Distribution of Causal Fusaria of Wheat and Barley Scab in Japan

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Abstract

Fusarium species were isolated from kernels or glumes of scab heads of wheat and barley collected from 202 fields in seven different districts of Japan from 1979 to 1987. Virulence to wheat heads of 273 isolates of five *Fusarium* species and six cultivars of *F. roseum* from the kernels and glumes was tested by spray-inoculation. *F. roseum* 'Graminearum', 'Avenaceum', 'Culmorum', 'Acuminatum', *F. nivale* and *F. tricinctum* caused scab symptoms on the inoculated wheat heads, and virulent isolates of *F. tricinctum* belonged to *F. sporotrichioides* and *F. tricinctum* based on Booth's descriptions. *F. roseum* 'Graminearum' causing scab symptoms was distributed all over Japan. *F. roseum* 'Avenaceum' virulent to wheat heads, however, was isolated from the samples in Hokkaido and Chugoku-Shikoku, while the respective virulent isolates of 'Culmorum' and 'Acuminatum' were distributed in Hokkaido and Tohoku, and in Tohoku, Hokuriku, Kanto-Tosan and Chugoku-Shikoku, respectively. *F. nivale* and *F. tricinctum* virulent to wheat heads were encountered in the samples from Hokkaido and Hokuriku, and from Hokkaido, Tohoku, Hokuriku and Chugoku-Shikoku, respectively.

(Received March 2, 1990)

Key words: distribution, causal fungi, scab, wheat and barley.

INTRODUCTION

Fusarium roseum Link emend. Snyder et Hansen f. sp. *cerealis* (Cooke) Snyder et Hansen 'Graminearum' (= *F. graminearum* Schwabe²⁾), 'Culmorum' (= *F. culmorum* (W.G. Smith) Saccardo²⁾), 'Avenaceum' (= *F. avenaceum* (Fries) Saccardo²⁾) and *F. nivale* (Fries) Cesati f. sp. *graminicola* (Berkeley et Broome) Snyder et Hansen are known to be the causal fungi of scab of cereals in North America⁴⁾, Europe³⁾ and China⁵⁾. In Japan, however, *F. roseum* f. sp. *cerealis* 'Graminearum'¹⁶⁾ and 'Avenaceum'²⁴⁾ were only reported as the pathogens of wheat and barley scab before this research started. The purpose of this investigation is to analyze the distribution of the causal fungi of wheat and barley scab in Japan.

MATERIALS AND METHODS

Samples. Samples of light-grains casted from the combines or scab heads of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) were collected from 202 fields in seven

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Table 1. Numbers of sampling fields, kernels and scab heads of wheat and barley used for *Fusarium* isolation and year of sampling

District	No. of fields sampled	No. of kernels and scab heads	Year
Hokkaido	32	960	1983
Tohoku	13	571	1979, 1985
Kanto-Tosan	32	844	1979, 1981, 1985
Hokuriku	7	270	1985
Tokai-Kinki	44	822	1979, 1981, 1985, 1987
Chugoku-Shikoku	50	1,122	1979, 1981, 1987
Kyushu	24	232	1980, 1981, 1987
Total	202	4,821	

different districts of Japan in 1979, 1981, 1983, 1985 and 1987 (Table 1) and kept at 4°C until examination.

Isolation and identification of *Fusarium*. Twenty to ninety kernels or glumes of one to eleven scab heads of wheat or barley were taken at random from each sample for fungal isolation. They were sterilized for 3 min in 1% aqueous sodium hypochlorite and washed three times in sterile water, then placed on potato-sucrose agar (PSA) or 2% water agar containing 100 mg/l of streptomycin sulfate in petri dishes. Five kernels or five pieces of glumes from the heads per dish were plated and incubated at 25°C. *Fusarium* colonies developed from them were transferred to PSA slants and single-spore isolation was made. All isolates were identified to species according to the descriptions of Snyder and Hansen¹⁹⁻²²⁾ and Matuo¹²⁾. However, some isolates identified as *F. tricinctum* (Corda) Saccardo emend. Snyder et Hansen were re-identified following the Booth's descriptions²⁾ since two out of the Booth's²⁾ four species, *F. poae* (Perk) Wollenweber, *F. sporotrichioides* Sherbakoff, *F. fusarioides* (Fragoso et Ciferri) Booth and *F. tricinctum* (Corda) Saccardo, which constitute *F. tricinctum* were reported virulent to wheat heads^{1,23,27)}.

Test plants. Three wheat seeds (cv. Fujimikomugi or Omasekomugi) were sown 30 cm apart in 240 cm rows at 60 cm intervals at the end of October from 1979 to 1989 in a 15 × 5 m-field plot. In April, wheat plants before heading in the plot were covered with a plastic-film house shaded with a black net over the roof (shielding rate of light, 0.52). The plants at the flowering stage were used for inoculation tests.

Virulence tests. Virulence of the 210 typical isolates from fields and the 63 stock cultures from the National Institute of Hygienic Sciences was tested from 1980 to 1989. To produce inoculum, each isolate was inoculated on oatmeal agar in two petri plates at 25°C for 5 to 7 days, and then the aerial mycelium was washed off with a paintbrush in sterilized water. The washed plates were exposed under black-light-blue lamps (maximum effective wave-length, 360 nm) to induce sporulation at 20°C for 3 days. The culture was then scraped with a paintbrush in sterilized water to harvest the spores and the suspension was filtered through tissue paper. Spore suspension (10^{4-6} spores/ml) of each isolate was prepared.

To prevent contamination between the isolates in a test field, every third plant was used and about twenty heads of each selected stand were covered with a plastic-film bag (45 × 65 cm) before inoculation. The heads of the plants were sprayed with 30 ml of the spore suspension from the lower slit of the bag. The plastic-film house was moistened by three humidifiers (1.5 l/hr, MG-type, Sanken Inc., Tokyo) for 2 days after inoculation and then the bags were removed. Symptoms of the inoculated heads of the plants were examined 10 to 15 days after inoculation. Reisolation from the scab heads of the inoculated plants was performed after observation.

RESULTS

Isolation of Fusarium species

Fusarium species were isolated from 936 (19.4%) of 4,821 kernels and scab heads of wheat and barley in Japan. They were *F. roseum* 'Graminearum', 'Avenaceum', 'Culmorum', 'Acuminatum', 'Equiseti', 'Semitectum', *F. nivale*, *F. tricinctum*, *F. oxysporum* Schlechtendahl and unidentified *Fusarium* spp. The most common species was *F. roseum* 'Graminearum', followed by *F. nivale*, *F. roseum* 'Avenaceum' and *F. tricinctum* (Table 2).

F. nivale predominated over the other *Fusarium* species in Hokkaido in 1983 when it was a cool summer, while *F. roseum* 'Graminearum' did in other districts. *F. nivale* was also isolated from the samples in Tohoku and Hokuriku. *F. roseum* 'Avenaceum' was associated with wheat and barley kernels in Hokkaido and Chugoku-Shikoku, while *F. roseum* 'Culmorum' was encountered among isolates from the Hokkaido and Tohoku samples. From wheat and barley kernels in Tohoku and Hokuriku, *F. roseum* 'Acuminatum' was isolated, and *F. tricinctum* was obtained from the Hokkaido, Tohoku, Hokuriku and Chugoku-Shikoku samples. Other species, *F. roseum* 'Equiseti', 'Semitectum' and *F. oxysporum*, were isolated from the samples in Tohoku and Kanto-Tosan, in Hokkaido, and in Hokkaido and Tohoku, respectively (Table 2).

Virulence tests

Table 3 shows the results of virulence tests. Symptoms on inoculated wheat heads were divided into four groups: "S", "M", "W" and "N". "S" indicates symptoms with discolored spikelets with mycelia on the whole or a part of heads, "M" partially discolored spikelets with mycelia on a part of heads, "W" partially browned spikelets, and "N" no symptoms. The "S" and "M" symptoms were recognized identical to scab symptoms.

Scab symptoms on wheat heads were caused by *F. roseum* 'Graminearum', 'Avenaceum', 'Culmorum', 'Acuminatum', *F. nivale* and *F. tricinctum* (Fig. 1). *F. roseum* 'Equiseti', 'Semitectum', *F. oxysporum* and *F. moniliforme* Sheldon did not cause scab symptoms. Among the isolates of *F. tricinctum*, those belonging to *F. sporotrichioides* and *F. tricinctum* of Booth's species²⁾ were virulent to wheat heads (Table 3). Cultures of the virulent *Fusarium* species re-isolated from kernels of the inoculated wheat heads were identical with the original isolates of the species.

Distribution of causal Fusarium of wheat and barley scab in Japan

Figure 2 shows the distribution of *Fusarium* species which caused scab symptoms on wheat heads after spray-inoculation. Virulent isolates of *F. roseum* 'Graminearum' were distributed all over the country. *F. roseum* 'Avenaceum' virulent to wheat heads, however, was isolated from the samples in Hokkaido and Yamaguchi Prefecture (a part of Chugoku-Shikoku) (Fig. 2).

F. roseum 'Culmorum' caused scab symptoms was associated with wheat kernels from three fields: two fields in Hokkaido and one field in Iwate Prefecture (a part of Tohoku), and isolates of *F. nivale* from the samples in Hokkaido and Toyama Prefecture (a part of Hokuriku) were virulent to wheat heads (Fig. 2).

F. roseum 'Acuminatum' was distributed in Tohoku, Kanto-Tosan, Hokuriku, Tokai-Kinki and Chugoku-Shikoku. The isolates of the cultivar causing scab symptoms on wheat heads were obtained from wheat and barley kernels in all the districts except Tokai-Kinki (Fig. 2).

F. tricinctum was isolated from wheat and barley kernels and glumes in Hokkaido, Tohoku, Kanto-Tosan, Hokuriku, Chugoku-Shikoku, and 10 out of 11 virulent isolates belonged to *F. sporotrichioides* based on Booth's descriptions²⁾. These 10 isolates were distributed in Hokkaido and Iwate (a part of Tohoku), Ishikawa (a part of Hokuriku) and Kagawa (a part of Chugoku-Shikoku) Prefectures. While the other virulent isolate, which belonged to *F. tricinctum* by Booth's taxon²⁾, was obtained from a barley kernel in Ishikawa Prefecture (Fig. 2).

Table 2. Distribution of *Fusarium* species on kernels and scab heads of wheat and barley in Japan

Species	Hokkaido			Tohoku			Kanto-Tosan			Hokuriku			Tokai-Kinki			Chugoku-Shikoku			Kyushu			Total		
	PF ^{a)}	PS ^{b)}		PF	PS		PF	PS		PF	PS		PF	PS		PF	PS		PF	PS		PF	PS	
<i>F. roseum</i>																								
‘Graminearum’	56.3	5.6 (23.6)		92.3	25.9 (87.1)		65.6	15.8 (95.7)		85.7	26.3 (53.4)		43.2	11.8 (100)		46.0	6.2 (94.6)		79.2	40.5 (100)		57.9	13.8 (71.3)	
‘Avenaceum’	25.0	2.4 (10.0)														2.0	0.2 (2.7)					4.5	0.5 (2.7)	
‘Culmorum’	3.1	0.1 (0.4)		7.7	0.2 (0.6)																1.0	+ ^{c)} (0.2)		
‘Acutinatum’				7.7	0.9 (2.9)					14.3	0.7 (1.5)										1.0	0.1 (0.7)		
‘Equiseti’				7.7	0.2 (0.6)		6.3	0.5 (2.9)													1.5	0.1 (0.5)		
‘Semitectum’	3.1	0.1 (0.4)																			0.5	+ (0.1)		
<i>F. nivale</i>	71.9	13.5 (56.8)		15.4	1.0 (3.5)					28.6	21.4 (43.6)										13.4	4.0 (20.7)		
<i>F. tricinatum</i>	37.5	1.8 (7.4)		23.1	0.7 (2.4)					14.3	0.7 (1.5)					2.0	0.1 (1.4)				8.4	0.5 (2.6)		
<i>F. oxysporum</i>	3.1	0.1 (0.4)		7.7	0.5 (1.8)															1.0	+ (0.4)			
<i>Fusarium</i> spp. (unidentified)	6.5	0.2 (0.8)		7.7	0.4 (1.1)		6.3	0.2 (1.4)								2.0	0.1 (1.4)				4.5	0.2 (0.7)		
Total		23.8 (100)		29.8 (100)			16.5 (100)			49.3 (100)			11.8 (100)				6.6 (100)			40.5 (100)			19.4 (100)	

a) PF: Percentage of fields where each *Fusarium* species was isolated to the total number of fields examined.b) PS: Percentage of kernels and scab heads of wheat and barley yielding each *Fusarium* species to the total number of kernels and scab heads examined.Number in parenthesis indicates the percentage of the kernels and heads yielding each *Fusarium* species to the kernels and heads yielding all the *Fusarium* species.

c) +: Less than 0.1%.

Fig. 1. Symptoms on heads of wheat cv. Fujimikomugi or Omasekomugi after inoculation with *Fusarium roseum* 'Graminearum' (A), 'Avenaceum' (B), 'Culmorum' (C), 'Acuminatum' (D), *F. nivale* (E) and *F. tricinctum* (= *F. sporotrichioides*) (F).

DISCUSSION

F. roseum 'Graminearum', 'Avenaceum', 'Culmorum' and *F. nivale* are generally recognized as the pathogens of wheat and barley scab^{3,4,28}. In Japan, however, *F. roseum* 'Graminearum'¹⁶, 'Avenaceum'²⁴ were reported to be the causal fungi of wheat and barley scab, and *F. nivale* was also recognized to be the causal fungus when this research proceeded^{9,11}. This paper first showed wheat and barley scab caused by *F. roseum* 'Culmorum' and the scab symptoms of wheat heads caused by *F. roseum* 'Acuminatum' and *F. tricinctum* in Japan.

F. roseum 'Acuminatum' and *F. tricinctum* are not generally regarded as the causal agents of wheat and barley scab^{3,4,28}. The China research team on wheat scab¹, however, reported that *F. acuminatum* (= *F. roseum* 'Acuminatum') isolated from blighted wheat heads showed a medium level of pathogenicity against wheat heads, and similar results were reported in North America^{13,23}. In the current experiment, 12 among the 14 isolates of *F. roseum* 'Acuminatum' from kernels of wheat and barley caused scab symptoms on the inoculated wheat heads (Table 3). Though *F. roseum* 'Acuminatum' did not reduce yield in wheat¹⁰, it induced discoloration

Table 3. Virulence^{a)} of 273 isolates of five *Fusarium* species and six cultivars from kernels and scab heads of wheat and barley to wheat heads

Species	No. of isolates examined	Symptoms ^{b)}			
		S	M	W	N
<i>F. roseum</i> 'Graminearum'	128 (14 ^{c)})	125	2	1	
" 'Avenaceum'	15 (0)	2	11	2	
" 'Culmorum'	4 (2)	3	1		
" 'Acuminatum'	14 (9)	2	10	1	1
" 'Equiseti'	7 (4)			7	
" 'Semitectum'	5 (4)			1	4
<i>F. nivale</i>	52 (0)	2	15	27	8
<i>F. tricinctum</i> (= <i>F. sporotrichioides</i> ^{d)})	11 (7)		10	1	
" (= <i>F. tricinctum</i> ^{d)})	3 (3)		1	1	1
" (= <i>F. poae</i> ^{d)})	18 (18)			18	
"	10 (0)			10	
<i>F. oxysporum</i>	4 (0)				4
<i>F. moniliforme</i>	2 (2)			2	

a) For ca. 20 heads of wheat (cv. Fujimikomugi or Omasekomugi) at the flowering stage inoculated, the symptoms were examined 10 to 15 days after inoculation.

b) Symptoms on inoculated wheat heads were separated into the four groups: S, discolored spikelets with mycelia on the whole or a part of heads; M, partially discolored spikelets with mycelia on a part of heads; W, partially browned spikelets; N, no symptoms. The S and M symptoms were identical to those of scab.

c) Number in the parenthesis indicates the stock isolates from the National Institute of Hygienic Sciences.

d) Species after Booth (1971)²⁾.

of the kernels on the inoculated wheat heads¹⁰⁾, and a mycotoxin was detected from the discolored kernels⁶⁾. From the above facts, we suggest that *F. roseum* 'Acuminatum' should be included in the causal agents of wheat scab.

Vargo and Baumer²⁷⁾ reported that symptoms of wheat heads inoculated with *F. sporotrichioides* (= *F. tricinctum*) were identical with those of scab. Stack and McMullen²⁸⁾ showed that *F. graminearum*, *F. culmorum*, *F. sporotrichioides* (= *F. tricinctum*) and *F. acuminatum* caused wheat head necrosis beyond the site of inoculation. Our 10 isolates belonging to *F. sporotrichioides* (Booth's taxon²⁾) in *F. tricinctum* (Snyder and Hansen's taxon²¹⁾) also caused scab symptoms on the inoculated wheat heads (Table 3). *F. sporotrichioides* did not reduce yield in wheat⁷⁾, but the species induced discoloration of the kernels on the inoculated wheat heads⁷⁾ as *F. roseum* 'Acuminatum' did. These suggest that *F. sporotrichioides* is also a pathogen of wheat scab.

The China research team on wheat scab¹⁾ also reported that *F. tricinctum* (Booth's taxon²⁾) showed a medium level of pathogenicity to wheat heads, and one out of the three isolates examined in this study, which belonged to *F. tricinctum* (Booth's taxon) in *F. tricinctum*, exhibited similar virulence (Table 3).

The difference in the geographic distribution among some causal *Fusarium* species of wheat and barley scab in this study was similar to that indicated by Cook⁴⁾. Temperature may affect the distribution⁴⁾. *F. nivale* was distributed in Hokkaido, Tohoku and Hokuriku districts with snow cover on the ground in winter. Most of our Hokkaido, Tohoku and Hokuriku isolates caused snow mold symptoms to wheat seedlings⁸⁾. Lesions of leaf blades, leaf sheaths and heads of wheat caused by *F. nivale* were also observed in the fields of Hokkaido^{14,17,26)} and Tohoku¹⁵⁾ and similar lesions of barley were reported in the fields where snow mold occurred severely in Hokuriku⁸⁾. It is thus evident that the causal fungus of snow mold, *F. nivale*, infected leaves and heads of wheat and barley, and caused the scab of the plants in Hokkaido, Tohoku and Hokuriku.

Fig. 2. Distribution of causal fungi of wheat and barley scab in Japan. *Fusarium* species causing scab symptoms to inoculated wheat heads (● and ▲), with a low pathogenicity (□ and △), without inoculation (○) and the stock strains of the National Institute of Hygienic Sciences (triangles) were isolated from locations marked with respective symbols.

Although the classification of *F. nivale* is controversial^{18,25}, we used this species name here.

We wish to thank the personnel concerned of the Agricultural Experiment Stations and Plant Protection Offices throughout Japan for supplying samples.

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和 文 摘 要

小泉信三・加藤 肇・吉野嶺一・林 長生・一戸正勝：ムギ類赤かび病菌のわが国における種類と分布

1979～87年に全国202地点で採集したムギ類の粒あるいは赤かび病類似症の穂から *Fusarium* 菌を分離・同定した。これらと国立衛生試験所保存のムギ類粒から分離された *Fusarium* 菌を開花盛期のコムギ穂に噴霧接種した。5種 (*F. roseum* の6 cultivarを含む) 273菌株のうちコムギ穂に赤かび病症状を呈したのは *F. roseum* ‘Graminearum’, ‘Avenaceum’, ‘Culmorum’, ‘Acuminatum’, *F. nivale* および *F. tricinctum* であった。ただし、*F. tricinctum* のうち、Booth (1971) の分類により細分される *F. sporotrichioides* と *F. tricinctum* には病原性のあるものが存在した。コムギ穂に病原性を示す ‘Graminearum’ は全国各地に分布したが、‘Avenaceum’ は北海道と山口県、‘Culmorum’ は北海道と岩手県の試料のみから分離された。また、病原性のある ‘Acuminatum’ は東北・北陸・関東・四国、*F. nivale* は北海道・北陸、*F. tricinctum* は北海道・東北・北陸・四国におおの分布した。本研究により本病原菌としてわが国では既知の *F. roseum* ‘Graminearum’, ‘Avenaceum’ の他に *F. nivale*, *F. roseum* ‘Culmorum’ が存在し、*F. roseum* ‘Acuminatum’, *F. tricinctum* も本病原菌となりうる事が明らかとなった。