Effects of Postemergence Herbicides on *Cercospora arachidicola* Hori and Early Leaf Spot of Peanut[1]

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ABSTRACT

Early leaf spot is a common disease of peanut caused by the fungus *Cercospora arachidicola* Hori. Experiments were conducted to evaluate the effect of postemergence herbicides on the conidial germination of *C. arachidicola* and on the incidence of early leaf spot disease in peanut (*Arachis hypogaea* L.) in a greenhouse. Conidial germination was enhanced (≥ 100%) at concentrations of 1, 100, and 1000 mg/L of 2,4-DB compared with the untreated control. Lactofen reduced conidial germination by 42% compared with the control at concentrations as low as 100 mg/L and completely inhibited germination at concentrations ≥ 5000 mg/L. A concentration of 10,000 mg/L acifluorfen and 2,4-DB completely inhibited conidial germination. Acifluorfen, acifluorfen plus 2,4-DB, and lactofen decreased the sporulation of early leaf spot lesions. Lactofen reduced leaf spot incidence 12% and decreased sporulation of lesions 22% compared with the control. None of the herbicides increased the incidence of early leaf spot on peanut plants or the number of early leaf spot lesions per leaflet when compared with plants that received no herbicide.

Key Words: Herbicide-plant disease interaction, leaf spot severity.

Weed and disease management are essential for profitable peanut production (7, 23). Composition of weed species and accompanying weed management systems vary across peanut growing regions of the U.S. A common disease problem throughout peanut-producing areas of the U.S. is early leaf spot, caused by the fungus *Cercospora arachidicola* Hori. Early leaf spot is a poly-

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cyclic disease which may occur early (within 3 to 5 wk after planting) and persists throughout the growing season (17). Early leaf spot can be very destructive to the plant and is considered one of the most serious diseases of peanut worldwide (17). Cultural practices that reduce the initial inoculum can be used to partially manage early leaf spot (23, 24); however, properly timed fungicide applications are required to prevent yield loss (27).

Postemergence (POST) herbicides are applied to peanut during the early part of the growing season for weed control and may precede or coincide with early leaf spot. Peanut growers in Oklahoma rely on POST herbicides to control weeds that germinate after planting and escape preplant soil herbicide applications. The biological activity of herbicides is not restricted to weeds and may affect other organisms including plant pathogens. Certain herbicides increase plant disease while others have decreased disease incidence (2, 3, 4, 5, 8, 13, 15, 22, 30, 31).

In vitro laboratory studies provide reliable information concerning the fungitoxicity of herbicides against specific pathogens (25). Shennan and Fletcher (26) reported that *in vitro* growth of various species of fungi, yeast, bacteria, actinomycetes, and green algae, were not inhibited in the presence of 2,4-D or 2,4-DB at concentrations comparable to field rates of application; however, at concentrations ≥ 500 mg/L, 2,4-DB was highly toxic and inhibited growth, whereas 2,4-D had no effect on disease development.

In vitro tests (2) revealed that 25 commercial crop production herbicides stimulated the growth of *Rhizoctonia solani* Kühn at concentrations up to 1000 mg/L. In these tests, 12 of the 25 herbicides had little effect on fungal growth at a concentration of 10,000 mg/L, while two herbicides inhibited growth completely. In another study (18), the fungal growth of *C. arachidicola* was completely inhibited by 73.5 mg/L aqueous preparation of the acaricide, cyhexatin.

Several POST herbicides used in peanut cause leaf burn or leaf bronzing (6, 9, 29, 32). Affected peanut leaves have areas of necrotic tissue accompanied by some chlorosis. Injury from these contact herbicides has been reported in other broadleaf crops, but the injury was apparently temporary due to the initiation of new growth and no effect on yield (11, 14, 16).

Katan and Eshel (15) discussed possible mechanisms involved in the increase of disease incidence due to herbicide application as the (a) direct stimulatory effect on the pathogen, (b) increased virulence of the pathogen, (c) increased susceptibility of the host, and (d) suppression of microorganisms antagonistic to the pathogen. A decrease in disease incidence due to herbicides might be a result of the reversal of any one of the four mechanisms involved in disease increase (15).

Peanut growers in Oklahoma and researchers have expressed concern about the possibility of increased incidence of early leaf spot resulting from POST herbicide use. Knowledge of herbicide activity on peanut and its effects on early leaf spot will help peanut growers make decisions that will optimize weed and disease management.

There is limited information on the effect of POST herbicides on *C. arachidicola* and early leaf spot disease of peanut. The objectives of this research were to investigate the effect of commercially formulated herbicides on the conidial germination of *C. arachidicola* and on disease incidence under greenhouse conditions.

Materials and Methods

Effect on Conidial Germination. Laboratory experiments were conducted to determine the germination of conidia of *C. arachidicola* in different concentrations of five foliar-applied herbicides commonly used in peanut production and one herbicide being developed for such use. Herbicide treatments included acifluorfen, bentazon, acifluorfen plus bentazon (Storm®, 159 g/L acifluorfen and 320 g/L bentazon, BASF Corp., Research Triangle Park, NC), lactofen, imazethapyr, and 2,4-DB.

Conidia of *C. arachidicola* were obtained from infected peanut plants grown in a greenhouse (19). Leaflets from plants with mature lesions were placed in petri dishes lined with moist Whatman #1 filter paper and incubated at 25 C for 2 to 3 d. The leaflets were then placed in minimal amounts of distilled water and gently agitated to dislodge conidia from the mature lesions. The concentration of conidia in the suspension was determined with a hemacytometer and then adjusted to 40,000/mL.

The germination of conidia was tested in distilled water and with each herbicide treatment at concentrations of 1, 100, 1000, 5000, and 10,000 mg/L using the depression slide technique (25). Each herbicide-conidia suspension was pipetted into two wells per slide and replicated four times. Slides were placed into petri dishes that were lined with moist Whatman #1 filter paper and covered to provide a humid environment to prevent water from evaporating and drying the wells. Dishes containing the slides were placed in an incubation chamber at 27 C (1). Conidial germination (%) at 72 hr in each well was determined at four microscope grids.

Data Analysis. Experiments had a complete randomized design and were conducted twice. Data were subjected to analysis of variance and mean separation was performed with a protected Least Significant Difference (LSD) Test at $P \leq 0.05$. The analysis of variance indicated no experiment by treatment interaction, therefore data presented are the pooled means of each herbicide treatment at a given concentration. Germination of conidia is expressed as the percentage of conidia germinated compared with germination of conidia in the distilled water control (18, 26).

Effect on Components of Early Leaf Spot. In the greenhouse, peanut cultivars that are susceptible to early leaf spot do not perform well when inoculated with *C. arachidicola* (Melouk, unpubl. data, 1991). Early leaf spot is severe in the warm, moist environment of the greenhouse and susceptible cultivars rapidly defoliate, thus hindering data collection. The runner-type peanut cultivar Okrun is slightly resistant to early leaf spot compared to other peanut cultivars grown in Oklahoma; therefore, it was used in these experiments. Seed were germinated on moist paper towels at 29 C for 48 hr prior to planting in pots (12-cm diameter) containing a mixture of soil, sand, and peat (1:1:2; v/v/v). Pots were placed in a greenhouse maintained at 28 C during the day and 22 C at night.

Four weeks after planting, main stems and horizontal branches of individual plants were marked with string and a water-resistant, permanent marker at the growing points. The number of peanut leaflets on each plant was counted and recorded. Peanut plants were then treated with POST herbicides using a laboratory table sprayer equipped with an 8002 even flat fan (Spraying Systems Co., Wheaton, IL) nozzle delivering 140 L/ha. Herbicide treatments included acifluorfen at 0.56 kg ai/ha, bentazon at 0.84 kg/ha, premixed acifluorfen at 0.28 kg/ha plus bentazon at 0.56 kg/ha, imazethapyr at 0.071 kg/ha, lactofen at 0.21 kg/ha, 2,4-DB at 0.45 kg/ha, acifluorfen plus 2,4-DB at 0.28 and 0.15 kg/ha, and bentazon plus 2,4-DB at 0.43 and 0.15 kg/ha. Crop oil concentrate (COC, Cornbelt Chemical Co., McCook, NE) was added to the bentazon treatment at 1.25% (v/v) of the spray volume. The other treatments, except 2,4-DB alone, were applied with a nonionic surfactant (NIS, Triton AG-98®, Rohm and Haas Co., Philadelphia, PA) at 0.25% (v/v).

One week after herbicide treatment, peanut plants were inoculated with conidia of *C. arachidicola* obtained from infected plants grown in the greenhouse. A conidial suspension (2×10^4 conidia/mL) was prepared in 100 mL of distilled water containing 2 drops of Amway all-purpose surfactant (Amway Corp., Ada, MI). The abaxial and adaxial surfaces of peanut leaflets were misted with the conidial suspension using a DeVilbiss No. 152 atomizer (The DeVilbiss Company, Somerset, PA) (10, 21). Plants were placed into a dew chamber and maintained in the dark for 96 hr at 21 C and 100% relative humidity, then maintained with a 12 hr light cycle for 72 hr at 27 C and 60-70% RH. Plants were removed from chambers and returned to the greenhouse for a 2-wk period prior to evaluation.

Leaflets that were treated with herbicide and inoculated with *C. arachidicola* were sampled 3 wk after inoculation and placed into petri-dishes lined with Whatman #1 filter paper moistened with water to maintain 100% relative humidity for 4 d to promote sporulation of the fungus in the lesions. The total number of lesions and sporulated lesions per leaflet were counted and recorded. The following disease parameters for each treatment were determined:

a) Leaf spot incidence (LSI) = no. of leaflets with leaf spot lesions/total no. of leaflets;

b) Leaf spot severity (LSS) = total no. of lesions/no. of leaflets with leaf spot lesions;

c) Degree of sporulation (DS) = no. of sporulating lesions/total no. of lesions;

d) Potential available inoculum index (PAI) = LSS * DS.

The PAI indicated how each herbicide treatment affected available inoculum for secondary infection cycles. A similar index was used to determine genotype reactions to leaf spot (20).

Data Analysis. Experiments had a randomized complete block design with four replications and were conducted three times. All data were analyzed following standard procedures for analysis of variance, and means were separated using a protected LSD test at $P \leq 0.05$. The analysis of variance indicated no experiment by treatment interaction, therefore data from all three experiments were pooled in analysis.

Results and Discussion

Effect on Conidial Germination. Treatments of acifluorfen plus bentazon, bentazon, and imazethapyr did not inhibit the germination of conidia at any concentration (Table 1). The acifluorfen plus bentazon and the bentazon mixtures enhanced conidial germination at a concentration of 1000 mg/L when compared with the control. Concentrations of ≤ 1000 mg/L of 2,4-DB and 1000 and 5000 mg/L of acifluorfen stimulated conidial germination. However, concentrations of 2,4-DB at > 1000 and acifluorfen at > 5000 mg/L were greatly inhibitory. Shennan and Fletcher (26) reported similar results with various species of fungi and MCPA at different concentrations. They suggested that certain species of fungi utilized the herbicide as an additional carbon source. Altman (2) reported that numerous herbicides stimulated *in vitro* growth of *R. solani* and that the fungus possibly utilized the herbicides as a source of energy.

Table 1. Effect of herbicides at different concentrations on the germination of *Cercospora arachidicola* conidia *in vitro* after 72 h.

Herbicide[b]	mg ai/L[a] 1	100	1000	5000	10,000
	% of control				
Acif.+Bent.	102 bc	88 c	129 b	119 ab	100 a
Bentazon	106 b	100 bc	133 b	113 ab	119 a
Imazethapyr	117 b	117 b	119 bc	115 ab	108 a
Lactofen	77 c	58 d	13 d	0 c	0 b
2,4-DB	146 a	158 a	163 a	8 c	0 b
Acifluorfen	96 bc	104 bc	171 a	144 a	0 b
Control[c]	100 bc	100 bc	100 c	100 b	100 a

[a]Means presented for each concentration are a percentage of the control. Values within columns followed by the same letter are not different at ($P \leq 0.05$) according to a protected LSD test.

[b]Acif. = acifluorfen, Bent. = bentazon.

[c]Percent germination in distilled water.

In 10,000 mg/L mixtures, percent conidial germination of acifluorfen plus bentazon was greater than acifluorfen alone (Table 1). The addition of bentazon to the mixture reduced the inhibitory effect of acifluorfen on conidia. However, due to the ratio of acifluorfen in the acifluorfen plus bentazon treatment, the actual concentration of acifluorfen is lower as compared to acifluorfen alone at 10,000 mg/L.

Lactofen reduced conidial germination at all concentrations and completely inhibited germination at concentration ≥ 5000 mg/L. Microscopic observations of conidia in 10,000 mg/L mixtures of lactofen, 2,4-DB, and acifluorfen revealed that plasmolysis of individual conidial cells had occurred. Similarly, plasmolysis of conidial cells of *Drechslera sorokiniana* (Sacc.) Subram. & Jain occurred after exposure to 1000 mg/L 2,4-D, 2,4,5-T, dicamba, and mecoprop (13). It is possible that the use of lactofen, 2,4-DB, and acifluorfen in the field may reduce the initial inoculum of *C. arachidicola* and possibly reduce the number of fungicide applications needed to manage the disease.

Recommended use rates of acifluorfen, acifluorfen

plus bentazon, bentazon, and 2,4-DB for peanut range from 5000 to 10,000 mg/L, while lactofen and imazethapyr rates range from 1000 to 5000 mg/L (based on 187 L/ha spray volume). Lactofen is not currently registered for use in peanut with the U.S. Environmental Protection Agency. Use rates of lactofen were derived from previous experimental trials not mentioned in this report. The range of herbicide concentrations used in this study were equal to or less than that recommended for peanut production (12). In this study, acifluorfen plus bentazon, bentazon, and imazethapyr had no significant inhibitory effect on conidial germination. However, these results indicate lactofen, 2,4-DB, and acifluorfen at a concentration of 10,000 mg/L could minimize the germination of conidia present on peanut leaves at time of application.

Effect on Components of Early Leaf Spot. Plants with no herbicide (control) produced equivalent or higher disease parameter values (increased disease incidence) than did herbicide-treated plants (Table 2). The LSI in peanut treated with acifluorfen, bentazon, acifluorfen plus bentazon, imazethapyr, 2,4-DB, acifluorfen plus 2,4-DB, and bentazon plus 2,4-DB were not significantly different from the control. There was a decrease in LSI of peanut treated with lactofen (12%) compared with the control. However, the LSI of peanut treated with lactofen was not significantly different from the other herbicide treatments.

Peanut injury (leaf burn) occurred after application of acifluorfen, acifluorfen plus bentazon, acifluorfen plus 2,4-DB, and lactofen treatments (data not shown). Leaf necrosis was most severe with acifluorfen alone, acifluorfen plus bentazon, and lactofen treatments. The damaged and reduced area of healthy peanut leaf tissue may explain the decrease in disease incidence because there were no leaf spot lesions observed on necrotic tissue. Due to this reduction in healthy leaf tissue, conidia may not have survived on necrotic leaf tissue, thus reducing the infection process of the disease cycle.

The LSS and PAI of all herbicide treatments were not significantly different from the control (Table 2). Leaf spot lesions present on leaflets treated with acifluorfen, acifluorfen plus 2,4-DB, or lactofen had the lowest DS values (< 53%) compared with the control. The reduction in sporulation is epidemiologically important because it may reduce the apparent infection rate or the rate of disease increase during the growing season (28). Leaf spot lesions on untreated peanut leaflets produced the highest DS value (69%) and had a high PAI (4.3) value.

This study did not address the effects of inoculum density or variable rates of the individual herbicides. Also, this study addressed only one disease cycle of early leaf spot in which the amount of sporulation was measured to predict the possible effect of herbicides on the secondary inoculum potential. The results of this research did not support our hypothesis that POST applied herbicides cause an increase in early leaf spot disease. It can be concluded that the POST herbicides investigated in this experiment did not increase disease.

In actual field situations, variable inoculum densities, environmental conditions, disease cycles, and number of herbicide applications may have an effect on the amount of disease. However, if the results of these experiments can be repeated in the field, peanut growers might be able to select a herbicide that will reduce the amount of disease and available inoculum or at least have some assurance that the use of a POST herbicide will not cause greater problems from foliar diseases.

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Table 2. The effect of postemergence herbicides on the components of early leaf spot of peanut caused by *Cercospora arachidicola*[a].

Herbicide	Additive	Rate	LSI	LSS	DS	PAI
		kg ai/ha				
Acifluorfen	NIS	0.56	0.59 ab	5.6 a	0.52 bc	2.9 a
Bentazon	COC	0.84	0.62 ab	6.6 a	0.61 ab	4.0 a
Acif.+ Bent.	NIS	0.28 + 0.56	0.57 ab	5.2 a	0.59 ab	3.1 a
Imazethapyr	NIS	0.071	0.59 ab	6.2 a	0.63 ab	3.9 a
Lactofen	NIS	0.21	0.54 b	5.2 a	0.47 c	2.4 a
2,4-DB	None	0.45	0.63 ab	4.6 a	0.59 abc	2.7 a
Acif.+2,4-DB	NIS	0.28 + 0.15	0.58 ab	5.9 a	0.52 bc	3.1 a
Bent.+2,4-DB	NIS	0.43 + 0.15	0.60 ab	5.4 a	0.59 ab	3.2 a
Control	None	None	0.66 a	6.2 a	0.69 a	4.3 a

[a]Acif. = acifluorfen, Bent. = bentazon. NIS = nonionic surfactant (0.25% v/v) and COC = crop oil concentrate (1.25% v/v). LSI = leaf spot incidence (no. treated leaflets with lesions/total no. treated leaflets), LSS = leaf spot severity (total no. lesions/total no. treated leaflets with lesions), DS = degree of sporulation (no. sporulating lesions/total no. lesions), and PAI = potential available inoculum (LSS * DS). Values within columns followed by the same letter are not different at ($P \leq 0.05$) according to a protected LSD test.

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