# Evaluation of Endemic Foliar Fungi for Potential Biological Control of Johnsongrass (*Sorghum halepense*): Screening and Host Range Tests

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**ABSTRACT**

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Diseased johnsongrass leaves were collected during 1985–1986 in North Carolina in a search for leaf-infecting fungi as potential biocontrol agents of johnsongrass. Seven pathogenic fungi were isolated and tested. After preliminary evaluation of virulence, dew requirements, and sporulation capacity, *Exserohilum turcicum, Colletotrichum graminicola*, and *Gloeocercospora sorghi* were selected for second-stage tests. Multiple isolates of these three fungi were compared in separate tests; isolates of each fungus differed in their virulence and host specificity. Data on *E. turcicum* isolates suggests physiologic specialization with regard to virulence to sorghum hybrids and johnsongrass from different seed collections. The most virulent isolate, with the best or average specificity to johnsongrass, was selected for each fungus for subsequent broad-spectrum host range tests. Data on leaf sporulation from inoculated plants indicate that *G. sorghi* was more host-specific than the other two fungi; it was compatible only with *Sorghum* spp., whereas *E. turcicum* and *C. graminicola* were compatible with *Sorghum* and corn. Moderate to high levels of leaf damage were observed in most compatible reactions. Dicotyledonous species were immune to all three fungi. Among the graminaceous plants, oats, wheat, and barley were undamaged or the least damaged, whereas all *Sorghum* spp. (sorghum, johnsongrass, sudangrass, and shattercane) were severely injured by inoculation with high spore concentrations suitable for mycoherbicide application. Overall results suggest that the three tested fungi are too virulent to be used as mycoherbicides in corn and sorghum but may be safe in dicotyledonous crops.

Johnsongrass (*Sorghum halepense* (L.) Pers.) is a large, perennial grass that propagates by seeds and rhizomes. A native of the Mediterranean region, johnsongrass has become well established in agricultural areas of the world. This tall, fast-growing grass can cause heavy losses of crop yields and is extremely difficult to eradicate once established. It has been considered one of the world's 10 worst weeds (13). Johnsongrass was introduced into the United States in the early 19th century for use as a potential forage crop and is now widely distributed (22,25). In southern states, it is a major weed in corn, soybean, cotton, and sorghum (2,18,23). Chemical, mechanical, and cultural methods have been recommended for johnsongrass control (2,3,16). Compared with other grass weeds, johnsongrass is difficult to control with selective preemergence herbicides, which do not control it from rhizomes. Foliage-applied herbicides are partially effective on established johnsongrass, with repeated treatments. Also, foliage-applied herbicides are often used only under certain conditions or on certain crops, because of the inadequate selectivity of these chemicals.

Johnsongrass and six other troublesome weeds that are hard to control by traditional methods in crop fields are target weeds for biocontrol in the southern states (8). The bioherbicide strategy employs endemic pathogens and has been proposed for control of these weeds in crop fields. In this strategy, large amounts of artificially produced inoculum are applied directly on target weeds to produce rapid, extensive disease that can kill or severely damage the weeds in a short time. The classical biocontrol strategy, which involves the release of a biocontrol agent with subsequent reliance upon natural epidemics to suppress the weed, is not suitable for intensively cropped areas that demand rapid and high levels of control (9,33,36).

Current theories and practices of mycoherbicide strategy have been developed from research involving dicotyledonous weeds (8,31–34). Studies on graminaceous weeds are rare. Of the more than 30 species of weeds that have been studied as targets for potential biocontrol in recent years, only wild oats (*Avena fatua* L.), fall panicum (*Panicum dichotomiflorum* Michaux), and johnsongrass are graminaceous. Research on these three grass weeds is in early evaluation stages (32). One reason for this limited interest in biocontrol of grass weeds is the concern that grass pathogens may damage cereal crops and other economic grass plants (9). The host ranges of graminicolous fungal pathogens vary considerably, but fungi specific to genus, species, or subspecies taxa have been reported (4,6,7,15,20,26,29,30). Thus it may be possible to find host-specific fungi for selective grass weed control. Most of the information on the pathogenicity, physiologic specialization, and host range of major fungal pathogens of graminaceous plants has been derived primarily from studies of isolates from crops. To understand the potential of host-specific fungi for grass weed control, isolates from weeds should be evaluated. Biotrophic fungi such as the rust and mildew fungi are not the preferred choices for mycoherbicides, because inocula of these fungi are difficult to produce on a large scale (34).

The purposes of this study were to collect and screen leaf-infecting fungi as potential mycoherbicides for control of johnsongrass; to select virulent, johnsongrass-specific fungal isolates; and to characterize the host specificity of promising fungi.

## MATERIALS AND METHODS

**Collection and preliminary evaluation.** Several trips through the Coastal Plain, Piedmont, and mountain regions of North Carolina were made from July to mid-September of 1985 and 1986 to collect diseased johnsongrass leaves. Johnsongrass plants from the seedling stage to maturity were found during the collection period. As the eventual goal was to control johnsongrass at early growth stages, our sampling emphasized young plants. Diseased leaves were collected, dried under paper presses, cut to appropriate sizes, and stored at 4 C in envelopes.

Leaf pieces with lesions were surface-sterilized with 0.05% sodium hypochlorite solution and incubated on V-8 agar (20% V-8 juice and 0.02% $CaCO_3$) or potato-dextrose agar (PDA) with 2% dextrose. Fungi that grew from the lesions were isolated and tested for pathogenicity. Mycelial plugs with conidia were placed

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on detached johnsongrass leaves in incubation boxes. Isolates that infected and colonized the detached leaves were further evaluated on intact johnsongrass seedlings in a greenhouse. Koch's postulates were applied for most samples shortly after each collection trip. Promising pathogenic fungi were further evaluated individually or in groups for virulence, dew requirements, and sporulation capacity under more defined conditions, described later. Pathogenic fungi were grown on V-8 agar in disposable petri plates (9-cm diameter) at 25 C in an incubator with alternating 12-hr periods of darkness and fluorescent lighting. Conidia harvested from plates were observed, to evaluate the sporulation capacity of isolates.

Conidial suspensions used for inoculation were filtered through cheesecloth to remove large mycelial fragments. The conidia were counted in a hemacytometer, and deionized water containing 0.05% Tween 20 was added to adjust the suspensions to designated concentrations. Suspensions of 20,000 and 500,000 conidia per milliliter (cpm) were used for fungi with large and small spores, respectively. For each inoculation, six to eight 20-day-old johnsongrass seedlings were sprayed to complete wetness with a conidial suspension. The inoculated plants were enclosed in clear plastic bags and placed under greenhouse benches to shade them from direct sunlight. At the end of the dew treatment, the bags were removed, and the plants were placed on top of the benches. The dew period was 24 hr, except in several tests in which a 12-hr period was used to evaluate dew requirements. A relative dew requirement index was used to assess the dew requirements. This index was the ratio (percentage) of disease severity in inoculated johnsongrass seedlings with a 12-hr dew period relative to severity in seedlings with a 24-hr dew period, inoculated with the same fungus. The plants were observed until 3–4 wk after inoculation. Greenhouse temperatures ranged from 23 to 32 C; the relative humidity was 60–90%. Unless otherwise described, inoculum preparations, inoculation procedures, and numbers and stages of test plants were the same in the isolate virulence test and the host range test.

*Exserohilum turcicum* (Pass.) Leonard & Suggs., *Colletotrichum graminicola* (Ces.) G. W. Wils., and *Gloeocercospora sorghi* Bain & Edgerton were selected for the isolate and host range tests. Monoconidial subcultures were prepared. Isolates were maintained either as frozen conidial suspensions in 15% glycerol at −70 C or on dried infected leaves stored at 4 C.

**Isolate virulence test.** Sixteen isolates of *E. turcicum* were tested on johnsongrass collection MI (seeds originated in Mississippi) and three sorghum hybrids (*S. vulgare* Pers. 'Pioneer Brand 815,' 'Pioneer Brand 988,' and 'Pioneer Brand 8501'). The plants were inoculated with a suspension of 20,000 cpm and exposed to a 12-hr dew period. In a second test, three isolates of *G. sorghi* and six isolates of *C. graminicola* were evaluated on johnsongrass (MI), wheat (*Triticum aestivum* L. 'Caldwell'), corn (*Zea mays* L. 'Pioneer 3389'), oats (*Avena sativa* L. 'Coker 716'), and barley (*Hordeum vulgare* L. 'Boone'). These plants were inoculated with a $10^6$-cpm suspension and exposed to a 24-hr dew period. All conidia used in these two tests were obtained from the first subculture from infected leaves. Inoculated plants were observed daily, and a final rating was made 14 days after inoculation. On the basis of lesion type and size, plant response was rated at four levels: 0 = lesions absent; 1 = small, unexpanded lesions; 2 = slightly to moderately expanded lesions; and 3 = large lesions. Maximum lesion size differed among pathogen-host combinations; so the lesion type was rated relative to the most susceptible response of the host to the same fungal pathogen. When inoculated leaves were too severely damaged for individual lesions to be rated at the time of the final observation, the rating made before the final observation was used.

**Host range test.** On the basis of the isolate test, three virulent isolates were selected for tests of host specificity: ET-6 (*E. turcicum*), GS-3 (*G. sorghi*), and CG-4 (*C. graminicola*). Three sets of test plants were inoculated, and a fourth set served as an uninoculated check. Each set of test plants consisted of 27 entries: corn (Pioneer Brand 3165, Pioneer Brand 3369, Pioneer Brand 3535, Silver Queen, and B73), sorghum (Pioneer Brand 855F, Pioneer Brand 931, Pioneer Brand 947, Pioneer Brand 8300, and Pioneer Brand 8515), johnsongrass (NC-1, NC-2, NC-3, NC-4, and MI), sudangrass (*S. vulgare* var. *sudanense* (Piper) Hitchc.), shattercane (*S. bicolor* (L.) Moench), goosegrass (*Eleusine indica* (L.) Gaertn.), yellow foxtail (*Setaria glauca* (L.) Beauv.), broadleaf signalgrass (*Brachiaria platyphylla* (Griseb.) Nash), oats (Coker 716), barley (Boone), wheat (Caldwell), soybean (*Glycine max* (L.) Merr. 'Ransom'), peanut (*Arachis hypogea* L. 'Florigiant'), pumpkin (*Cucurbita pepo* L. 'Mammoth Gold'), and collards (*Brassica oleracea* L. var. *acephala* DC.). All corn and sorghum entries were hybrids except for the inbred line B73. The shattercane and one entry of johnsongrass were from Missouri and Mississippi, respectively. All other entries of weeds were collected in North Carolina. Conidial suspensions and dew treatments were the same as in the isolate test, with the exception that a suspension of $5 \times 10^4$ cpm was used for *E. turcicum*. Check plants were sprayed with water containing 0.05% Tween 20 and exposed to a 24-hr dew period.

Pathogen-plant interactions were evaluated by two parameters, disease severity and sporulation. Disease severity, rated 7 days after inoculation, was expressed as the percentage of the leaf area damaged on inoculated leaves, including only the second and third true leaves of graminaceous plants and the first and second true leaves of dicotyledonous plants. Areas of necrosis, wilting, dehydration, or excessive discoloration were considered damaged. Disease severity was rated on a scale of 0–5, with the ratings corresponding to percentages of leaf damage: 0 = no disease, 1 = 1–20%, 2 = 21–40%, 3 = 41–60%, 4 = 61–80%, and 5 = 81–100%.

After disease severity was rated, four inoculated leaves were detached from plants of each entry. These leaves were surface-sterilized, rinsed, and then incubated on wetted paper in plastic boxes. Sporulation was examined with a stereo microscope after 24, 48, and 72 hr of incubation. Sporulation was rated at three levels: − = no sporulation; + = light sporulation; ++ = moderate to heavy sporulation. Time and the level of sporulation were used to characterize the compatibility of host-pathogen interactions. Isolates that did not sporulate within 72 hr on a host were designated incompatible with that host. Sporulation was not observed on any intact plant or leaves before incubation. Both sclerotia and conidia were considered in rating sporulation.

## RESULTS

**Collection and preliminary evaluation.** Seven pathogenic fungi—*E. turcicum, E. rostratum* (Drechs.) Leonard & Suggs, *Bipolaris sorghicola* (Lefebvre & Sherwin) Alcorn, *C. graminicola, G. sorghi, Cercospora sorghi* Ell. & Ev., and an unknown *Bipolaris* sp.—were isolated from infected leaves of johnsongrass. The unknown *Bipolaris* sp. was morphologically similar to *B. maydis* (Nisik. & Miyake) Shoemaker and *B. zeicola* (Stout) Shoemaker but failed to mate with either of these two fungi and was considered an unreported species (5). The other six pathogens have been reported on sorghum or johnsongrass, or both, in North Carolina (7,10,29,30). Northern leaf blight, caused by *E. turcicum*, and anthracnose, caused by *C. graminicola*, were the two most common and widespread diseases of johnsongrass in North Carolina. Zonate leaf spot, caused by *G. sorghi*, and target leaf spot, caused by *B. sorghicola*, were common in the Piedmont area. Diseases caused by *E. rostratum, G. sorghi*, and the unknown *Bipolaris* sp. were not often found during collections and generally did not cause severe damage. In the field, severe damage of entire johnsongrass plants was mainly associated with *E. turcicum* and *B. sorghicola*.

All pathogenic fungi except *G. sorghi* induced severe damage to johnsongrass seedlings. However, the damage appeared primarily on inoculated leaves and exposed leaf sheaths. Inoculated leaves were often destroyed within 2 wk after inoculation. Johnsongrass growth was reduced after inoculation, but as new leaves developed, these plants gradually recovered. *C. graminicola* and *G. sorghi* required longer dew periods for infection than *Exserohilum* and *Bipolaris* spp. Dew requirement indices for *Exserohilum* and *Bipolaris* spp. were greater than 80, whereas those for *C. graminicola* and *G. sorghi* were approximately 10 and 40, respectively. For all tested fungi, lesions on johnsongrass seedlings were smaller than those on larger field plants. Characteristic zonate lesions of *G. sorghi* and *B. sorghicola* seldom appeared on inoculated johnsongrass seedlings in our tests.

**Isolate test.** After the inoculation of johnsongrass with *E. turcicum*, flecks appeared within 24 hr, and expanded lesions began to appear after 4–5 days; four of 16 isolates failed to induce expanded lesions. Of the 12 isolates that produced expanded lesions on johnsongrass, only nine induced wilting and large, necrotic lesions (Table 1), which are typical of the virulent-susceptible reaction (11,12,14,20). The three sorghum hybrids responded differently to the set of 16 *E. turcicum* isolates, but all isolates induced expanded lesions on one or more sorghum hybrids. On the basis of their ability to induce large, necrotic lesions on johnsongrass and sorghum, the 16 *E. turcicum* isolates were classified in four types: virulent to both hosts (ET-1, 2, 4, 5, 7, 10, 12, and 13), virulent to johnsongrass (ET-6), virulent to sorghum (ET-3, 8, 9, and 14), and weakly virulent or avirulent to both hosts (ET-11, 15, and 16). According to guidelines established in other studies (11,12,14,20,26), isolate ET-6 could be defined as johnsongrass-specific.

Lesions induced by *C. graminicola* appeared on johnsongrass 2–3 days after inoculation, and the number of lesions increased for several days. Expanded lesions appeared 4–5 days after inoculation. Lesions on corn, barley, and oats were also observed, but these lesions did not expand. No lesions developed on wheat. Five of the six *C. graminicola* isolates induced expanded lesions on johnsongrass; isolate CG-4 was the most virulent (Table 2).

Lesions induced by *G. sorghi* appeared on johnsongrass within 24 hr after inoculation; expanded or coalesced lesions appeared 2–3 days after inoculation. Many fewer lesions were observed on leaves inoculated with *G. sorghi* than on leaves inoculated with *C. graminicola*, although the inoculum suspensions contained equal concentrations of conidia. Two isolates induced lesions only on johnsongrass; the most virulent isolate (GS-3) also caused lesions on corn (Table 2).

**Host range test.** No lesions appeared on any check plants. At the time of disease rating, 7 days after inoculation, natural senescence occurred only on the first true leaf of some corn and sorghum entries. Thus the leaf damage observed on the second and third true leaves, on which the severity rating was based, was caused by fungal infection. Lesions were not observed on inoculated soybean, peanut, pumpkin, or collards, and sporulation did not occur on leaves from these plants incubated up to 72 hr. These four dicotyledonous plants were immune to the three tested fungi (Table 3).

Lesions developed on all graminaceous plants inoculated with *E. turcicum*. All members of the genera *Sorghum* and *Zea* were moderately to severely damaged. Considerable damage was also observed on yellow foxtail and broadleaf signalgrass. Oats, wheat, barley, and goosegrass were less affected by *E. turcicum* (Table 3).

Sporulation on incubated leaves showed a different pattern of interaction between *E. turcicum* and these hosts. Sporulation appeared only on detached leaves of corn and *Sorghum* spp.; none occurred on the other graminaceous plants, regardless of the extent of leaf damage. Within 24 hr, conidia were produced on incubated leaves of sudangrass, shattercane, and all johnsongrass entries. Sporulation occurred on all sorghum and four corn hybrids after 24–48 hr of incubation. This suggests the *E. turcicum* isolate from johnsongrass is more compatible with johnsongrass than with sorghum or corn.

*C. graminicola* caused moderate to severe leaf damage on all *Sorghum* spp. (Table 3); yellow foxtail, broadleaf signalgrass, and three corn entries also were considerably damaged. Oats and barley were affected slightly. Sporulation within 24 hr occurred only on incubated leaves of *Sorghum* spp. Sporulation occurred on corn and yellow foxtail, but mainly after 48–72 hr of incubation. Both disease severity and sporulation data indicate that *C. graminicola* isolate CG-4 was more compatible with *Sorghum* spp. than other graminaceous plants, with little specificity to different *Sorghum* spp.

Oats, barley, and wheat were not affected by *G. sorghi* (Table 3). Corn, goosegrass, yellow foxtail, and *Sorghum* spp. were damaged to the same degree by *G. sorghi*. Mycelial growth and sclerotia appeared on incubated leaves of all *Sorghum* spp. but not other species. This indicates that *G. sorghi* was compatible only with *Sorghum* spp.

**Table 1.** Host specificity of *Exserohilum turcicum* isolates from johnsongrass on inoculated johnsongrass and sorghum[a]

| | Host response[b] | | | |
|---|---|---|---|---|
| | | Sorghum | | |
| Isolate | Johnsongrass | P 815[c] | P 988 | P 8501 |
| ET-1 | 3 | 3 | 3 | 3 |
| ET-2 | 3 | 3 | 3 | 3 |
| ET-3 | 1 | 3 | 3 | 1 |
| ET-4 | 3 | 1 | 3 | 1 |
| ET-5 | 3 | 3 | 3 | 1 |
| ET-6 | 3 | 2 | 2 | 1 |
| ET-7 | 3 | 2 | 3 | 1 |
| ET-8 | 1 | 3 | 3 | 3 |
| ET-9 | 1 | 1 | 3 | 1 |
| ET-10 | 3 | 3 | 3 | 3 |
| ET-11 | 2 | 1 | 1 | 2 |
| ET-12 | 3 | 1 | 3 | 3 |
| ET-13 | 3 | 3 | 3 | 3 |
| ET-14 | 2 | 1 | 3 | 1 |
| ET-15 | 1 | 2 | 2 | 1 |
| ET-16 | 2 | 1 | 2 | 2 |

[a] In a greenhouse test, 20-day-old seedlings were inoculated with a suspension of $2 \times 10^4$ conidia per milliliter and subjected to a 12-hr dew period.
[b] Host response was rated before and at 14 days after inoculation: 1 = flecklike lesions; 2 = slightly to moderately developed lesions; 3 = large, necrotic lesions.
[c] P = Pioneer Brand.

**Table 2.** Host specificity of isolates of *Gloeocercospora sorghi* and *Colletotrichum graminicola* from johnsongrass on inoculated grass species[a]

| | | Host response[b] | | | | |
|---|---|---|---|---|---|---|
| Fungus | Isolate | Johnsongrass | Corn | Barley | Oats | Wheat |
| *Gloeocercospora sorghi* | GS-1 | 2 | 0 | 0 | 0 | 0 |
| | GS-2 | 2 | 0 | 0 | 0 | 0 |
| | GS-3 | 3 | 1 | 0 | 0 | 0 |
| *Colletotrichum graminicola* | CG-1 | 1 | 0 | 0 | 0 | 0 |
| | CG-2 | 2 | 1 | 1 | 1 | 0 |
| | CG-3 | 2 | 1 | 1 | 1 | 0 |
| | CG-4 | 3 | 1 | 1 | 1 | 0 |
| | CG-5 | 2 | 1 | 1 | 1 | 0 |
| | CG-6 | 2 | 1 | 1 | 1 | 0 |

[a] In a greenhouse test, 20-day-old seedlings were inoculated with a suspension of $10^6$ conidia per milliliter and subjected to a 24-hr dew period. The johnsongrass originated in Mississippi. The cultivars were Pioneer Brand 3389 corn, Boone barley, Coker 716 oats, and Caldwell wheat.
[b] Host response was evaluated before and at 14 days after inoculation: 0 = lesions absent; 1 = small, flecklike lesions; 2 = slightly to moderately expanded lesions; 3 = large lesions.

## DISCUSSION

Virulence, host specificity, environmental requirements, and sporulation capacity on artificial media are some of the major criteria for evaluating potential mycoherbicides (8,9,31–33,36). Ecological effectiveness is not a prime requirement in selecting mycoherbicides, because inundative application can overcome or compensate for natural constraints on epidemics (31,36). Our preliminary evaluations of several foliar pathogens of johnsongrass indicate that these fungi can be manipulated to induce moderate to severe foliar damage on johnsongrass seedlings. Abundant fungal sporulation on artificial media also suggests that large-scale inoculum production would

**Table 3.** Disease severity and sporulation on plants inoculated with isolate ET-6 of *Exserohilum turcicum*,[a] isolate CG-4 of *Colletotrichum graminicola*,[b] and isolate GS-3 of *Gloeocercospora sorghi*[b]

| Cultivar | Leaf stage at inoculation[c] | Disease severity[d] | | | Sporulation[e] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 24 hr | | | 48 hr | | | 72 hr | | |
| | | ET-6 | CG-4 | GS-3 | ET-6 | CG-4 | GS-3 | ET-6 | CG-4 | GS-3 | ET-6 | CG-4 | GS-3 |
| **Corn (*Zea mays* L.)** | | | | | | | | | | | | | |
| P 3165[f] | 4+ | 4 | 0 | 0 | − | − | − | ++ | − | − | ++ | ++ | − |
| P 3369 | 4 | 3 | 0 | 0 | − | − | − | − | − | − | − | − | − |
| P 3535 | 4+ | 4 | 3 | 2 | − | − | − | ++ | − | − | + | − | − |
| Silver Queen | 4 | 4 | 3 | 1 | − | − | − | ++ | − | − | ++ | ++ | − |
| B73 | 4 | 5 | 2 | 0 | − | − | − | ++ | + | − | ++ | ++ | − |
| **Sorghum (*Sorghum vulgare* Pers.)** | | | | | | | | | | | | | |
| P 855F | 4+ | 5 | 5 | 2 | − | + | − | ++ | ++ | + | ++ | ++ | + |
| P 931 | 4+ | 5 | 4 | 4 | − | + | − | ++ | ++ | + | ++ | ++ | + |
| P 947 | 5 | 5 | 3 | 2 | + | − | − | ++ | + | − | ++ | ++ | + |
| P 8300 | 5 | 4 | 3 | 2 | − | + | − | ++ | ++ | + | ++ | ++ | + |
| P 8515 | 4+ | 5 | 3 | 2 | − | + | − | ++ | ++ | + | ++ | ++ | + |
| **Johnsongrass (*S. halepense* (L.) Pers.)** | | | | | | | | | | | | | |
| NC-1 | 4 | 5 | 5 | 2 | ++ | + | − | ++ | ++ | + | ++ | ++ | + |
| NC-2 | 4 | 5 | 5 | 2 | ++ | + | − | ++ | ++ | + | ++ | ++ | + |
| NC-3 | 4 | 5 | 3 | 3 | ++ | − | − | ++ | ++ | + | ++ | ++ | + |
| NC-4 | 4+ | 5 | 4 | 2 | ++ | + | − | ++ | ++ | + | ++ | ++ | + |
| MI | 4+ | 5 | 5 | 4 | ++ | + | − | ++ | ++ | + | ++ | ++ | + |
| **Sudangrass (*S. vulgare* var. *sudanense* (Piper) Hitchc.)** | | | | | | | | | | | | | |
| | 3+ | 4 | 5 | 2 | ++ | + | − | ++ | ++ | + | ++ | ++ | + |
| **Shattercane (*S. bicolor* (L.) Moench)** | | | | | | | | | | | | | |
| | 4 | 3 | 3 | 2 | + | + | − | ++ | ++ | + | ++ | ++ | + |
| **Goosegrass (*Eleusine indica* (L.) Gaertn.)** | | | | | | | | | | | | | |
| | 4 | 1 | 0 | 2 | − | − | − | − | − | − | − | − | − |
| **Foxtail, yellow (*Setaria glauca* (L.) Beauv.)** | | | | | | | | | | | | | |
| | 3+ | 4 | 3 | 1 | − | − | − | − | − | − | − | ++ | − |
| **Broadleaf signalgrass (*Brachiaria platyphylla* (Griseb.) Nash)** | | | | | | | | | | | | | |
| | 3 | 2 | 3 | 0 | − | − | − | − | − | − | − | − | − |
| **Oat (*Avena sativa* L.)** | | | | | | | | | | | | | |
| Coker 716 | 2+ | 1 | 1 | 0 | − | − | − | − | − | − | − | − | − |
| **Barley (*Hordeum vulgare* L.)** | | | | | | | | | | | | | |
| Boone | 2+ | 1 | 1 | 0 | − | − | − | − | − | − | − | − | − |
| **Wheat (*Triticum aestivum* L.)** | | | | | | | | | | | | | |
| Caldwell | 3+ | 1 | 0 | 0 | − | − | − | − | − | − | − | − | − |
| **Soybean (*Glycine max* (L.) Merr.)** | | | | | | | | | | | | | |
| Ransom | 1st trifoliate | 0 | 0 | 0 | − | − | − | − | − | − | − | − | − |
| **Peanut (*Arachis hypogaea* L.)** | | | | | | | | | | | | | |
| Florigiant | 2nd trifoliate | 0 | 0 | 0 | − | − | − | − | − | − | − | − | − |
| **Pumpkin (*Cucurbita pepo* L.)** | | | | | | | | | | | | | |
| Mammoth Gold | 2nd true leaf | 0 | 0 | 0 | − | − | − | − | − | − | − | − | − |
| **Collards (*Brassica oleracea* L. var. *acephala* DC.)** | | | | | | | | | | | | | |
| | 2nd true leaf | 0 | 0 | 0 | − | − | − | − | − | − | − | − | − |

[a] Plants were inoculated with a suspension of $5 \times 10^4$ conidia per milliliter and subjected to a 12-hr dew period.
[b] Plants were inoculated with a suspension of $10^6$ conidia per milliliter and subjected to a 24-hr dew period.
[c] 4+ = Fourth leaf fully unrolled and fifth leaf partially opened from whorl.
[d] Disease severity was rated on the basis of the percentage of damaged area on the second and third leaves, for all graminaceous species: 0 = no disease; 1 = 1–20%; 2 = 21–40%; 3 = 41–60%; 4 = 61–80%; 5 = 81–100%.
[e] Sporulation on detached leaves after 24, 48, and 72 hr of incubation under saturated moisture: − = no sporulation; + = sparse sporulation; ++ = moderate to heavy sporulation. Interactions that failed to sporulate within 72 hr were designated incompatible.
[f] P = Pioneer Brand.

not be a limitation if other factors are suitable. Some fungi tested required long dew periods for adequate infection. Recent formulation and application techniques indicate that this factor may be overcome (25).

Because a high level of inoculum is used in the mycoherbicide strategy, safety to nontarget plants is a major consideration. The host specificity of potential pathogens must be characterized in the early stage of biocontrol programs (34,36).

In several previous studies, *E. turcicum* from johnsongrass was avirulent to corn (11,15,27). Hamid and Aragaki (11) described isolates of *E. turcicum* pathogenic to johnsongrass but not to corn or sorghum. On the basis of tests with isolates collected from corn, sorghum, and johnsongrass in Hawaii, Hamid and Aragaki (11) proposed three formae speciales of *E. turcicum*: ff. sp. *zea, sorghi*, and *complex*. Isolates specific to johnsongrass or sorghum, or both, were classified as *E. t.* f. sp. *sorghi*. All these three types of "f. sp. *sorghi*" can be identified in our data (Table 1). In addition, a forma specialis avirulent or weakly virulent to both johnsongrass and sorghum (obtained from infected johnsongrass leaves having necrotic lesions) can also be observed (Table 1). Nine of 16 *E. turcicum* isolates produced typical virulent-susceptible necrotic lesions on inoculated johnsongrass seedlings from a Mississippi seed source. On corn, at least three pathogenic races of *E. turcicum* have been identified (17,28). Avirulence to johnsongrass suggests that the johnsongrass tested was different from the plants from which the fungal isolates were obtained; it also implies physiologic specialization between *E. turcicum* and johnsongrass genotypes. According to McWhorter (21), many ecotypes of johnsongrass occur in the United States. For fungi used in the biocontrol of weeds, a broad spectrum of virulence to diverse populations of the target weed is desirable.

Physiologic specialization of *C. graminicola* and *G. sorghi* has been reported; isolates from one grass species were frequently found to be nonpathogenic to other species (1,7,19,24,37). Host range test results in this study indicate that johnsongrass isolates of these fungi differed in virulence or host specificity (Table 2). Most johnsongrass isolates of *C. graminicola* induced lesions on oats, a species that was reported to be immune to corn isolates (24,37). The most virulent isolate of *G. sorghi* was less host-specific than two other isolates; this demonstrates that it may not always be possible to select isolates with the optimum combination of virulence and specificity.

Corn and sorghum are the only two major graminaceous crops that require johnsongrass control. Our data indicate that the three fungi tested are not specific enough for use as mycoherbicides in these two crops. However, the immune response observed on dicotyledonous plants suggests these fungi could be safely used on nongraminaceous plants.

Lesion type, sporulation, and disease severity are parameters frequently used in characterizing host range and physiologic specialization of fungal pathogens (1,6,11,12,15,20,25,27,35). Isolate ET-6 of *E. turcicum* was chosen as the most johnsongrass-specific isolate on the basis of its ability to induce large, necrotic lesions on johnsongrass but not sorghum. However, in the host range test this isolate caused high levels of leaf damage on johnsongrass, sorghum, and corn. Several incompatible interactions (lack of sporulation) were associated with severe leaf damage (Tables 3). Therefore ratings based on lesion type or sporulation, or both, may not provide sufficient information to predict actual damage to plants exposed to mycoherbicide applications. If the eventual acceptability of a mycoherbicide is determined by absolute effectiveness against weeds and nontarget plants, then it might be necessary to characterize the host response of the potential fungal pathogen in terms of disease severity. Because of the quantitative nature of this parameter (disease severity), host specificity tests should be conducted using the same conidial concentration in suspension that would be employed in control of the weed. Potential damage to nontarget plants may be underestimated if low inoculum concentrations are used in host range tests.


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## LITERATURE CITED


1. Ali, M. E. K., and Warren, H. L. 1987. Physiological races of *Colletotrichum graminicola* on sorghum. Plant Dis. 71:402-404.
2. Anonymous. 1976. Johnsongrass as a weed. Farmers Bull. 1537. 18 pp.
3. Anonymous. 1986. North Carolina Agricultural Chemicals Manual. School of Agriculture and Life Sciences, North Carolina State University, Raleigh. 312 pp.
4. Bergquist, R. R., and Masias, O. R. 1974. Physiologic specialization in *Trichometasphaeria turcica* f. sp. *zea* and *T. turcica* f. sp. *sorghi* in Hawaii. Phytopathology 64:645-649.
5. Chiang, M.-Y., Van Dyke, C. G., and Leonard, K. J. 1986. Leaf spot of johnsongrass caused by an unidentified *Bipolaris* species. (Abstr.) Phytopathology 76:1111.
6. Ferreira, A. S., and Warren, H. L. 1982. Resistance of sorghum to *Colletotrichum graminicola*. Plant Dis. 66:773-775.
7. Frederiksen, R. A., ed. 1986. Compendium of Sorghum Diseases. American Phytopathological Society, St. Paul, MN. 82 pp.
8. Freeman, T. E., and Charudattan, R. 1980. Biological control of weeds with plant pathogens: Prospectus 1980. Pages 293-299 in: Proc. Int. Symp. Biol. Control Weeds 5th. E. S. Delfosse, ed. 649 pp.
9. Goeden, R. D. 1977. Biological control of weeds. Pages 43-47 in: Research Methods in Weed Science, 2nd ed. B. Truelove, ed. Southern Weed Science Society, Champaign, IL. 211 pp.
10. Grand, L. F., ed.. 1985. North Carolina Plant Disease Index. N.C. Agric. Res. Serv. Tech. Bull. 240 (rev.). 157 pp.
11. Hamid, A. H., and Aragaki, M. 1975. Inheritance of pathogenicity in *Setosphaeria turcica*. Phytopathology 65:280-283.
12. Hilu, H. M., and Hooker, A. L. 1963. Monogenic chlorotic lesion resistance to *Helminthosporium turcicum* in corn seedlings. Phytopathology 53:909-912.
13. Holm, L. G., Plucknett, D. L., Pancho, J. V., and Herberger, J. P. 1977. The World's Worst Weeds: Distribution and Biology. University Press of Hawaii, Honolulu. 609 pp.
14. Hooker, A. L. 1963. Inheritance of chlorotic-lesion resistance to *Helminthosporium turcicum* in seedling corn. Phytopathology 53:660-662.
15. Hooker, A. L., Nelson, R. R., and Hilu, H. M. 1965. Avirulence of *Helminthosporium turcicum* on monogenic resistant corn. Phytopathology 55:462-463.
16. Keeley, P. E., Thullen, R. J., and Caster, C. H. 1987. Repeated annual applications of herbicides for control of rhizome johnsongrass (*Sorghum halepense*) in cotton (*Gossypium hirsutum*). Weed Sci. 35:75-79.
17. Lim, S. M., Kinsey, J. G., and Hooker, A. L. 1974. Inheritance of virulence in *Helminthosporium turcicum* to monogenic resistant corn. Phytopathology 64:1150-1151.
18. Lorenzi, H. J., and Jeffery, L. S. 1987. Weeds of the United States and Their Control. Van Nostrand Reinhold, New York. 355 pp.
19. Luttrell, E. S. 1954. Diseases of pearl millet in Georgia. Plant Dis. Rep. 38:507-514.
20. Masias, O. R., and Bergquist, R. R. 1974. Host-specific forms of *Trichometasphaeria turcica* in relation to homokaryons and heterokaryons in nature. Phytopathology 64:436-438.
21. McWhorter, C. G. 1971. Growth and development of johnsongrass ecotypes. Weed Sci. 19:141-147.
22. McWhorter, C. G. 1971. Introduction and spread of johnsongrass in United States. Weed Sci. 19:496-500.
23. McWhorter, C. G., and Chandler, J. M. 1982. Conventional weed control technology. Pages 5-27 in: Biological Control of Weeds with Plant Pathogens. R. Charudattan and H. L. Walker, eds. John Wiley & Sons, New York. 293 pp.
24. Naylor, V. D. 1975. Temperature relations, overwintering, host range, and virulence of *Colletotrichum graminicola*, cause of corn anthracnose. M.S. thesis, North Carolina State University, Raleigh. 64 pp.
25. Quimby, P. C., Jr., and Fulgham, F. E. 1986. Formulating and applying a weed pathogen to bypass dew. (Abstr.) Pages 25-26 in: Pestic. Formulations Applic. Syst. Symp. 7th
26. Robert, A. L. 1960. Physiologic specialization in *Helminthosporium turcicum*. Phytopathology 50:217-220.
27. Rodriguez, A. E., and Ullstrup, A. J. 1962. Pathogenicity of monoascosporic progenies of *Trichometasphaeria turcica*. Phytopathology 52:599-601.
28. Smith, D. R., and Kinsey, J. G. 1980. Further physiologic specialization in *Helminthosporium turcicum*. Plant Dis. 64:779-781.
29. Sprague, R. 1950. Diseases of Cereals and Grasses in North America. Ronald Press, New York. 538 pp.
30. Tarr, S. A. J. 1962. Diseases of Sorghum, Sudangrass and Broom Corn. Commonwealth Mycological Institute, Kew, Surrey, England. 380 pp.
31. TeBeest, D. O., and Templeton, G. E. 1985. Mycoherbicides: Progress in the biological control of weeds. Plant Dis. 69:6-10.
32. Templeton, G. E. 1982. Status of weed control with pathogens. Pages 29-44 in: Biological Control of Weeds with Plant Pathogens. R. Charudattan and H. L. Walker, eds. John Wiley & Sons, New York. 293 pp.
33. Templeton, G. E., TeBeest, D. O., and Smith, R. J., Jr. 1979. Biological weed control with mycoherbicides. Annu. Rev. Phytopathol. 17:301-310.
34. Templeton, G. E., Weideman, G. J., and Smith,

R. J., Jr 1986. Biological weed control. Pages 99-109 in: Research Methods in Weed Science, 3rd ed. N. D. Camper, ed. Southern Weed Science Society, Champaign, IL. 486 pp.
35. Walker, H. L. 1982. Seedling blight of sicklepod caused by *Alternaria cassiae*. Plant Dis. 66:426-428.
36. Wapshere, A. J. 1982. Biological control of weeds. Pages 47-56 in: Biology and Ecology of Weeds. W. Holzner and N. Numata, eds. Dr. W. Junk Publishers, The Hague.
37. Wheeler, H., Politis, D. J., and Poneleit, C. G. 1974. Pathogenicity, host range, and distribution of *Colletotrichum graminicola* on corn. Phytopathology 64:293-296.