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# A Seedborne Fungus *Bipolaris spicifera* Detected from Imported Grass Seeds

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**Seedborne fungus *Bipolaris spicifera*, which has not been previously reported in Korea, was detected from imported grass seeds in the country. The most frequently detected fungi from the seeds were *Fusarium* species, *Ulocladium atrum*, *B. spicifera*, *Alternaria*, and *Cuvularia lunata* among 17 different seed samples of the family Gramineae. Detection frequencies of *B. spicifera* were 11, 8, 5% in Bermuda grass, tall fescue, and mixed lawn grass imported from USA, respectively, and 9% in mixed lawn grass imported from Italy. This suggests that important seedborne pathogen could be spread between countries through seed sources. The pathogen was seed-transmitted causing damping-off of Bermuda grass seedlings and showed strong pathogenicity to rice, corn, Bermuda grass, sorghum, and tall fescue. However, it did not infect wheat and blue grass.**

***Keywords*** : *Bipolaris spicifera*, conidia, grass seeds, seedborne fungi

Recent importation of agricultural products including seeds into Korea has tremendously increased (KNPQ, 1994-1997), and so has the potential threat of seedborne pathogens being introduced into the country. Importation of grass seeds such as Bermuda grass and mixed grass into Korea has increased from 4,829 metric ton (MT) in 1994 to 5,964 MT in 1997 for use in the production of silage and lawn for golf course (KNPQ, 1994-1997). However, seedborne pathogens, which contaminate grass seeds, have not been studied in detail. One such pathogen, *Bipolaris spicifera* (Bainier) Subramanian, which has not been previously reported in Korea (List of Plant Diseases in Korea, 1998), is an important potential plant pathogen.

*B. spicifera* (teleomorph: *Cochliobolus spicifer* Nelson) is distributed worldwide and occurs mainly in tropical and subtropical regions (Sivanesan and Holliday, 1981; Alcorn, 1988). This fungus was first named as *Brachucladium spoiciferum* Bainier in 1908 (Sivanesan and Holliday, 1981; Alcorn, 1988), renamed as *Curvularia spicifera* (Bainier) Boedijin in 1933 (Sivanesan and Holliday, 1981; Alcorn, 1988), then *Helminthosporiun spiciferum* (Bainier) Nicot in 1953 (Sivanesan and Holliday, 1981; Alcorn, 1988), followed by *Drechslera spicifera* (Bainier) Arx in 1970 (Sivanesan and Holliday, 1981; Alcorn, 1988), and renamed again as *B. spicifera* (Bainier) by Subramanian in 1971 (Sivanesan and Holliday, 1981; Alcorn, 1988). *B. spicifera* has a wide host range infecting more than 77 plant species including 51 grass genera and could be isolated from soil and air (Domsch et al., 1980). Sivanesan and Holliday (1981) reported that seeds were the main sources for the spread of this pathogen.

The objective of this study was to determine seedborne fungi from imported grass seeds in Korea. Pathogenicity test of *B. spicifera* detected in this study was also conducted.

## Materials and Methods

**Detection of seedborne fungi on seeds.** Imported seed samples were collected from the Seoul Branch Station of the National Plant Quarantine Service of Korea located at the Kimpo Airport. There were 17 seed samples belonging to 12 grass species of the family Graminiae. Two hundred seeds per sample were surface sterilized in 1% freshly made sodium hypochlorite solution for 1 minute. Seeds were rinsed with sterilized distilled water and then plated onto three layers of moisturized filter paper (Whatman No. 2.) in Petri dishes and incubated at 25°C for 7 days under alternating near ultra violet light (nuv) and dark condition (nuv/dark, 12 hours/12 hours). The fungi that sporulated on seeds were observed under stereo and compound microscopes to classify them into species.

**Identification of seedborne fungi.** Where *B. spicifera* was particularly identified on the quarantined seeds, further observation was made on morphological characteristics such as pattern of formation of conidia and conidiophore, and the germination process of conidia under stereo and compound microscopes. More than 20 conidia per isolate were measured. Identification was determined according to Sivanesan (1987). Other fungi were identified by the methods used in *B. spicifera* according to relevant references (Barnett and Hunter, 1998; Ellis, 1971; Sutton, 1980).

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**Fungal culture.** Single conidia of *B. spicifera* were isolated from the seeds of Bermuda grass and maintained on potato dextrose agar (PDA) for the studies of pathogenicity.

**Seed transmission of *B. spicifera*.** One hundred seeds disinfested with 1% NaOCl were planted into test tubes (one seed per tube) containing 25 ml of 1% water agar, incubated at 25°C for 30 days, and monitored for disease development. One hundred untreated seeds were planted as control.

**Pathogenicity.** *B. spicifera* was grown to produce conidia on PDA under nuv and dark condition (12 hours/12 hours) for 2 weeks. Conidia were harvested on PDA with 3% Tween-20 adjusted with sterile distilled water. The conidia ($1\times10^5$/ml) were sprayed onto twenty 21-day-old plants of *Oryza sativa* L., *Hordeum vulgare* L., *Triticum aestivum* L., *Zea mays* L., sorghum *Sorghum bicolor* (L.) Moench, *Phleum pratense* L., *Poa* sp. L., *Cynodon dactylon* L., *Festuca arundinacea* Schreber, *Dactylis glomerata* L. in the restricted glasshouse of the Korean National Quarantine Service. The control was sprayed with sterile distilled water. The inoculated plants were humidified at humidity chamber for 48 hours and grown in the greenhouse. Plants with symptoms were observed 7 days after inoculation. Symptoms were arbitrarily scored as: – for none, + for below 50%, and ++ for above 50% disease development based on diseased area.

## Results

The most frequently detected fungi based on sporulation from contaminated seeds were *Fusarium* spp., *Ulocladium atrum*, *Alternaria* spp., *B. polaris*, and *C. lunata* observed 7, 5, 5, 4 and 3 times out of 17 different seed samples of Gramineae, respectively (Table 1). *B. spicifera* infected 11, 8, and 5% of Bermuda grass, tall fescue, and mixed lawn grass imported from USA, respectively, and 9% of mixed lawn grass imported from Italy (Table 1).

Conidiophores of *B. spicifera* rising in singles or groups were moderate to dark brown, slender, long, cylindrical, and flexuous with septa and scars (Figs. 1A, B, C and D). Conidia were produced on the apices of conidiophores (Figs. 1C, D), about 1-12 but mostly 5-8, in short or long distances from conidiophores. Spores were straight, oblong, cylindrical, and brown with three distosepta. The size was 20-31×7.5-12.5 μm (mean 25.5×10.5 μm). The comparison of the characteristics of conidia and conidiophores of *B. spicifera* observed in this study with that described by Sivanesan (1987) was the basis for the identification of *B. spicifera* (Table 2).

The coleoptiles and roots that developed from contaminated seeds were often infected with pycnidia, resulting in damping-off and death (11%). However, seedlings from healthy and surface sterilized seeds with 1% sodium hypochlorite resulted to 0 and 1% damping-off, respectively (Table 3).

Symptom caused by *B. spicifera* developed starting with brown spots with leaves at 7 days after inoculation. *B.*

**Table 1.** Fungal pathogens detected from the imported seeds of Gramineae crops

| Seed[a] | Imported country | Fungi detected[b] (Detection frequency, %) |
|---|---|---|
| Oat | Kazakhstan | *Alternaria dianthi* (4), *Ulocladium atrum* (6) |
| Rye grass | Netherlands | *Fusarium semitectum* (3), *Phoma glomerata* (2) |
| | U.S.A. | *F. solani* (3), *Puccinia* spp. (1) |
| | | *Stemphylium botrysum* (1) |
| Bent grass | U.S.A | *S. botrysum* (4), *U. atrum* (1) |
| Blue grass | U.S.A. | *Colletotrichum gloeosporioides* (2) |
| | | *P. glomerata* (3) |
| Sudan grass | Australia | *Curvularia lunata* (7) |
| Orchard grass | U.S.A. | *F. oxysporum* (2), *F. semitectum* (4) |
| Joysia grass | U.S.A. | *C. falcatum* (2), *C.lunata* (5) *F. solani* (4) |
| | Japan | *A. dianthi* (2), *U. atrum* (6) |
| | P.R.O.C. | *A. dianth i*(3), *C. lunata* (4) |
| | | *F. solani* (3), *U. atrum* (2) |
| Tall fesque | U.S.A. | *Bipolaris spicifera* (8), *F. solan i*(4) |
| Perenial rye grass | U.S.A | *P. glomerata* (3), *Puccinia* spp. (5) |
| Kentucky blue grass | U.S.A. | *A.dianthi* (3), *F. oxysporum* (5), |
| | | *F. semitectum* (2) |
| Mixed grass | U.S.A. | *B. spicifera* (5), *P. glomerata* (4), *Puccinia* spp. (3) |
| | Italy | *B. spicifera* (9) |
| Bermuda grass | U.S.A | *B. spicifera* (11), *U. atrum* (2) |
| | Japan | *A. dianthi* (5) |

[a] Seeds were collected from the samples at inspection and immersed in 1% freshly made sodium hypochlorite for 1 minute.
[b] One hundred seeds per sample were tested by blotter method. The plates were incubated at 25°C for 7 days (dark/near-uv, 12 hours/12 hours).

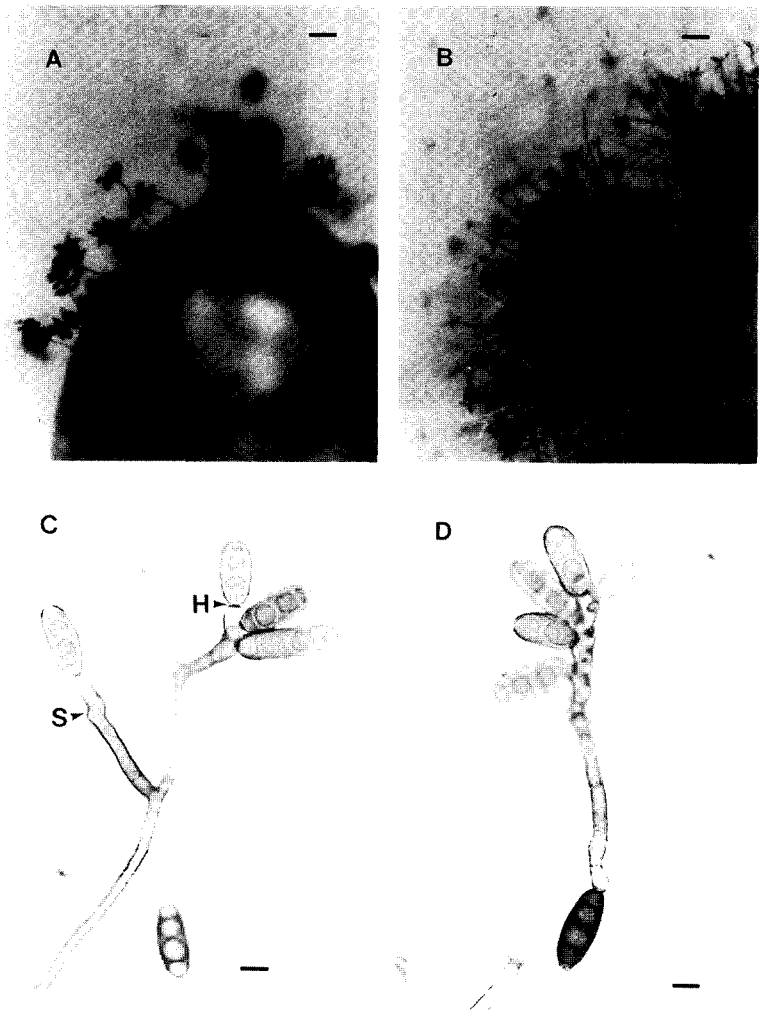


**Fig. 1.** Characteristics of conidia produced on conidiophores of *Bipolaris spicifera*. (**A**) and (**B**) conidiophores were produced in singles or groups. (**C**) Conidia were produced on the apices of conidiophores, which had septa and scar. (**D**) Occasionally, conidiophores were directly produced from conidia. S, scar; H, helium; Scale bar = Fig. 1A, 110 μm; B, 136 μm; C, D, 14 μm.

*spicifera* showed strong pathogenicity with above 50% disease development each plant in rice, Bermuda grass, maize, sorghum, tall fescue, and intermediate pathogenicity of below 50% disease development in timothy, orchard grass, and barley. However, the pathogen did not cause any symptom in wheat and blue grass (Table 4).

## Discussion

All of the samples tested were contaminated with various fungi, i.e., *Fusarium* spp., *U. atrum*, *Alternaria* spp., and *Cuvularia* spp., and *B. spicifera*. These seedborne fungi could reduce not only seed quality but also cause yield loss (Neergaard, 1979). Also, Neergaard (1979) described that reduction of yield could be caused by seedborne fungi as a result of poor development, which is not necessarily seed-transmitted. Halfon-Meiri (1970) reported that seedborne fungi such as *A. brassicola* and *Phoma ligam* in crops belonging to family Gramineae could shrink and reduce seeds. Many seedborne fungi could cause seed rot either in crops or during germination (Neergaard, 1979), i.e., *Fusarium* species in cereals including *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. moniliforme*, *F. nivale,* and *F. semitectum*.

Many species in *Fusarium* are major plant pathogens, and many are seedborne (Neergaard, 1979). *F. oxysporum*, an agent of Fusarium wilt of many hosts, is the most important plant pathogenic species of *Fusarium*, having wide host range, and including numerous *formae speciales*, some of which contain two or several pathogenic races causing devastating wilt disease (Neergaard, 1979). Many of these *F. oxysporum* are seedborne (Noble and Richardson, 1968), and the Anderson (1974) index includes hosts such as *Allium* spp., *Glycine max, O. sativa, Solanum melongena, S. vulgare, Cucumis sativa*, and *Pisum sativum. F. semitectum*

**Table 2.** Morphological characteristics of *Bipolaris spicifera* detected from seeds of Bermuda grass

| Morphological characteristics | Present study[a] | Sivanesan[b] |
|---|---|---|
| Conidiophores | | |
| Shape | flexuous | flexuous |
| Color | mid to dark brown | mid to dark brown |
| Length | 105-705 μm | up to 300 μm long or more |
| Width | 4.3-5.7 μm | 4 μm-9 μm |
| Conidia | | |
| Shape | straight, oblong or cylindrical | straight, oblong or cylindrical |
| Color | brown | golden brown |
| Size | 20-31×7-12.5 μm | 20-40×9-14 μm |
| Hilum width | 2-3 μm | 2-3 μm |
| Septum | 3-distoseptate | 3-distoseptate |

[a] Seeds were plated on moisturized three layers of filter papers (Whatman No 2; U.S.A.). Morphological characteristics of the pathogen were studied through stero and light microscopy. Twenty conidia were observed for determination of the size.
[b] Sivanesan. 1987. Graminicolous species of *Bipolaris, Curvularia, Drechslera, Exserohilum* and teleomorphs.

**Table 3.** Infection rate of seedlings of Bermuda grass

| Treatment[a] | Germination rate (%) | Damping-off (%) |
|---|---|---|
| Healthy seed | 60 | 0 |
| Contaminated seed | 56 | 11 |
| Contaminated seed followed by bleach[b] | 55 | 1 |

[a] One hundred (100) seed were plated on water agar, one for each test tube.
[b] Naturally contaminated seeds were surface sterilized with 1% sodium hypochlorite for 1 minute.

is predominantly a secondary invader of plant tissues and rather common seeds of many hosts including *O. sativa, Z. mays*, and *S. vulgare,* and prevailed in tropical and subtropical regions (Neergaard, 1979). *F. solani* is a ubiquitous pathogen and occurs on seed of a wide range of hosts such as *Capsicum annuum, Coriandrum sativum, Cuminum cyminum, Lycopercon esculentum, O. sativa, Phaseolus vulgaris,* and *S. vulgare* (Ram Nath et al., 1970). Noble and Richardson (1968) also listed *Allium, Cucurbita, Trifolium pratense,* and *Zea mays*. The pathogen causes foot rot in many hosts such as broad bean, Phaseolus bean, and pea (Neergaard, 1979).

The different species of *Ulocladium* are found in low percentage as saprophyte in seeds of many hosts (Groves and Skolko, 1944; Neergaard, 1979).

Most *Alternaria* species such as *A. brassicae* and *A. chrysanthemi,* except for *A. triticina,* have never been reported in grass seeds (Neergaard, 1979). However, *A. dianthi* was detected from oat and Bermuda grass in this study. *A. dianthi* was reported to cause leaf spot and leaf blight from carnation seeds and was seedborne, although rather infrequently.

**Table 4.** Pathogenicity of *Bipolaris spicifera* to some gramineae species

| Plant[a] | Pathogenicity[b] |
|---|---|
| Bermuda grass | ++ |
| Rice | ++ |
| Maize | ++ |
| Sorghum | ++ |
| Wheat | - |
| Barley | + |
| Timothy | + |
| Orchard grass | + |
| Blue grass | - |
| Tall fescue | ++ |

[a] Twenty plants were spray-inoculated at a concentration of $10^5$ conidia/ml suspension.
[b] Pathogenicity was determined 7 days after inoculation. – : not pathogenic, +: mild virulence, ++: severe virulence.

Benoit and Mathur (1970) described habit characters of *Curvularia* species on seed, and reviewed their occurrence as pathogens mainly on cereals. *C. lunata* detected from Sudan grass and zoysia grass in this study was reported on rice seed (Benoit and Mathur, 1970). In addition to *C. lunata, C. cymbopogonis, C. eragrostidis, C. geniculata, C. inaequalis, C. intermedia, C. oryzae, C. palleescens, C. siddiquii, C. trifolii,* and *C uncinata,* all were recorded on rice seeds (Benoit and Mathur, 1970).

*Colletotrichum gloeosporioides* is reported in seeds of *S. vulgare* (Neergaard, 1979), *Acacia decurrens* var. *dealbata* (Hashimoto, 1968), *Trifolium pratense* (Butler, 1953b), *Capsicum* species (Smith and Crossan, 1958; Grover and Bansal, 1971), and *Nicotiana tabacum* (Bates, 1958).

*Stemphylium botrysum* detected in rye grass and bent grass in this study is ubiquitous saprophyte or weak pathogen, occurring commonly but usually in low percentage in seeds of many hosts (Neergaard, 1979).

*Puccinia* species is obligate parasite and attacks many families of angiosperms (Neergaard, 1979). *P. alli* occurs in seeds of *Allium* spp. (Alcock, 1931; Campacci, 1950), while black stem rust occurs profusely as sori in seeds of wheat (Hungerford, 1920). *P. striiformis* (*P. glumarum*) causes yellow rust in wheat (Naumova, 1960). However, seed transmission of *P. graminis* and *P. striiformis* has not been proven, although suspected (Neergaard, 1979).

It is particularly noted that *B. spicifera* has never been previously reported in Korea (List of Korean Plant Disease,

1988). *B. spicifera* was detected at 8-11% from Bermuda grass, tall fescue, and mixed lawn grass imported from different countries, indicating that seeds could cause spread of this pathogen between countries. Bermuda seeds contaminated with *B. spicifera* germinated but with damping-off, indicating that the fungus can infect the host. *B. spicifera* was further determined to be pathogenic to Bermuda grass, rice, maize, sorghum, timothy, orchard grass, tall fescue, and barley but not to wheat and blue grass, suggesting that the fungal pathogens could be spread through seed sources in world trade. This result was different from that of wheat in pre-emergence, which was most susceptible to *B. spicifera*, causing dark root, and reduction of emergence and fresh and dry weight (Gonzalez and Trevathan, 2000), suggesting that virulence of *B. spicifera* might be different among isolates of *B. spicifera*. Richardson (1979) reported that *B. spicifera* was transmitted from seeds of oat, buffalo grass, Bermuda grass, Japanese millets, soybean, cotton, rice, millet, bluegrass, sugarcane, eggplants and sorghum.

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