# Effects of Herbicide Antidotes on Sorghum Downy Mildew

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Cooperative investigations of the Agricultural Research Service, USDA, and the Texas Agricultural Experiment Station, College Station. Approved for publication as Technical Article 22157 of the Texas Agricultural Experiment Station.

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This study supported in part by a grant from the Ciba-Geigy Corporation.

Accepted for publication 15 April 1987.

## ABSTRACT

Craig, J., Frederiksen, R. A., Odvody, G. N., and Szerszen, J. 1987. Effects of herbicide antidotes on sorghum downy mildew. Phytopathology 77:1530-1532.

Relationships between herbicide antidotes and incidences of sorghum downy mildew observed in field plantings of sorghum were studied in greenhouse tests. Seed treatment with the herbicide antidote CGA 92194 caused significant increases in downy mildew incidence in susceptible sorghum hybrids grown in soil infested with oospores of *Peronosclerospora sorghi* or inoculated with conidia of the pathogen. CGA 92194 did not increase disease incidence in resistant sorghum genotypes. Seed treatment with the herbicide antidote flurazole had no significant effect on disease incidence of susceptible or resistant sorghum genotypes planted in oospore-infested soil. Flurazole seed treatment significantly reduced disease incidence in two of three susceptible sorghum genotypes inoculated with conidia of *P. sorghi*. The field observations of increased downy mildew incidence in sorghum treated with CGA 92194 and the greenhouse test results indicate that CGA 92194 could increase the severity of downy mildew damage in susceptible sorghum genotypes.

Sorghum downy mildew, caused by *Peronosclerospora sorghi* (Weston & Uppal) C. G. Shaw, is an important disease of sorghum (*Sorghum bicolor* (L.) Moench) and corn (*Zea mays* L.) in several countries. In the United States, the disease causes economically significant losses in sorghum production in Texas (6). Environmental factors such as soil moisture, temperature, and soil pH affect the level of disease incidence when susceptible host plants are exposed to *P. sorghi* (1,11). Recent observations in Texas indicated that herbicide antidotes (chemicals used to protect plants from herbicide damage) also affected the incidence of downy mildew in sorghum. In many regions of sorghum production, the use of herbicide antidotes is required because the only herbicides that provide effective control of grassy weeds in sorghum fields are toxic to sorghum.

An increase in downy mildew induced by a herbicide antidote would constitute an iatrogenic disease, a disease caused or made more severe by a chemical applied to protect the plant (8). Such diseases are relatively common (7). An example is the increased severity of corn root rot caused by the herbicide picloram (10). In addition to herbicides, chemical agents involved in iatrogenic diseases include insecticides, fungicides, and growth regulators (7).

To our knowledge, the first indication that a herbicide antidote could serve as an iatrogenic agent occurred in a farmer's field near Laward, TX, in 1984. The farmer planted sorghum seed treated with herbicide antidotes because of his use of herbicides toxic to sorghum for weed control. Two seed lots of the sorghum hybrid Dinero (Taylor Evans Seed Co., Tulia, TX) had been planted in alternating strips. One seed lot had been treated with herbicide antidote CGA 92194 (α[(1,3-dioxolan-2-yl-methoxy)imino] benzeneacetonitrile) produced by Ciba-Geigy Ltd., Basle, Switzerland, and the other seed lot had been treated with another herbicide antidote, flurazole (5-thiozolecarboxylic acid, benzyl ester, 2-chloro-4-(trifluoromethyl)) produced by Monsanto Chemical Co., St. Louis, MO. At 3 wk after planting, the incidence of sorghum downy mildew, determined by counts at four sites in the field, was 60% in plots planted with CGA-92194-treated seeds. In contrast, only 15% of the plants from seeds treated with flurazole were diseased (J. Craig, *unpublished*). The difference in downy mildew incidence between the two sorghum populations suggested that either CGA 92194 increased the incidence of the disease or that flurazole protected sorghum from downy mildew. This study was conducted to determine the relationships between these herbicide antidotes and the incidence of infection in sorghum plants exposed to *P. sorghi*.

## MATERIALS AND METHODS

Tests of the effect of herbicide antidotes on the incidence of downy mildew induced by oospores of *P. sorghi* were conducted in the greenhouse with oospore-infested soil from the field where the herbicide antidote effects were noted. The sorghum hybrids used in these trials were Dinero and Tophand (Conlee Seed Co., Waco, TX). Tophand is susceptible to all known pathotypes of *P. sorghi* in Texas (J. Craig, *unpublished*) (3,4). Dinero is resistant to pathotypes 1 and 2, and susceptible to pathotype 3 (J. Craig, *unpublished*). The reactions of sorghum lines used to identify pathotypes indicated that the population of *P. sorghi* in the collected soil contained pathotype 3. Pathotypes 1 or 2 may or may not have been present; these pathotypes cannot be indentified by the reactions of the sorghum genotypes used as differentials if pathotype 3 is present (4).

A completely randomized experimental design was used to test each hybrid. The test treatments were seed treated with CGA 92194, seed treated with flurazole, and untreated seed as a control. The herbicide antidotes were applied to the sorghum seeds at the manufacturers' recommended rate of 1.25 g a.i./kg of seed.

Peat pots, 6 cm square and 6 cm high were filled two-thirds full with oospore-infested soil. Ten sorghum seeds were placed on the surface of the soil in each pot and covered by filling the pot with oospore-infested soil. Nine pots of each treatment were planted. The pots were placed in trays and watered by adding water to the trays as needed. The pots were placed in a 20 ± 1 C environment

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chamber. The lighted period was 18 hr (intensity 84 $\mu E \cdot m^{-2}s^{-1}$) followed by 6 hr of darkness. After 7 days, the chamber temperature was increased to 22 ± 1 C.

Fourteen days after planting, the peat pots of sorghum were removed from the environment chamber and planted in 10-cm-diameter clay pots. The plants were grown in the greenhouse for 14 days and observed daily for the chlorotic-leaf symptom indicative of sorghum downy mildew (6). The diseased plants were removed from the pots when symptoms appeared. Plants without disease symptoms after 14 days of observation were assumed to have escaped infection. The surviving plants in each treatment were counted, and the incidence of downy mildew was calculated. The trial was repeated twice.

Further tests of herbicide antidote effects were conducted with oospore-infested soil collected from a sorghum disease nursery near Skidmore, TX. The results of tests of sorghum genotypes used to differentiate pathotypes of *P. sorghi* (4) indicated that only pathotype 1 was present in this soil. The sorghum hybrids used in the herbicide antidote trials were Dinero, resistant to pathotype 1, and Paymaster R920 (Paymaster Seeds, Plainview, TX), susceptible to pathotype 1 (J. Craig, *unpublished*).

The experimental design and treatments were as described above. At planting, peat pots were half filled with Baccto Potting Soil (Michigan Peat, Houston, TX) and 15 $cm^3$ of oospore-infested soil was placed on the surface of the potting soil in each pot. Fifteen sorghum seeds were placed in each pot and covered by filling the pots with oospore-infested soil. The pots were placed in a 26 ± 1 C environment chamber with an 18-hr light period (intensity 84 $\mu E \cdot m^{-2}s^{-1}$) followed by 6 hr of darkness. Fourteen days after planting, the pots were removed from the chamber, planted in 10-cm-diameter clay pots, and the plants were thinned to 10 plants per pot. The plants were grown in the greenhouse for 14 days and observed and scored as described above. The trial was repeated twice.

Greenhouse tests were conducted to determine the effect of herbicide antidotes on downy mildew incidence induced by inoculating sorghum seedlings with conidia of *P. sorghi*. The sorghum hybrids Dinero, Tophand, and W839DR (Warner Seed Co., Waco, TX) were used in these trials. The hybrid W839DR is resistant to pathotypes 1 and 2 and susceptible to pathotype 3 (J. Craig, *unpublished*). The experimental design and seed treatments were as those described above for the oospore inoculum tests. The sorghum seeds were germinated on moist paper and planted in peat pots of Baccto Potting Soil, five germinated seeds per pot. Nine pots were planted per treatment. The plants were grown in the greenhouse and inoculated at the 1.5–2-leaf stage of growth with conidia of pathotype 3.

The conidial inoculum was obtained from leaves of diseased sorghum seedlings by using a previously described method for production and storage of conidia (2). An aqueous suspension of conidia was adjusted to $3 \times 10^4$ conidia per milliliter. The conidial suspension was sprayed over the sorghum seedlings with an aerosol sprayer (Crown Spra-Tool, Fisher Scientific, Pittsburgh, PA) at the rate of 0.25 ml per plant. The inoculated plants were placed in a 20 ± 2 C, 100% RH environment chamber for 24 hr and then planted in 10-cm-diameter clay pots. The inoculated plants were grown in the greenhouse for 21 days after inoculation. The plants were observed for symptoms of downy mildew and the treatments scored for disease incidence as described above in tests with oospore inoculum. The test of each hybrid was repeated twice.

## RESULTS AND DISCUSSION

Seed treatment with CGA 92194 induced significantly higher ($P = 0.01$) incidences of downy mildew than the control treatment in the sorghum hybrids planted in oospore-infested soil from Laward, TX (Table 1). The hybrid Tophand had higher incidences of disease than Dinero. This may indicate that Tophand is more susceptible to pathotype of *P. sorghi* than Dinero. However, as stated earlier, the presence or absence of pathotypes 1 and 2 in this soil could not be verified. These pathotypes are virulent to Tophand and avirulent to Dinero, and their presence would have increased the probability of infection for Tophand. Seed treatment with flurazole did not differ significantly in disease incidence from the control treatment in either hybrid (Table 1).

Seed treatment with CGA 92194 did not affect the incidence of downy mildew in Dinero exposed to oospores of pathotype 1 in the soil from Skidmore (Table 1). Pathotype 1 is avirulent to Dinero, and the low incidence of downy mildew in the herbicide antidote treatments and the control treatments could reflect either heterogeneity of the seed lot or a low frequency of pathotype 3 in the pathogen population.

CGA 92194 seed treatment increased downy mildew incidence significantly ($P = 0.01$) in the pathotype 1-susceptible hybrid R920 planted in the Skidmore soil. There was no significant difference in disease incidence between the flurazole treatment and the control treatment in either Dinero or R920 (Table 1).

In comparisons of control treatment and herbicide antidote treatments, disease incidences induced by conidial inoculation were significantly higher ($P = 0.05$) in the CGA 92194 treatments of Tophand and W839DR (Table 2). No significant difference between CGA 92194 and control treatments was found in Dinero (Table 2). In contrast, seed treatment with flurazole produced significantly lower ($P = 0.05$) incidences of downy mildew in Dinero and W839DR, but no significant difference was found in Tophand (Table 2).

The results of this study indicate that CGA 92194 causes significant increases in the incidence of sorghum downy mildew in susceptible sorghum hybrids attacked by oospores of *P. sorghi*. However, CGA 92194 did not reduce the resistance to downy mildew conferred by an incompatible relationship between host and pathogen such as that between Dinero and pathotype 1 of *P. sorghi*. The relationship between CGA 92194 and the severity of downy mildew induced by conidial inoculation with pathotype 3 was not clearly defined in the hybrid Dinero because of the high incidence of downy mildew in the untreated control plants. However, the results with Tophand and W839DR indicate that CGA 92194 increases downy mildew incidence from conidial inoculum in situations that limit disease incidence in untreated sorghum.

TABLE 1. Effects of herbicide antidotes CGA 92194 and flurazole on the incidence of sorghum downy mildew induced by oospores of *Peronosclerospora sorghi*, pathotypes 1 and 3, in the sorghum hybrids Dinero, Tophand, and R920

| Herbicide antidote | Disease incidence (%)[a] | | | |
|---|---|---|---|---|
| | Pathotype 1 | | Pathotype 3 | |
| | Dinero | R920 | Dinero | Tophand |
| Control | 2 | 42 | 5 | 34 |
| Flurazole | 2 | 34 | 3 | 30 |
| CGA 92194 | 5 | 90**[b] | 50** | 84** |

[a] Frequency of diseased plants; each treatment value is the mean of three trials, 90 plants per trial.
[b] Asterisks indicate herbicide antidote values significantly different from the control treatment according to Student's *t* test ($P = 0.01$).

TABLE 2. Effects of herbicide antidotes CGA 92194 and flurazole on the incidence of sorghum downy mildew induced by conidial inoculation of sorghum hybrids Dinero, Tophand, and W839DR with *Peronosclerospora sorghi* pathotype 3

| Herbicide antidote | Disease incidence (%)[a] | | |
|---|---|---|---|
| | Dinero | Tophand | W839DR |
| Control | 78 | 56 | 35 |
| Flurazole | 17*[b] | 25 | 16* |
| CGA 92194 | 91 | 86* | 93* |

[a] Frequency of diseased plants; each treatment value is the mean of three trials, 45 plants per trial.
[b] Asterisk indicates herbicide antidote values significantly different from the control value according to Student's *t* test ($P = 0.05$).

The relationship between flurazole and the incidence of sorghum downy mildew appears complex. Flurazole had no effect on the incidence of disease caused by oospores in the greenhouse tests. Paradoxically, flurazole reduced downy mildew incidence significantly in two of the three hybrids inoculated with conidia and caused a nonsignificant 55% reduction of disease incidence in the third compared to control treatments. It is probable that flurazole would reduce downy mildew incidence induced by conidia of *P. sorghi* when conditions were conducive to high levels of disease in untreated plants. However, flurazole seed treatment would not be an effective means of controlling downy mildew in Texas, where most of the disease is caused by oospore inoculum (6).

Acetanilide-type herbicides such as metolachlor control the annual grassy weeds that frequently reduce yields of grain sorghum, but these herbicides are toxic to sorghum. CGA 92194 and flurazole are herbicide antidotes that protect sorghum from the acetanilide herbicides (5,9) and thus permit their use for selective weed control in sorghum fields. The mechanism by which these antidotes protect sorghum from herbicide injury is not known. Ketchersid and Merkle (9) reported that CGA 92194 and flurazole reduced respiration and growth rates in treated sorghum. They suggested that the herbicide antidotes were mildly toxic to sorghum and stimulated defensive metabolic systems that protected the seedlings from herbicide injury.

The mechanisms by which these herbicide antidotes influence the incidence of downy mildew are unknown. Chemical agents involved in iatrogenic diseases influence disease severity by affecting the host plant, the pathogen, or the ecosystem (7). It is possible that CGA 92194 makes the ecosystem more favorable to disease development by inhibiting microbial antagonists of *P. sorghi* in the soil. CGA 92194 could affect the pathogen by improving the germination of oospores of *P. sorghi* in soil. However, neither supposition accounts for the increased disease incidence in CGA-92194-treated plants inoculated with conidia (Table 2). The available information suggests that CGA 92194 affects downy mildew incidence by inducing changes in the host plant that favor disease development. Flurazole could cause changes in the sorghum plant that are unfavorable to disease development. If so, these unfavorable changes do not occur early enough to limit the disease induced by oospores that infect the seedlings shortly after seed germination.

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