Diclofop-methyl interactions with soil-borne fungal pathogens in wheat
by Mary M Kleis

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology
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Abstract:
Herbicide applications can influence disease development. There is some evidence that applications of diclofop-methyl to diseased wheat may increase expected levels of crop injury. Using the soil-borne fungal pathogens Bipolaris sorokiniana, Cephalosporium gramineum,. Fusarium culmorum, and Gaeumannomvdes graminis, this research evaluated: (1) Changes in virulence and growth alterations in response to diclofop-methyl, (2) Existence of interactions in wheat under field conditions, (3) Effects of diclofop on wheat root growth in the presence of G. graminis.

Field evaluations compared wheat response to diclofop-methyl in artificially inoculated plots versus uninoculated plots. Wheat was treated with 0, 1.12, and 2.24 kg ai/HA diclofop-methyl. Fungitoxicity tests measured the effect of diclofop-methyl at 0, 1, 10, 100, and 1,000 mg/1 on mycelial growth. Changes in virulence were evaluated after pathogen exposure to 100 mg/l diclofop-methyl. Interactions between diclofop-methyl and (G. graminis were evaluated in a hydroponic system, where root length, dry weight and volume were measured.

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# DICLOFOP-METHYL INTERACTIONS WITH SOIL-BORNE FUNGAL PATHOGENS IN WHEAT

by

MARY M KLEIS

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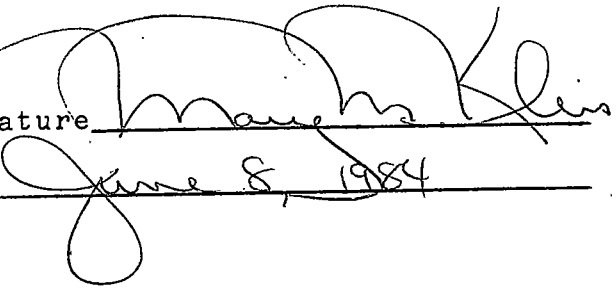
Signature 

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## ABSTRACT

Herbicide applications can influence disease development. There is some evidence that applications of diclofop-methyl to diseased wheat may increase expected levels of crop injury. Using the soil-borne fungal pathogens Bipolaris sorokiniana, Cephalosporium gramineum, Fusarium culmorum, and Gaeumannomyces graminis, this research evaluated: (1) Changes in virulence and growth alterations in response to diclofop-methyl, (2) Existence of interactions in wheat under field conditions, (3) Effects of diclofop on wheat root growth in the presence of G. graminis.

Field evaluations compared wheat response to diclofop-methyl in artificially inoculated plots versus uninoculated plots. Wheat was treated with 0, 1.12, and 2.24 kg ai/HA diclofop-methyl. Fungitoxicity tests measured the effect of diclofop-methyl at 0, 1, 10, 100, and 1,000 mg/l on mycelial growth. Changes in virulence were evaluated after pathogen exposure to 100 mg/l diclofop-methyl. Interactions between diclofop-methyl and G. graminis were evaluated in a hydroponic system, where root length, dry weight and volume were measured.

Data from field studies showed no increases in expected levels of herbicide or disease injury with diclofop-methyl applications to infected wheat. Conversely, B. sorokiniana-diclofop-methyl interactions resulted in yield increases. In fungitoxicity tests diclofop-methyl inhibited fungal growth, except in the case of G. graminis where growth stimulation was noted at 10 mg/l diclofop-methyl. However, results from G. graminis-diclofop-methyl hydroponic studies showed no increased root injury due to diclofop-methyl application to infected wheat. Further field evaluations of G. graminis-diclofop-methyl interactions are necessary. No changes in virulence of these pathogens were noted after diclofop-methyl exposure.

# INTRODUCTION

Selective herbicides are chemicals which alter the growth of plants through disruption of biochemical processes. In addition to effects on higher plants, herbicides can affect other organisms including fungi. The interrelationship between fungal pathogens and herbicides may lead to changes in plant disease incidence or severity. Disease epidemiology can be influenced by the direct effect of herbicides on individual pathogens, as well as by the indirect activity of herbicides on the host plant and in the soil environment (Katan and Eshel, 1973; Altman and Campbell, 1977).

In cereal crops less research had been conducted on pathogen-herbicide interactions than with other higher value crops. This is probably due to the fact that in temperate zones fewer pesticides are applied to cereals relative to other crops such as cotton, peas, bean, tomatoes, potatoes, etc. Most herbicide-pathogen interaction studies conducted in cereals have emphasized the effect of phenoxy herbicides on cereal diseases.

In Montana, phenoxy herbicides are commonly applied to wheat for the control of broadleaf weeds (Nissen,

1983). In addition to broadleaf herbicides, a substantial acreage is treated with one of several non-phenoxy herbicides for the control of annual grassy weeds. Diclofop-methyl (methyl 2-(4-(2',4'-dichlorophenoxy)phenoxy) propanoate, hereafter referred to as diclofop, is one of the newest grass herbicides registered for use in cereals. Use of diclofop is increasing in Montana as well as in other wheat producing areas of the world (E. Faust, personal communication). This herbicide selectively controls annual grassy weeds in cereals including, wild oat (Avena fatua), green foxtail (Setaria viridis), yellow foxtail (Setaria lutecens), and annual ryegrass (Lolium multiflorum).

Field observations have indicated that there may be interactions between diclofop and some common soil-borne cereal diseases. An increased incidence of Take-All of wheat in diclofop-treated fields has been observed in Chili (R. Madariaga, personal communication). In Oregon increased diclofop damage in wheat may have been related to interactions with unidentified soil-borne pathogens (P. Olson, personal communication).

Diclofop can reduce root growth in wheat and other grasses. Growth restrictions have been observed in adventitious roots (Donald et al., 1982). Affected roots

are shorter and thicker than normal roots. The nubbed appearance of the roots is often referred to as root pruning. Since pruned roots do not resume normal growth after initial herbicide exposure, diclofop applications could decrease the absorptive capacity of the plant and reduce crop vigor (Morrison et al., 1981). Although wheat roots can be damaged by diclofop exposure, yield reductions are not generally observed. However, when combined with other root growth reducing factors such as plant disease, the additive effects of herbicide and disease may significantly affect wheat growth. Diclofop root pruning when combined with pathogen infection may account for the increase in crop injury which has been observed with diclofop applications to diseased wheat (Madariaga and Olson). Yield losses due to herbicide-disease interactions may be greater than those caused individually by either diclofop or disease.

Those plant pathogens likely to interact with diclofop to cause increased crop damage are the soil-borne root infecting fungi. In Montana the pathogens commonly infecting wheat and reducing root growth and/or water utilization include Cephalosporium gramineum, Fusarium culmorum, Bipolaris sorokiniana, and Gaeumannomyces graminis var. tritici (Dubbs and Mathre,

1979).

Cephalosporium gramineum is a vascular pathogen of winter wheat. Infection results in a physical reduction in internal water movement, and causes localized water shortages within the plant (Morton and Mathre, 1980). Infected plants are stunted with chlorotic leaves, and eventually produce shriveled kernels. Both Bipolaris sorokiniana and Fusarium culmorum can infect wheat roots, causing the disease known as dryland (common) root rot. These organisms cause root, crown, and subcrown internode necrosis leading to a reduction in plant vigor. The effects of infection are thought to be severe under dry conditions when the diseased roots are unable to obtain sufficent water to sustain plant growth. Severe subcrown internode necrosis can disrupt water movement from the seminal roots to the leaves, causing internal water deficits which may be an important yield reducing factor under hot dry conditions (Wiese, 1977). Take-All disease, caused by Gaeumannomyces graminis var. tritici, can be severe under irrigation or in high rainfall areas. The fungus causes necrosis of the roots and crown, thereby inhibiting water and nutrient transport (Wiese, 1977).

Since the relationships between soil-borne pathogens and diclofop are poorly understood, the intent of

this research was to investigate these possible interactions. The fungi evaluated were: B. sorokiniana, F. culmorum, C. gramineum and G. graminis, with the objectives of: (1) Determination of pathogen virulence and growth alterations in response to diclofop exposure, (2) Evaluation of diclofop and pathogen interactions in wheat under field conditions, (3) Investigation of wheat root responses to diclofop in the presence and absence of G. graminis.

# LITERATURE REVIEW

## Herbicide-Pathogen Interactions

The first selective herbicide developed for use in agronomic crops was 2,4-dichlorophenoxy acetic acid (2,4-D). This new management tool became available to American farmers in 1945. Since the introduction of 2,4-D, selective herbicides have become an integral part of modern agriculture. With the development of herbicides that alter the growth of higher plants, came a parallel interest in their effects on other organisms, including plant pathogens. As early as 1947 Fenner and Fate (1947) reported that 2,4-D treatment of Ceratocystis ulmi, the causal agent of Dutch Elm Disease, caused the formation of abnormally large and misshapen coremial masses. Richards (1949) reported that 2,4-D and 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) caused irregularities in mycelial growth and spore germination of several fungal pathogens when grown on herbicide amended media. However, the application of 2,4-D or 2,4,5-T to crop plants did not necessarily affect disease development. In a field study conducted by Sackston (1948), it was determined that 2,4-D applications to flax had no effect on the incidence or severity of disease caused by Septoria

linicola or Melampsora lini.

As continued research led to the development of various classes of herbicides, the study of the effects of these new materials on plant pathogens became widespread. Herbicide interactions with plant pathogens are expressed as effecting an increase, decrease or no change in plant disease. Katan and Eshel (1973) have proposed three mechanisms by which herbicides can influence disease development. These include direct inhibitory or stimulatory effects on the pathogen, changes in host susceptability and alterations in competitive interactions.

Direct effects of herbicides on pathogens are best studied in the laboratory. There are numerous examples of herbicides causing changes in the growth and development of pathogenic organisms. These effects vary with the herbicide, its concentration, and the organism studied. Many of these studies have been reviewed by Katan and Eshel (1973) as well as by Altman and Campbell (1977).

Katan and Eshel (1972) have postulated that herbicides could effect pathogen virulence by altering fungal metabolism or growth. However, experimental evidence of

herbicide induced changes in pathogen virulence is limited. An inconclusive report by Hsia and Christensen (1951) found that virulence of Helminthosporium sativum on wheat increased when the fungus was cultured on media containing 2,4-D. However, changes in disease severity could have been the result of direct effects of 2,4-D in small amounts on the host, rather than on herbicide-induced changes in the pathogen.

The mechanisms of action of herbicides on plant pathogens may be similar to those operative in higher plants. Sahid and Lyon (1981) showed that paraquat caused disruption of membrane integrity of several fungi. Membrane disruption caused by peroxidation of lipids has been shown to be paraquat's mechanism of action in higher plants (Hatzios and Pfenner, 1982).

Host susceptibility can be changed by herbicide treatment. Herbicides may alter morphological and physiological traits in host plants which can affect host-pathogen interactions (Altman, 1977, Katan and Eshel, 1973, Davis and Dimond, 1956). These may include effects on cell division, meristematic activity, cuticle production, cell wall thickness, membrane permeability, respiration, metabolism, as well as other plant characteristics and functions (Hatzios and Pfenner, 1982).

Alterations in competitive interactions may indirectly affect the pathogen. Understandably, organisms vary in their ability to metabolize herbicides (Wilkinson and Lucas, 1969b; Kurtz et al., 1982). Therefore, a herbicide could change the population of pathogens by favoring the development of one organism over the other. Such changes occur when the herbicide is more toxic to some members of the population than to others (Cerkauskas, 1982; Wilkinson and Lucas, 1969a,b).

The interactions between herbicides and plant disease are complex. Although a change leading to an increase in plant disease may be observed under laboratory conditions, a corresponding response may not necessarily be observed in the field. Although Johnston et al. (1980) found that in greenhouse studies several dinitroanaline herbicides could reduce disease severity in peas caused by a Fusarium complex, field data did not support these findings. In the field neither reductions in pea yield nor root rot severity where observed.

Timing of herbicide application may influence the outcome of pathogen interactions. Richardson (1959) found that 2,4-D applied to sand seven days before inoculation of tomatoes with Fusarium oxysporum f. lycopersicii decreased wilt, whereas applications after

inoculation increased disease severity. Soil type may also affect the interactions between herbicides and pathogens. Filo and Dhingra (1980) found that in a sandy clay loam soil dinoseb reduced populations of Macrophomina phaseolina by 96%, whereas in a sandy loam populations were reduced by 61%. Differences in disease interactions that vary with soil type are perhaps related to the herbicide reaction in the soil. Such characteristics as binding to soil colloids, solubility, pH reaction, and volatility can influence the exposure of soil borne pathogens to the herbicide in different soil types (Newman and Downing, 1958; Altman; 1977). Regardless of the means of application, all herbicides eventually reach the soil. Therefore, an organism living in the soil is likely to be influenced by a herbicide. Research on soil-borne diseases has been extensive. Increases as well as decreases in disease severity have been documented.

Hsia and Christensen (1951) showed that 2,4-D increased the incidence of seedling blight of wheat caused by Helminthosporium sativum. They concluded that the increase in disease was due to an increase in host susceptibility caused by herbicide application. However, Richardson (1957) found that 2,4-D, as well as monuron

and dalapon, reduced root rot infection in wheat. Of the herbicides studied, only maleic hydrazide increased disease severity. Conversely, Tinline and Hunter (1982) found no correlation between phenoxy herbicide application and the incidence or severity of common root rot in wheat. In laboratory studies Hodges (1977, 1981) showed that 2,4-D could increase Helminthosporium sativum mycelial growth and conidiospore germination. Although no reference was made to seedling diseases, Hodges found that leaf spot caused by H. sativum was increased due to foliar applications of 2,4-D in turfgrass (Hodges, 1977). Madson and Hodges (1982) found that MCPP, a phenoxy herbicide similar in mode of action to 2,4-D, decreased the content of sucrose and soluble sugars in treated plants. The low sugar levels were correlated with an increase of H. sativum leaf spot.

Increases in root exudation have been shown to increase the incidence of some soil-borne diseases. Altman (1972) showed that in sugarbeets pyrazon and cycloate increased damping off caused by Rhizoctonia solani by 50%. An increase in glucose exudate from herbicide treated roots caused increased sclerotia germination in the rhizosphere, which led to increased damping-off. Similarly Lee and Lockwood (1977) found that chloramben

enhanced soybean damping-off caused by Thielaviopsis basicola. In the field, plant stand and yield were reduced when chloramben was applied to infested soils. Laboratory experiments showed that germination of T. basicola spores was two to four times higher in the rhizospheres of treated soybean seedlings. Herbicide induced root exudation of amino acids stimulated spore germination which led to an increase in soybean disease severity.

Changes in inoculum potential as a result of herbicide application can affect disease development (Altman and Campbell, 1977). Duncan and Paxton (1981) found that although Phytophthora megasperma var. sojae growth in culture was inhibited by trifluralin, increased oospore production was observed. It was theorized that an increase in oospore production may lead to the increase in Phytophthora root rot noted in trifluralin treated soybeans. Nilsson (1973a) found that MCPP (mecroprop) increased perithecia and microspore production of Gaeumannomyces graminis in culture. Such changes may account for the increases in Take-All disease observed in wheat fields treated with MCPP (Nilsson, 1973b).

Herbicides may indirectly influence the populations of plant pathogens in the soil. Wilkinson and Lucas

(1969a) showed that herbicide residues in plant tissues can affect competition among fungi. In their study paraquat treated tissues were more condusive to colonization by Fusarium culmorum than by Trichoderma viride. Similar results were found for Rhizopus stolonifer and Aspergillus niger. Therefore herbicide treatment may lead to increases or decreases in plant disease by changing the competitive ability of plant pathogens.

The Herbicide Diclofop-methyl

Diclofop-methyl (methyl 2-(4-(2',4'-dichlorophenoxy)phenoxy) panoate), hereafter referred to as diclofop, is a diphenyl-ether herbicide that has both preemergence and post emergence activity. Symptoms on susceptible plants include chlorosis, necrosis, stunting, and restricted root growth. The herbicide can be absorbed through the foliage as well as through the roots. Both sensitive and tolerant species absorb significant amounts of herbicide (Boldt and Putnam, 1980), however, susceptible species are unable to detoxify the herbicide while tolerant species inactivate the herbicide metabolically (Shimabukuro et al., 1979).

Although, the specific mechanism of activity is not fully understood, Boldt and Putnam (1980, 1981) noted

irregularities in chlorophyll content and phloem transport as well as changes in the rates of photosynthesis and ATP production. Shimabukuro et al. (1978) implicated auxin antagonism as a primary mechanism of activity, whereas Brezeanu et al. (1976), Davis and Brezeanu (1979), and Crowley and Prendeville (1979) observed changes in membrane integrity which may be directly related to cell death leading to plant dysfunction.

As related to soil-borne disease, perhaps the most interesting aspect of diclofop activity in plants is decreased root growth. Root pruning has been observed in susceptible as well as in tolerant plants. In tolerant plants root pruning is more pronounced when soils are wet and temperatures are cool. Under such conditions roots may absorb more herbicide due to increased root exposure because wet conditions may concentrate the herbicide in the soil water around the root zone. Low temperatures may decrease the plant's metabolic rate, thereby reducing detoxification and increasing the concentration of the active herbicide in the tissue.

Chow and LaBerge (1978) suggested that root pruning is the result of a decrease in transportation of photosynthate from the leaves, rather than from any direct effect on the root. In more recent investigations,

Morrison et al. (1981) measured differences in wheat root growth with root exposure to diclofop. Similar results were obtained by Donald et al. (1982). In these studies reduction in adventitious root initiation as well as root length were detected. Histological studies conducted by Morrison et al. (1981) indicate that diclofop stops cell division in roots prior to mitosis, possibly during interphase. Within roots, other effects included tissue disruption in the central cylinder and structural deterioration of the epidermis.

Changes in root growth and morphology may increase wheat susceptibility to root infecting pathogens. A study by Nilsson (1973a) suggested that wheat roots damaged by the herbicide MCPP (Mecroprop) were more easily penetrated by Gaeumannomyces graminis. The roots in these studies were stunted and had bulbous tips due to herbicide application. Similar symptoms are seen with diclofop (Donald et al., 1982; Morrison et al., 1981), however the result of these changes on disease incidence or severity have not been investigated.

Aside from field observations, there have been no controlled studies conducted on wheat diseases and diclofop interactions. In fact the only research on the affect of diclofop on plant disease was conducted by

Ruppel et al. (1982). In a sugarbeet field trial, they found no significant interactions between diclofop and *Rhizoctonia solani*.

# MATERIALS AND METHODS

## The Pathogens

The pathogenic isolates of *B. sorokiniana*, *F. culmorum*, *G. graminis*, and *C. gramineum* were obtained from D.E. Mathre, Plant Pathology Department, Montana State University, Bozeman, Montana 59715. The isolates used in this research were *B. sorokiniana* isolate 214 and *F. culmorum* isolate 209. *G. graminis* and *C. gramineum* were isolations from infected wheat grown in Montana. All isolates were highly virulent.

## Field studies with Cephalosporium gramineum

Cephalosporium field trials were established in Bozeman, Montana at the Arthur H. Post Agricultural Research Field Laboratory and at the Central Montana Agricultural Research Center at Moccasin, Montana during the fall of 1981. Three winter wheat cultivars with differential susceptibility to Cephalosporium stripe were seeded. These were Redwin (CI 17844) and Winalta (CI 13670), both susceptible cultivars, and Winridge (CI 17902), a cultivar with only moderate susceptibility. To insure uniform disease development the plots were inoculated at planting with oat kernel inoculum at the rate of

1.5 grams per meter of row (Mathre and Johnston, 1975). Plots were seeded in Moccasin on September 11 and on September 17 in Bozeman using a cone-seeder. The seed and inoculum were added simultaneously to the row.

Treatments were arranged in a split plot design with four replications. Each of the cultivars was seeded in a block. Plots consisted of 12 rows with lengths of 3.3 meters at Bozeman, and 4.5 meters at Moccasin. Inoculations were split with six rows inoculated and six rows uninoculated. Diclofop was applied to 12 row plots at rates of 0, 1.12 and 2.24 kg. ai per HA. All treatments were randomized within a block design.

Diclofop was applied in the spring of 1982. At application, wheat was fully tillered, Zadoks stage 24 (Tottman, et al. 1979). Herbicide applications were made with a backpack sprayer calibrated to deliver 76 l/HA at a pressure of 2.39 kg/cm2. At both locations broadleaf weeds were controlled with Bronate (MCPA plus bromoxynil). To avoid diclofop-phenoxy antagonism, Bronate was applied to all plots 15 to 20 days after the diclofop applications. No other pesticides were applied.

Herbicide injury ratings were made 14 days after diclofop applications. Phytotoxicity ratings were based on visual estimation of percent stunting and degree of

leaf yellowing. A rating of over 20% injury was considered commercially unacceptable, while a rating of 100% indicated all plants dead. Additionally, plots were rated for disease severity by a visual estimation of percentage white heads per plot. These evaluations were made after flowering when the white heads were clearly evident.

At Moccasin, prior to harvest, rows were trimmed by mowing 0.3 meters from each end. The center four rows of each six row plot were harvested with a plot combine. The grain was weighed and yields recorded. At Bozeman, 2.5 meters of row were hand harvested from the middle of each of the center two rows. The heads were threshed and cleaned mechanically. The grain was weighed and yields recorded.

Field Studies with Fusarium culmorum and Bipolaris sorokiniana

Field trials were established in the spring of 1982 at Bozeman, Montana on the Arthur H. Post Agricultural Research Field Laboratory, and at the Central Montana Agricultural Reseach Center at Moccasin, Montana. In 1983 the experiment was repeated at Moccasin. The same design was used for all tests. Plots of Fortuna spring wheat (CI 13596) were artifically inoculated with either

B. sorokiniana or F. culmorum using oat kernel inoculum.

The procedure for making the inoculum was similar to that outlined by Mathre and Johnston (1975) for C. gramineum. Inoculum for field trials was made by culturing B. sorokiniana and F. culmorum on autoclaved oat kernels. In 1 liter glass jars were placed 150 grams oats with 100 ml distilled water. Jars were covered with Whatman qualitative filter paper, 7.0 cm in diameter. Metal lids with a 12 mm diameter hole in the center were then screwed onto the jars. The oats were autoclaved at 121 C for 20 minutes. After autoclaving the oats were allowed to cool at 21 C for 24 hours. After cooling, 8 mycelial plugs 1 cm in diameter were placed in each jar. Mycelial plugs were taken from B. sorokiniana and F. culmorum cultures growing on potato dextrose agar. Jars were shaken to distribute the mycelial plugs among the oat kernels. The oats were incubated three weeks at 21 C. After three weeks, when the oats were well covered by mycelia, the oat kernels were removed from the jars, spread on sheets of brown paper, and allowed to air dry at 21 C. After drying the inoculum was placed in paper sacks and stored at 5 C until used.

The inoculum was applied simultaneously with the seed at planting at a rate of three grams inoculum per

meter of row. A cone-seeder was used for planting. Treatments were arranged in a split plot design with four replications. Plots consisted of 12 rows with lengths of 3.3 meters at Bozeman and 6 meters at Moccasin. Inoculations were split with six rows inoculated and six rows uninoculated. Diclofop was applied to 12 row plots at rates of 0, 1.12 and 2.24 kg. ai/HA. B. sorokiniana and F. culmorum treatments were in separate blocks. All treatments within the block were randomized.

Diclofop was applied to tillering wheat, Zadoks stage 22. Herbicide applications were made with a backpack sprayer calibrated to deliver 76 l/HA at a pressure of 2.39 $kg/cm^2$. In all tests, Bronate (MCPA plus bromoxynil) was applied for broadleaf weed control. To avoid diclofop-phenoxy antagonism, Bronate applications were made 15 to 26 days after the diclofop application. No other pesticides were used.

Herbicide injury ratings were made 14 days after diclofop application. Phytotoxicity ratings were based on visual estimation of percent stunting and degree of yellowing. A rating of 20% injury was considered to be commercially unacceptable, while a rating of 100% indicated all plants were dead. To estimate the combined effects of disease and herbicide on crop vigor, a visual

assessment of percent injury, including height and stand reductions, was made at harvest. Yield data were collected following the procedure outlined for C. gramineum field plots.

As an indication of disease severity, subcrown internode ratings and stand counts were taken from B. sorokiniana and F. culmorum plots. For these ratings wheat plants from 0.3 meters of row were pulled and counted at harvest. The sample was taken from row three. The subcrown internode was rated for degree of necrosis. A scale of 0 to 3 was used. A rating of 0 represented healthy tissue without lesions; 1 represented lesions present, however, not coalescing around the internode tissue; 2 represented lesions coalescing but with no more than 50% necrosis; 3 represented lesions coalescing with greater than 50% necrosis.

As an inoculum control, prepared oat kernel inoculum was autoclaved to destroy the fungi. This autoclaved inoculum was added to row four in the uninoculated control plots. The rate for the autoclaved inoculum was also 3 grams per meter of row. At sampling disease ratings from rows three and four were compared.

Field Studies with Gaeumannomyces graminis

Field trials were established at the same locations

as C. gramineum field trials. Fortuna spring wheat was artifically inoculated using oat kernal inoculum. A rate of 1.6 grams of inoculum per meter of row was applied at planting. The experimental design and plot treatment were identical to that given in the above section for Fusarium and Bipolaris.

Greenhouse Studies with Gaeumannomyces graminis

Surface sterilized wheat seeds, cultivar Butte (CI 17681), were incubated seven to ten days at 21 C in plastic boxes lined with paper toweling. Seeds were sterilized by soaking for five minutes in 0.5% sodium hypochlorite. The seedlings were transferred to 1 liter opaque glass culture jars containing one-half strength Hoagland's solution, when the leaf reached the coleoptile tip, Zadoks stage 09. Styrofoam corks with holes for each seedling were used to cover the jars and support the seedlings. Each jar contained two seedlings.

The seedlings were maintained in the greenhouse. The day length was extended to 14 hours using supplementary fluorescent lighting. Temperatures averaged 8 C during the night and 24 C during the day. After seven days the nutrient solution was replaced with full strength Hoagland's solution enriched with 4 mg/l chelated iron. The nutrient solution was changed weekly

for the duration of the experiment. Additional nutrient solution was added as needed to maintain the liquid in the culture jars at 1 liter. The nutrient solution was continuously aerated with compressed air.

When the seedlings reached two leaves, Zadoks stage 12, 50% of them were inoculated with G. graminis. Inoculation was accomplished by attaching a 1 cm diameter mycelial plug to the shoot just above the seed. Mycelial plugs were removed from G. graminis cultures grown on PDA. These plugs were attached to the seedling by wrapping them to the plant with moistened cotton strands. The inoculum was positioned just above the liquid in the culture jars.

At tillering, Zadoks stage 22, the wheat was treated with diclofop to reach a final concentration of 3 uM for 48 hours. The herbicide was added directly to the culture jars. According to Shimabukuro (1982), a 3 uM diclofop solution will alter wheat root growth with a 48 hour exposure time. After 48 hours the solutions in all the culture jars were replaced with fresh Hoagland's solution.

Treatments consisted of an untreated check, G. graminis, diclofop, and G. graminis plus diclofop. Treatments consisted of eight plants, planted two plants per

jar. The jars were arranged on the greenhouse bench in a completely randomized design.

At heading, Zadoks stage 59, roots were clipped from the plants. Secondary root length was evaluated by averaging the root length of the uppermost five roots. Live root volume was measured volumetrically by emersing the root mass in a known volume of water and noting the change in volume of the water. The roots were then oven dried at 55 C for 24 hours and weighed.

Analysis of variance was used to detect differences among treatments. Comparisons among means were made using Student Newman Keuls test (SNK) for equal means.

Studies on the Fungitoxicity of Diclofop

The effect of diclofop on the growth of *B. sorokiniana*, *F. culmorum*, *C. gramineum*, and *G. graminis* was evaluated by culturing these fungi on diclofop-amended potato dextrose agar (PDA). Difco PDA was prepared as directed. The autoclaved PDA was cooled to 45 C. A commercial diclofop formulation containing 360 grams per liter diclofop was then added to the PDA to produce amended PDA with diclofop concentrations of 0, 1, 10, 100, and 1,000 mg/l. Amended PDA was poured into plastic Petri plates and cooled.

To compare the effects of the solvents, surfactants

and other compounds found in the commercial diclofop formulation, a blank formulation containing no diclofop was compared to the formulated herbicide at volumes equal to those required to produce 1, 10, 100, and 1,000 mg/l concentrations of diclofop. Both the commercial herbicide and the blank formulation were supplied by American Hoechst Corporation, Somerville, NJ.

All fungi were maintained on PDA. A 10 mm mycelial plug taken from the outer edge of an actively-growing culture was placed in the center of each Petri plate containing PDA or amended PDA. All transfers for each fungus were taken from the same culture plate. Each treatment was replicated five times.

Culture plates were maintained at 21 C. Radial mycelial growth was measured when the mycelia in one of the treatments reached the edge of the Petri plate. A two factor analysis of variance was used to determine differences among treatments. Treatment means were compared using an LSD at the 5% level.

Studies on Diclofop Induced Virulence Changes

To determine if diclofop exposure produces physiological or genetic changes within the pathogens that may alter virulence, virulence tests were conducted. A 10 mm mycelial plug was taken from cultures of *C. gramineum*, *B.*

sorokiniana, G. graminis, and F. culmorum growing on PDA amended with 100 mg/l diclofop. The plugs were transferred to PDA. This transfer was done to eliminate the effects of diclofop contained in the amended media on the wheat. Simultaneously, a mycelial plug from an unamended PDA culture of the same age was transferred to PDA.

The cultures of all but C. gramineum were incubated 10 days at 21 C. After incubation a 22 mm mycelial plug was cut from the media and placed in a Cone-tainer 3 cm in diameter, which had been filled with moistened vermiculite. Pre-germinated Butte spring wheat seeds, Zadoks scale 05, were placed on top of the mycelial plug and covered with vermiculite. Controls from cultures not exposed to diclofop, as well as uninoculated controls were also included. Each treatment was replicated 10 times.

In the case of C. gramineum the cultures were incubated 26 days at 21 C, to allow for adequate mycelial growth. After incubation eight 10 mm mycelial plugs were mixed with sufficient moistened vermiculite to fill a 10 cm pot. Two Butte spring wheat plants at the one node stage, Zadoks scale 30, were placed in each pot. Prior to transplanting seedlings had been grown in vermiculite. In order to facilitate infection the wheat root mass was

trimmed to 12 cm at transplanting. A control from cultures not exposed to diclofop was included, as well as an uninoculated control. Treatments were replicated four times with two plants per pot, i.e. eight plants per treatment.

The Cone-tainers and pots were maintained in the greenhouse, with day temperatures of 18 C and night temperatures of 8 C. Plants were fertilized as needed with full strength Hoagland's solution.

At four weeks after inoculation each plant was rated for disease severity. A rating scale of 0 to 5 was used with 0 representing healthy, and with 5 representing complete leaf necrosis. With C. gramineum, plant height was measured and leaf striping noted.

# RESULTS

## Field Studies with Cephalosporium gramineum

The application of diclofop to winter wheat had no effect on yield of either C. gramineum infected plants or healthy plants. However, significant ($P<0.05$) yield differences between infected and healthy plants were found at both locations (Table 1). Although the yield difference between healthy and infected wheat was greatest with no herbicide and decreased with herbicide application, this trend was not statistically significant ($P<0.05$) (Table 2 ). An analysis of variance was used to compare treatments. No significant differences in other parameters were found. Neither visual disease assessment or visual herbicide injury assessment showed any difference in reaction between healthy and diseased wheat to diclofop.

Table 1. Effect of Cephalosporium gramineum on yield of three winter wheat cultivars at Bozeman and Moccasin, Montana in 1982.

| | Yield (kg/HA)[1] | | | |
|---|---|---|---|---|
| Inoculation | Redwin[2] | Winridge[2] | Winalta[2] | Mean |
| Uninoculated | 3703[a] | 4444[a] | 3771[a] | 3973[a] |
| Inoculated | 2896[b] | 3367[b] | 2963[b] | 3097[b] |

[1]Averaged across 0, 1.12, and 2.24 kg ai/HA diclofop.

[2]Yields are the mean of 4 replications at 2 locations; values in the same column are different when followed by different letters, LSD at 5%.

Table 2. Effect of diclofop-methyl on yield of winter wheat uninoculated or inoculated with Cephalosporium gramineaum.

| | Yield (kg/HA)[1, 2] | | |
|---|---|---|---|
| Diclofop-methyl (kg ai/HA) | Uninoculated | Inoculated | Difference |
| 0.0 | 3990[a] | 2929[a] | 1061[a] |
| 1.12 | 4027[a] | 3178[a] | 849[a] |
| 2.24 | 3879[a] | 3118[a] | 761[a] |

[1]Yields are a mean of 4 replications at 2 locations, averaged across 3 cultivars.

[2]Values in the same column are different when followed by different letters, LSD at 5%.

Field Studies with Fusarium culmorum and Bipolaris sorokiniana.

Dryland root rot field trials over three location years indicated that wheat in both inoculated and uninoculated plots responded similarly to diclofop treatment. Yield decreases due to inoculation with these two pathogens were significant ($P<0.05$) for B. sorokiniana in 1982, and for F. culmorum in 1983. These yield responses were significant only at Moccasin. Otherwise, inoculation with these two pathogens caused no effect on yield (Table 3).

Table 3. Effect of Bipolaris sorokiniana or Fusarium culmorum inoculation on yield of Fortuna spring wheat at Moccasin (Mc) and Bozeman (Bz), Montana.

| | Yield (kg/HA)[1, 2] | | | | | |
|---|---|---|---|---|---|---|
| | B. sorokiniana | | | F. culmorum | | |
| Inoculum Added | 1982 | | 1983 | 1982 | | 1983 |
| | Mc | Bz | Mc | Mc | Bz | Mc |
| No | 1468[a] | 3113[a] | 2249[a] | 1297[a] | 3311[a] | 2269[a] |
| Yes | 1257[b] | 3125[a] | 2211[a] | 1246[a] | 3266[a] | 2215[b] |

[1]Yields are a mean of 4 replications averaged across 0, 1.12 and 2.24 Kg ai/HA diclofop.

[2]Values in the same column are different when followed by different letters, LSD at 5% level.

When comparing plots inoculated with F. culmorum to non-inoculated plots, no significant interactions ($P<0.05$) between diclofop application and yield were detected (Table 4). However, with B. sorokiniana, yields in inoculated plots increased significantly ($P<0.1$) with diclofop applications, when compared to uninoculated plots. Herbicide induced yield increases in inoculated plots were noted at both Moccasin and Bozeman in 1982, but no interactions were detected in 1983 (Table 5).

Table 4. Effect of diclofop-methyl on yield of Fortuna spring wheat uninoculated (U) or inoculated (I) with Fusarium culmorum.

| | Yield (kg/HA)[1, 2] | | | | | |
|---|---|---|---|---|---|---|
| | 1982 | | | | 1983 | |
| Diclofop (kg ai/HA) | Moccasin | | Bozeman | | Moccasin | |
| | U | I | U | I | U | I |
| 0.0 | 1374[a] | 1253[a] | 3717[a] | 3131[a] | 2303[a] | 2256[a] |
| 1.12 | 1205[a] | 1185[a] | 3051[a] | 3252[a] | 2310[a] | 2208[a] |
| 2.24 | 1313[a] | 1300[a] | 3165[a] | 3414[a] | 2195[a] | 2182[a] |

[1]Yields are mean of 4 replications.

[2]Values in the same column are different when followed by different letters, LSD at 5%.

Table 5. Effects of diclofop-methyl on yield of Fortuna spring wheat uninoculated (U) or inoculated (I) with Bipolaris sorokiniana.

| Diclofop-methyl (kg ai/HA) | Yield (kg/HA)[1, 2] 1982 Moccasin U | 1982 Moccasin I | 1982 Bozeman U | 1982 Bozeman I | 1983 Moccasin U | 1983 Moccasin I |
|---|---|---|---|---|---|---|
| 0.0 | 1421[a] | 1044[a] | 3360[a] | 2842[a] | 2269[a] | 2155[a] |
| 1.12 | 1542[a] | 1212[a] | 3138[a] | 3508[b] | 2242[a] | 2249[a] |
| 2.24 | 1441[a] | 1515[b] | 2842[a] | 3024[b] | 2236[a] | 2229[a] |

[1]Yields are a mean of 4 replications.

[2]Values in the same column are different when followed by different letters, LSD at 5%.

For B. sorokiniana at Bozeman in 1982 disease ratings based on subcrown internode necrosis, showed that disease severity was greater in the inoculated plots than in the uninoculated plots ($P<0.1$). Due to background levels of B. sorokiniana, no differences in disease severity were noted at Moccasin (Table 6). There was no difference in subcrown internode ratings between plants in rows with autoclaved inoculum compared to uninoculated rows ($P<0.1$). Wheat stand was reduced by inoculation at both locations (Table 6).

Table 6. Effects of Bipolaris sorokiniana inoculation on percent disease (D)[1], percent severe disease (SD)[2], and wheat population (P) and yield at Bozeman and Moccasin, Montana in 1982.[3, 4]

| | Bozeman | | | Moccasin | | |
|---|---|---|---|---|---|---|
| | % D | % SD | P | % D | % SD | P |
| Inoculated | 1.4[b] | 0.5[b] | 19.9[b] | 5.1[a] | 1.5[a] | 12.3[b] |
| Uninoculated | 8.5[a] | 5.0[a] | 6.7[a] | 3.5[a] | 0.7[a] | 20.1[b] |

[1]Percent disease represents the percentage of subcrown internodes with lesions and/or some degree of necrosis.

[2]Percent severe disease represents the percentage of subcrown internodes with coalescing lesions and 50% or greater subcrown internode necrosis.

[3]Values represent an average of 4 replications, based on 0.3 meter row sample, averaged across 0, 1.12, and 2.24 kg ai/HA diclofop.

[4]Numbers in the same column are different when followed by different letters, LSD at 5%.

For F. culmorum in 1982 disease ratings based on subcrown internode necrosis showed that at both locations a greater number of plants were severely infected in inoculated plots compared to uninoculated plots (Table 7). The difference in percent overall infection between inoculated and uninoculated plots was significant at Bozeman ($P<0.05$), but not at Moccasin. As with B. sorokiniana, background levels of F. culmorum were responsible for a lack of response to inoculation in

overall disease rating. A reduction in wheat stand due to inoculation was noted at Bozeman (Table 7). There were no differences in subcrown internode ratings between plants in rows with autoclaved inoculum compared to uninoculated rows ($P<0.1$).

Table 7. Effects of Fusarium culmorum inoculation on percent disease (D)[1], percent severe disease (SD) and wheat population (P) at Bozeman and Moccasin, Montana in 1982.[3, 4]

| | Bozeman | | | Moccasin | | |
|---|---|---|---|---|---|---|
| | % D | % SD | P | % D | % SD | P |
| Inoculated | 50.4[a] | 4.7[a] | 16[a] | 34.18[a] | 2.9[a] | 20.5[a] |
| Uninoculated | 30.3[b] | 2.4[b] | 19[b] | 31.41[a] | 1.4[b] | 21.3[a] |

[1]Percent disease represents the percentage of subcrown internodes with lesions and/or some degree of necrosis.

[2]Percent severe disease represents the percentage of subcrown internodes with coalescing lesions and 50% or greater subcrown internode necrosis.

[3]Values represent an averge of 4 replications, based on 0.3 meter row sample, averged across 0, 1.12 and 2.24 kg ai/HA diclofop.

[4]Numbers in the same column are different when followed by different letters, LSD at 5%.

No visual herbicide injury was noted at the 1.12 kg ai/HA rate, either 14 days after application or at harvest. However, at 2.24 kg ai/HA, wheat injury of 15% was

observed 14 days after application at both locations in 1982. No injury was noted in 1983. No differences were apparent in degree of visual injury between inoculated and uninoculated plots.

Field Studies with Gaeumannomyces graminis

Inoculum density and favorable moisture conditions combined to reduce stands of Fortuna wheat by 90%. Due to severe disease losses no herbicide-G. graminis interactions could be evaluated. Similar tests in 1983 were destroyed by hail and animal grazing.

Greenhouse Studies with Gaeumannomyces graminis

In a hydroponic system, diclofop applications to healthy wheat reduced root volume and dry weight (Table 8). Conversely, diclofop applications to G. graminis infected wheat caused no differences in root volume or dry weight (Table 8). Since responses of healthy roots to diclofop were more pronounced than were those of diseased roots, an analysis of variance detected significant ($P<0.05$) interactions between G. graminis infection and diclofop treatment (Figure 1). These data indicate that the effect of diclofop on root volume and dry weight is less severe in diseased wheat than in healthy wheat. A significant reduction in root length due to diclofop

treatment was noted in both infected and healthy wheat (Table 8). Both diseased and healthy roots responded similarly to diclofop in respect to root growth reduction (Figure 2).

TABLE 8. Effects of treatment with diclofop-methyl on root volume, dry weight and length of Butte spring wheat, with or without *Gaeumannomyces graminis* inoculation.[1]

| Diclofop-Methyl Concentration | Wheat Root Response[2] | | | | | |
|---|---|---|---|---|---|---|
| | No Inoculation | | | With Inoculation | | |
| | Vol (cc) | Wt (mg) | Length (mm) | Vol (cc) | Wt (mg) | Length (mm) |
| 0 uM | 5.4[a] | 275[a] | 13.8[a] | 1.9[a] | 70[a] | 11.0[a] |
| 3 uM | 2.5[b] | 113[b] | 8.7[b] | 1.5[a] | 79[a] | 6.5[b] |

[1]Values in the same column are different when followed by different letters. Student Newman Keuls test at 5% level.

[2]Average of 8 replications with 1 plant per replicate.

*Diclofop Fungitoxicity Studies*

At the concentrations evaluated, diclofop inhibited pathogen growth except for *G. graminis* where growth stimulation was noted at 10 mg/l ($P<0.05$) (Table 9). *F. culmorum* was tolerant to diclofop. Significant *F. culmorum* growth reductions were noted only at 1000 mg/l.

Pathogen growth was also reduced significantly by exposure to the herbicide formulation without diclofop ($P<0.05$) (Table 10).

Table 9. Effects of diclofop-methyl (DM) and diclofop-methyl formulation blank (FB) on radial growth of *Gaeumannomyces graminis*, *Cephalosporium gramineum*, *Fusarium culmorum*, and *Bipolaris sorokiniana*.[1]

| Concentration mg/l | Growth (mm)[2] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *C. gramineum* | | *B. sorokiniana* | | *F. culmorum* | | *G. graminis* | |
| | DM | FB | DM | FB | DM | FB | DM | FB |
| 0 | 75[a] | 75[a] | 78[a] | 78[a] | 78[a] | 78[a] | 64[a] | 64[a] |
| 1 | 70[a] | 73[a] | 66[b] | 66[b] | 78[a] | 76[a] | 61[b] | 65[a] |
| 10 | 58[b] | 72[a] | 59[c] | 71[c] | 78[a] | 78[a] | 70[c] | 65[a] |
| $10^2$ | 38[c] | 55[b] | 36[d] | 36[d] | 77[a] | 64[b] | 57[d] | 46[b] |
| $10^3$ | 29[d] | 36[c] | 26[e] | 29[e] | 41[b] | 44[c] | 13[e] | 11[c] |

[1]Values represent an average of 5 replications.

[2]Values in the same column are different when followed by different letters, LSD at 5% level.