thresher. The harvested grain samples were cleaned manually. The percentage of discolored kernels (DK), thousand kernel weight (TKW, g), DON and NIV contents were measured for each grain sample. The DON and NIV contents were analyzed for 100 g of each grain sample using the previously described enzyme-linked immunosorbent assay (ELISA) system²⁵⁾ of the KM Assay Center, Kyowa Medex Co., Ltd. (Nagaizumi-cho, Shizuoka, Japan).

In both test years, fungicide application around the beginning of SAE resulted in the best efficacy for

Fig. 4. 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 on the cleistogamous barley in each study year¹⁰⁾. 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).

controlling FHB and mycotoxin content in the cleistogamous barley (Fig. 4). These results suggest that the optimal time for chemical control of FHB and mycotoxin contamination in cleistogamous barley is around the beginning of SAE, rather than at anthesis¹⁰. Our findings have already been confirmed by field trials by some prefectural researchers, and beginning of SAE is currently recommended as fungicide application timing for two-rowed, closed-flowering barley in Japan.

Study on wheat

For wheat, it is reasonable that anthesis is considered the optimal growth stage for fungicide application to control FHB. This is because wheat is most susceptible to FHB during anthesis^{2, 11-13}, at which time anthers extrude from the florets. However, the toxin-reducing effect of fungicide application is not sufficiently stable or high. We considered it is important to know when fungal toxin production mainly occurs during grain development because if we can control toxin production during this period, the final toxin level would be effectively reduced. Thus, we investigated the accumulation manner of DON and NIV in developing grain as well as the influence of infection timing on DON and NIV accumulation in wheat.

Four cultivars were tested in a greenhouse environment, where the plants were spray-inoculated at three different stages with a mixture of DON and NIV chemotypes of *F. graminearum*. The results indicated that

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 wheat cultivars in the greenhouse experiments in 2004⁹. 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.

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Fig. 6. Deoxynivalenol (DON) and nivalenol (NIV) concentration in grains at two developmental stages for the seven wheat cultivars in the field experiments in 2007 and 2008⁹⁾. 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.

high levels of DON and NIV were produced beyond 20 DAA, i.e., in the latter period of grain development, even by early infection (i.e., 0 and 10 DAA infection) (Fig. 5). The results of field experiments performed on seven cultivars, where inoculation was conducted using colonized maize kernel inoculum and mist irrigation, were consistent with the greenhouse results (Fig. 6). In addition, 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 spike⁹.

To date, strategies for controlling FHB and toxin contamination have been developed, mostly focusing on infection during the flowering stage of the host. However, our results indicated the importance of the late stages of grain development for toxin contamination in addition to the early stage in wheat. 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 previously infected fungus. Studies to test the effectiveness of such 'late control' for reducing toxin contamination are currently underway.

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大麦および小麦の赤かび病かび毒蓄積特性に基づいた薬剤散布適期の解明

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麦類の赤かび病によるかび毒低減対策として、特に麦の生産段階における適切な薬剤散布が重要である。従来、大麦の赤かび病防除適期は小麦の例に倣って開花期（またはほぼ同時期に相当する穂揃い期）とされてきたが、改めて検討した結果、閉花受粉性の二条大麦では、開花 10 日後頃の「葯殻抽出期」が赤かび病およびかび毒蓄積に対し急激に感受性が高まる時期であり、薬剤散布適期であることが明らかになった。また小麦では、登熟期間後半（開花 20 日後以降）に、穀粒中のかび毒蓄積量の大幅な増加が起こることが明らかになり、小麦のかび毒汚染低減には、開花期頃の感染抑制に加え、登熟期間後半のかび毒蓄積抑制が重要であることが示唆された。

キーワード：大麦, 薬剤防除, デオキシニバレノール, 赤かび病, ニバレノール, トリコテセン, 小麦