JARQ 35(4), 221 – 226 (2001) http://www.jircas.affrc.go.jp

Claviceps sorghicola and *C. africana*, the Ergot Pathogens of Sorghum, and their Cultural Control in Japan

Takao TSUKIBOSHI[1]*, Tadayuki SHIMANUKI[2] and Hironori KOGA[3]

[1] Natural Resources Inventory Center, National Institute for Agro-Environmental Sciences (Tsukuba, Ibaraki 305–8604, Japan)
[2] Department of Agro-Environmental Sciences, National Agricultural Research Center for Hokkaido Region (Sapporo, Hokkaido 062–8555, Japan)
[3] Research Institute of Agricultural Resources, Ishikawa Agricultural College (Nonoichi, Ishikawa 921–8836, Japan)

Abstract

Ergot pathogens of sorghum occurring in Japan were identified as *Claviceps sorghicola* and *C. africana*. *C. sorghicola* is the original fungus and it differs from the other *Claviceps* species infecting Sorghum in the color and texture of the sclerotia, morphology of stromata with clavicipitaceous stipes and capitula and the size of ascospores and conidia. It is predominant and widely distributed in Japan. *C. africana* is a pathogen with worldwide distribution which occurs mainly in the southern part of Japan. Host ranges of these fungi were restricted only to the genus *Sorghum*, based on the results of inoculation tests. Field tests showed that the method applied to sow the resistant sorghum lines at an earlier time was effective to avoid or control the occurrence of the ergot.

Discipline: Plant disease
Additional key words: sudangrass, resistance, seeding time

Introduction

Sorghum is an important forage crop in Japan and it is cultivated over about 28,000 ha mainly in the southern part of the country. Ergot of sorghum is a serious disease occurring worldwide due to recent expansion from Africa and Asia to the Americas and Australia[3,13]. Since ergot-infected flowers produce honeydew, followed by the production of sclerotia, the disease is a threat not only to seed production but also has health implications for livestock due to the potential toxicity of the alkaloids produced in sclerotia[2]. *Claviceps sorghi* Kulkarni, Seshadri & Hegde[11] and *C. africana* Frederickson, Mantle & De Milliano[9] were identified as the predominant ergot pathogens of sorghum occurring in India (*C. sorghi*) and Africa, Australia and the Americas (*C. africana*). The pathogens differ from each other in morphology and induce diseases with different symptomatologies[9].

In Japan, sorghum ergot was first observed in the Kyushu district, in southern Japan in 1985[17]. Ergot also occurs on sudangrass (*S. sudanense* (Piper) Stapf). The disease became more widely distributed in the 1990s and has begun to cause serious damage. We identified 2 types of sorghum ergot in Japan. Type I produces a light-brown honeydew in the infected flowers (Fig. 1A) and then hard black-purple sclerotia that are 0.5–2 cm in length, conical, and covered with white sphacelial tissues (Fig. 1C). Black mass of the saprophyte *Cerebella* [*Epicoccum*] sp. is often associated with the honeydew (Fig. 1B). Type II produces a transparent to pinkish honeydew, the surface of which is covered with a white mass of secondary conidia, and the whole infected head looks white and powdery (Fig. 2A). Ergot sclerotia are difficult to detect because they are produced between the glumes of the infected florets. We describe the morphology of both fungi with the method of control of the disease in fields.

Materials and methods

1) Collection and incubation of samples

Samples of the honeydew and sclerotia of Japanese ergot were collected from sorghum at 9 sites in 6 Prefectures in Japan (Tochigi, Chiba, Mie, Miyazaki, Kuma-

*Corresponding author: fax +81–298–38–8199, e-mail tuki@affrc.go.jp
Received 22 January 2001, accepted 24 April 2001.

Fig. 1.

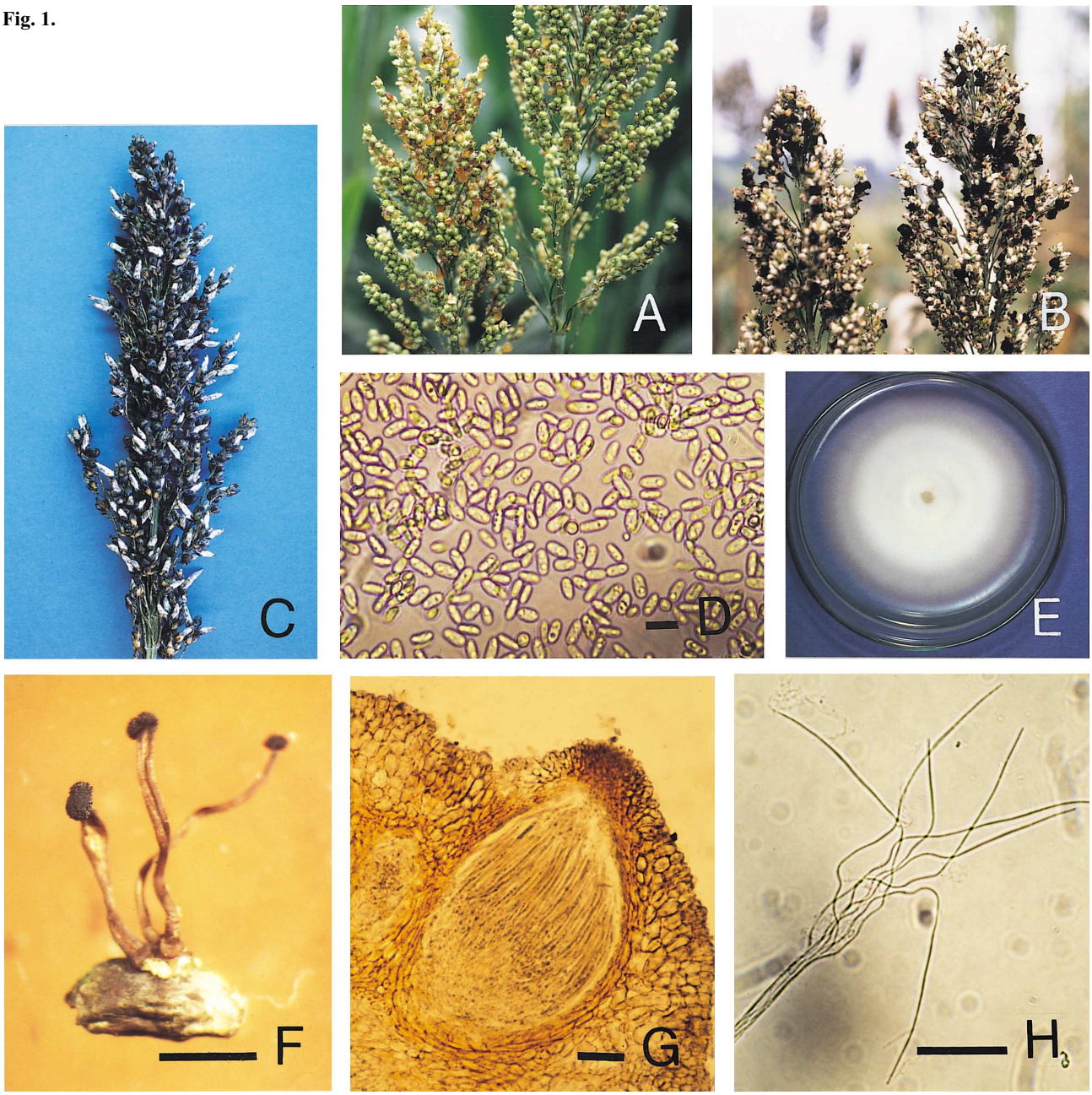
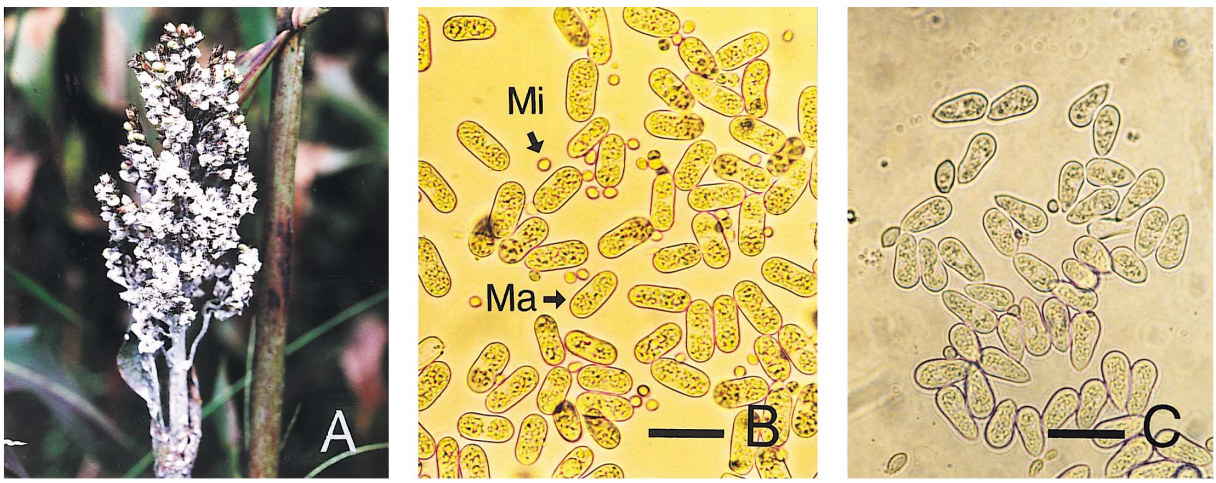


Fig. 2.



moto and Kagoshima Prefectures). Samples of *C. purpurea* (Fr.) Tul. from Italian ryegrass (*Lolium multiflorum* Lam.) were also collected in Tochigi Prefecture. Honeydew samples and sclerotia were stored dry on a filter paper in a refrigerator at 5ºC. Sclerotia collected from sorghum in Nishinasuno, Tochigi, were incubated on moist sand at 20–23ºC and under a daily cycle of light and darkness. Sclerotia were observed for germination after 2 months. Sclerotia of Type I were also placed on the soil in the sorghum field at Nishinasuno, late in May to observe germination under field conditions.

2) Inoculation

Dried honeydew of each type of sorghum ergot was diluted in sterile distilled water with 0.1% of a wetting agent (Tween 20) to approximately 10^6 spores / mL. The heads of sorghum (cv. P931, Pioneer Co.), in which approximately 50% of the florets were flowering, were atomized with about 3 mL aliquots. After inoculation, each head was covered with a plastic bag for 48 h. Following bag removal, the inoculated plants were kept in a greenhouse at 25–30ºC. After 2 weeks, honeydew produced from infected florets was collected and used for host range testing.

3) Host range tests

Eleven gramineous plants shown in Table 1, including sorghum (*Sorghum bicolor* (L.) Moench, cv. TX2727), sudangrass (*S. sudanense* (Piper) Stapf, cv. Piper), orchardgrass (*Dactylis glomerata* L., cv. Aonami), Italian ryegrass (*Lolium multiflorum* Lam., cv. Waseyutaka), perennial ryegrass (*L. perenne* L., cv. Friend), tall fescue (*Festuca arundinacea* Schreb., cv. Kentucky 31), meadow fescue (*F. elatior* L., cv. Fast), pearl millet (*Pennisetum americanum* (L.) Leeke, cv. unknown), rye (*Secale cereale* L., cv. Hatsuharu), Kentucky bluegrass (*Poa pratensis* L., cv. unknown), and silvergrass (Japanese plume grass, *Miscanthus sinensis* Anderss.) were cultivated in the greenhouse and their flowering heads were inoculated with fresh honeydew of Type I and Type II pathogens as described above. An isolate of *C. purpurea* from Italian ryegrass was also tested for pathogenicity as a control. The inoculated heads were observed for the production of honeydew and sclerotia 10 days and 1 month after inoculation, respectively.

4) Field tests

Field tests to develop a cultural method for the control of sorghum ergot were performed. One resistant sorghum/sudangrass hybrid (K-70, Kaneko Co.), one medium-resistant sorghum/sudangrass hybrid (P988, Pioneer Co.) and one susceptible cultivar (Sugar Graze, male sterile sorghum cultivar) were selected based on the results of the inoculation tests previously conducted[19]. Tested cultivars were sown from mid-May to late June at intervals of 3 weeks. The ergot severity caused by Type I was estimated under natural infection conditions in November with a disease rate of 1 for 1%, 2 for 5%, 3 for 10%, 4 for 20% and 5 for 35% of florets ergotized.

Results

1) Identification of the ergot fungi

We have already described Type I as a new species, *Claviceps sorghicola* Tsukiboshi, Shimanuki & Uematsu based on the morphology which is distinctly different from that of the already reported sorghum ergot pathogens[21]. Sclerotia are cylindrical or conical, curved or straight, hard, 2.5–20 × 1.9–3.5 mm in size. The surface of the sclerotium is purple-black to black under white sphacelial tissues with several longitudinal grooves. Sclerotia bear white sphacelial caps at the distal end. The incubated sclerotia germinate to produce stromata with clavicipitaceous stipes and capitula both in incubated and field samples (Fig. 1F). Stipes are bronze to brown and capitula are dark brown, globose to subglobose. The capitulum surface is distinctly papillate. Perithecia are ovate to pyriform, and filled with asci that are hyaline and cylindrical (Fig. 1G). Ascospores are hyaline, 8 per ascus, filiform, and measure 122.5–215 µm in length (Fig. 1H). Conidia from honeydew and the sphacelial tissues of the sclerotia, are hyaline, ellipsoidal to oval, aseptate and 5–11.3 × 2.5–3.8 µm in size (Fig. 1D). Microconidia and secondary conidia are never

Fig. 1. Symptoms caused by *Claviceps sorghicola*, the pathogen of sorghum ergot

A: Light brown honeydew exuding from sorghum flowers infected with *C. sorghicola*. B: Black mass of the saprophyte *Cerebella* sp. associated with the honeydew. C: Ergotized florets of sorghum, sphacelia protruding from infected florets. D: Conidia in the honeydew (bar: 10 µm). E: Colony morphology on PDA. F: Mature stromata with papillate capitula germinated from sclerotium (bar: 5 mm). G: Perithecia filled with asci produced on the surface of capitula (bar: 20 µm). H: Ascospores from a single ascus (bar: 20 µm).

Fig. 2. Symptoms caused by *Claviceps africana*, the pathogen of sorghum ergot

A: White powdery head with honeydew of infected sorghum. B: Macroconidia (Ma) and microconidia (Mi) in the honeydew (bar: 20 µm). C: Secondary conidia on the surface of honeydew (bar: 20 µm).

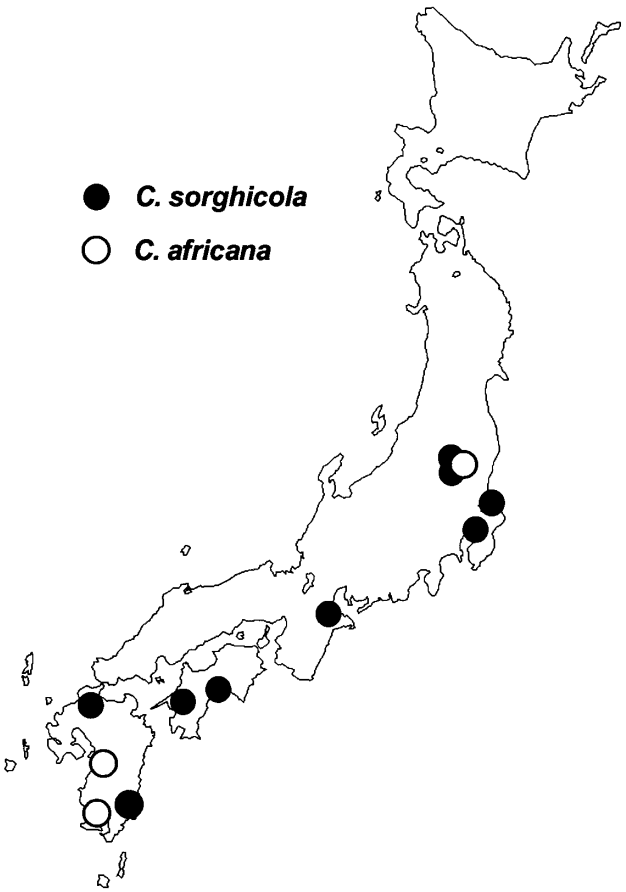


Fig. 3. Distribution of *Claviceps sorghicola* and *C. africana* in Japan

observed in nature. The conidia germinate with 4–5 germ tubes and soon produce hyphae in water after 16 h. *C. sorghicola* is distinct from *C. sorghi* and *C. africana* in the color and texture of the sclerotia, color and morphology of the stromata arising from the sclerotia, and size of the ascospores and conidia. *C. sorghicola* had already been confirmed to be different from the other *Claviceps* species based on differences in the nucleotide sequences of internal transcribed spacer (ITS) 1 and 5.8s rDNA regions[16,18].

Type II produces macroconidia and microconidia in honeydew (Fig. 2B). Macroconidia are hyaline, aseptate, oblong to oval, 8.8–16.3 × 5–8.8 μm in size and slightly constricted in the center. Microconidia are hyaline, aseptate, spherical to subspherical, 2.5–5 μm in diameter. Type II also produces secondary conidia with the conidiophores erupting through the surface of honeydew. Secondary conidia are hyaline, aseptate, pear-shaped, 11.3–18.8 × 5–7.5 μm in size and slightly constricted in the center (Fig. 2C). The teleomorph has never been produced *in vitro*. The fungus had already been identified as *C. africana* (anamorph: *Sphacelia sorghi* McRae) based on the morphology [20], the pattern of alkaloid production[13] and the sequence of rDNA ITS region (Tooley, P. W., personal communication).

2) Distribution of sorghum ergot in Japan

We observed that sorghum ergot caused by *C. sorghicola* is widely distributed in the region south from Tochigi (Kanto district) to Miyazaki (Kyushu district) (Fig. 3). The disease was first reported in Miyazaki Prefecture in 1985 and most recently in Ehime Prefecture in

Table 1. Results of inoculation of *C. sorghicola*, *C. africana* and *C. purpurea* to 11 gramineous plants

	C. sorghicola	*C. africana*	*C. purpurea*
C4-plants			
Sorghum bicolor	+	+	–
S. sudanense	+	+	–
Pennisetum americanum	–	–	–
Miscanthus sinensis	–	–	–
C3-plants			
Dactylis glomerata	–	–	+
Festuca arundinacea	–	–	+
F. elatior	–	–	+
Lolium multiflorum	–	–	+
L. perenne	–	–	+
Poa pratensis	–	–	+
Secale cereale	–	–	+

+: Infection occurred, –: Absence of infection.

2000. The damage on sorghum is most severe in the Kyushu and Shikoku districts in the southern part of Japan. Most of the plants in the field are affected by the disease and most of the flowers in the head are ergotized when infected severely. The occurrence of *C. africana* was first observed in Kumamoto Prefecture in 1991 and most recently Tochigi Prefecture in 1996. The distribution of the fungus and damage on sorghum production are rather restricted in Japan compared with *C. sorghicola*.

3) Host range

C. sorghicola and *C. africana* infected sorghum and sudangrass in the inoculation tests using conidia produced in the honeydew (Table 1). However, pathogenicity to other C4-plants such as pearl millet, silvergrass and C3-plants such as orchardgrass, ryegrass, bluegrass, fescue and rye was not detected. *C. purpurea* infected a wide range of C3-plants such as ryegrass, fescue, bluegrass and rye, as reported previously[12].

4) Field tests

In the field tests using the selected resistant and susceptible sorghum/sudangrass cultivars, slight infection occurred in the plots sown from mid-May to early June (flowering from late July to early September) under the natural infection conditions of *C. sorghicola* (Fig. 4). The disease rates were less than 0.40, regardless of the cultivars used. However, severe infection occurred in the plots sown in late June and on secondary shoots (flowering at late September to late October). In this case, the resistant line showed a distinctive resistance (disease rate: 0.01 to 0.10) compared with the susceptible cultivars (disease rate: 1.53 to 4.23). Germination with the stromata from the sclerotia of *C. sorghicola* was observed in late July in the field.

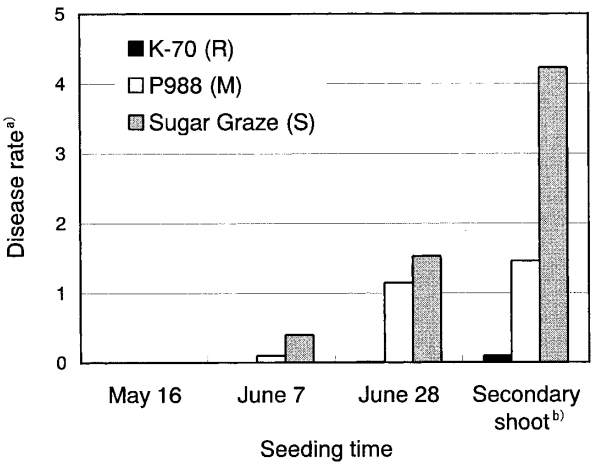


Fig. 4. Control of sorghum ergot caused by *Claviceps sorghicola* by modifying the seeding time and the use of resistant lines

a): 1, 1%; 2, 5%; 3, 10%; 4, 20%; 5, 35% of florets ergotized.

b): Secondary shoots in the plot seeded on May 16 were observed and the disease rate was estimated.

R: Resistant, M: Medium-resistant, S: Susceptible. Sorghum/sudangrass lines were used in the field.

Discussion

C. sorghicola has never been reported elsewhere in the world and is an original fungus causing sorghum ergot in Japan. The fungus is peculiar for the production of caffeine, an alkaloid never reported to be produced by fungi in sclerotia[5]. It is widely distributed in Japan and investigations on its distribution in Asia should be conducted in future. We also detected *C. africana* as a sorghum ergot pathogen mainly in the southern part of Japan. *C. africana* increasingly occurs worldwide, and was detected in Japan in addition to India[4], Taiwan[6] and so on.[3] *C. sorghi* mainly occurs in India and has never been observed in Japan.

C. sorghicola and *C. africana* have a narrow host range, infecting only the genus *Sorghum*. Generally the *Claviceps* species occurring on C4-plants are much more specific in their pathogenicity to their host, while *C. purpurea* infects a wide range of C3-plants[12]. Although *C. africana* was reported to infect pearl millet[10], this was not reconfirmed in this study.

We confirmed that the life cycle of *C. sorghicola* is initiated by the production of stromata from sclerotia on the soil, since the germination of sclerotia was observed in the field study. Ascospores ejected from the stromata are considered to form the primary inoculum. Secondary spread of the disease was assumed to occur mainly by the dispersal of primary conidia from honeydew, since secondary conidia, the main transmission propagules of *C. sorghi* and *C. africana*[3,8], have never been observed in nature.

C. sorghicola is strongly pathogenic to sorghum and sudangrass. Ergots produced by *C. sorghi* and *C. africana* mainly occur on male sterile sorghum and are a constraint on F1 hybrid seed production[3]. However, *C. sorghicola* occurs also on commercial fertile sorghum and sudangrass. As already reported[21], *C. sorghicola* grew rapidly and produced large colonies on PDA (Fig. 1E), while *C. sorghi* and *C. africana* were typically slow growers on PDA and other synthetic media. Frederickson and Mantle[7] indicated that the infection of *C. sorghi* occurs only in unfertilized gynoecia resulting from male sterility or unfavorable conditions for pollination. It is speculated that the fast hyphal growth of *C. sorghicola*

may lead to the infection of the stigmas where pollen tubes are elongating. This may explain why *C. sorghicola* causes infection in fertile and pollinating sorghum and sudangrass. McLaren and Wehner[14] reported that pre-flowering low temperature-induced sterility was the cause of the high ergot severity of *C. africana* in male normal sorghum plants. This may also explain the high ergot severity in fertile sorghum and sudangrass in Japan, which is located in the temperate region.

When the seeding time of sorghum was modified in the field, the disease rate decreased in the plots sown in the early season. In the case of *C. sorghi*, the decrease of the disease incidence by early seeding had already been reported[1]. And in this study, when the resistant cultivar was cultivated, the disease rate was suppressed more distinctly. We had already observed that most of the resistant lines to *C. sorghicola* were sudangrass and sorghum/sudangrass hybrids and their resistance was attributed to the shorter stigma exsertion at flowering, high seed setting rate and high coverage of the seed surface by glumes[19]. Sowing of the resistant lines in the early season was considered to be efficient to avoid or control the occurrence of ergot caused by *C. sorghicola*. We have not yet confirmed the effectiveness of the control method to *C. africana*. However, since the use of the resistant lines[15] and earlier seeding to avoid low temperature at flowering[14] were effective to avoid the disease occurrence by *C. africana*, our method is considered to be useful to control *C. africana* also in Japan.

References

1) Anahosur, K. H. & Patil, S. H. (1982): Effect of date of sowing on the incidence of sorghum ergot. *Indian Phytopathol*., **35**, 507–509.
2) Bailey, C. A. et al. (1999): Evaluation of sorghum ergot toxicity in Broilers. *Poult. Sci*., **78**, 1391–1397.
3) Bandyopadhyay, R. et al. (1998): Ergot; A new threat to sorghum in the Americas and Australia. *Plant Dis*., **82**, 356–367.
4) Bogo, A. & Mantle, P. G. (1999): *Claviceps africana* discovered in India. *Plant Dis*., **83**, 79.
5) Bogo, A. & Mantle, P. G. (2000): Caffeine; also a fungal metabolite. *Phytochemistry*, **54**, 937–939.
6) Chen, G. M., Cheng, A. H. & Yeh, C. C. (1991): Ergot disease of sorghum in Taiwan. *Plant Prot. Bull*., **33**, 223–226 [In Chinese with English summary].
7) Frederickson, D. E. & Mantle, P. G. (1988): The path of infection of sorghum by *Claviceps sorghi*. *Physiol. Mol. Plant Pathol*., **33**, 221–24.
8) Frederickson, D. E., Mantle, P. G. & de Milliano, W. A. J. (1989): Secondary conidiation of *Sphacelia sorghi,* a novel factor in the epidemiology of ergot disease. *Mycol. Res*., **93**, 497–502.
9) Frederickson, D. E., Mantle, P. G. & de Milliano, W. A. J. (1991): *Claviceps africana* sp.nov.; the distinctive ergot pathogen of sorghum in Africa. *Mycol. Res*., **95**, 1101–1107.
10) Frederickson, D. E. & Mantle, P. G. (1996): Pearl millet as an alternate host of the sorghum ergot pathogen, *Claviceps africana*. *Int. Sorghum Millet Newsl*., **37**, 83–85.
11) Kulkarni, B. G. P., Seshadri, V. S. & Hegde, R. K. (1976): The perfect stage of *Sphacelia sorghi* McRae. *Mysore J. Agric. Sci*., **10**, 286–289.
12) Loveless, A. R. (1971): Conidial evidence for host restriction in *Claviceps purpurea*. *Trans. Bri. Mycol. Soc*., **56**, 419–434.
13) Mantle, P. G. & Hassan Hanadi, A. G. (1994): Widening geographical distribution of *Claviceps africana*, an important ovary pathogen of grain sorghum. *Int. Sorghum Millet Newsl*., **35**, 97–98.
14) McLaren, N. W. & Wehner, F. C. (1992): Pre-flowering low temperature predisposition of sorghum to sugary disease (*Claviceps africana*). *J. Phytopathol.*, **135**, 328–334.
15) McLaren, N. W. (1992): Quantifying resistance of sorghum genotypes to the sugary disease pathogen (*Claviceps africana*). *Plant Dis*., **76**, 986–988.
16) Pazoutova, S. et al. (2000): Relations among sorghum ergot isolates from the Americas, Africa, India, and Australia. *Plant Dis*., **84**, 437–442.
17) Shimanuki, T., Kimigafukuro, T. & Tsukiboshi, T. (1988): Occurrence of ergot of sorghum caused by *Claviceps* sp. in Japan. *In* Abstr. 5th Int. Congr. Plant Pathol. 136.
18) Tooley, P. W. et al. (2000): Assessment of diversity in *Claviceps africana* and other *Claviceps* species by RAM and AFLP analysis. *Phytopathology*, **90**, 1126–1130.
19) Tsukiboshi, T. et al. (1998): Resistance of sorghum and sudangrass to ergot caused by *Claviceps* sp. and the cultural control of the disease. *Bull. Natl. Grassl. Res. Inst*., **56**, 28–35 [In Japanese with English summary].
20) Tsukiboshi, T., Shimanuki, T. & Koga, H. (1999): A New pathogen, *Sphacelia sorghi*, causing ergot of sorghum occurs in Japan. *Ann. Phytopathol. Soc. Jpn*., **65**, 318–320 [In Japanese with English summary].
21) Tsukiboshi, T., Uematsu, T. & Shimanuki, T. (1999): *Claviceps sorghicola* sp. nov., a destructive ergot pathogen of sorghum in Japan. *Mycol. Res*., **103**, 1403–1408.