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Pre-Emergence Herbicides Influence the Efficacy of Fungicides in Controlling Cotton Seedling Damping-Off in the Field

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Abstract: Two field experiments were conducted in Safford and Tucson, Arizona to investigate the impact of three pre-emergence herbicides (pendimethalin, prometryn and trifluralin) on the efficacy of commonly used fungicides (metalaxyl, triadimenol and thiram) against *Rhizoctonia solani* a soil borne fungus causing cotton seedling damping-off. Pendimethalin, prometryn and trifluralin were applied to the field soil at the recommended field concentrations of 0.9, 1.3 and 0.7 kg active ingredient (a.i.) ha^{-1}, respectively. In both field trials, seed treatment with fungicides caused a significant ($p<0.05$) decrease in fungal pathogenicity based on the number of emerged cotton seedlings, relative to the control (not treated with the fungicide). In Tucson trail, only one of the test herbicides (pendimethalin) interfered with the ability of fungicides to reduce the pathogenicity of *Rhizoctonia solani* on cotton seedlings 15, 25 and 50 days after sowing. In Safford trial, the effectiveness of fungicides on fungal pathogenicity was reduced in the presence of pendimethalin and prometryn, 15, 25 and 50 days after sowing. Trifluralin showed no significant effect on the activity of fungicides in both field trials.

Key words: *Rhizoctonia solani*, cotton, fungicides, herbicides, tucson, safford

INTRODUCTION

Cotton (*Gossypium hirsutum* L.,) seedling diseases caused by a number of soil-born fungi including *Rhizoctonia solani* Kuhn are important throughout the world. Diseased plants may be killed prior to or after emergence (Heydari *et al*., 2005; Naraghi *et al*., 2007). Seed treatment with protectant and systemic fungicides is the most common method for controlling cotton seedling damping-off (Moustafa-Mahmoud and Sumner, 1992). However, fungicides are not always effective in reducing disease incidence in the field perhaps due to the changes in their efficacy mediated by certain factors such as soil temperature, soil moisture, soil texture, planting date and the fungal isolate (Jones and Pettiet, 1987; Sumner, 1987; Colyer *et al*., 1991; Moustafa-Mahmoud and Sumner, 1992).

Fungicide efficacy in the field may also be affected by the presence of other pesticides including herbicides (Awadalla and El-Refaie, 1994; El-Khadem and Papavizas, 1984; Hans and Dodan, 1982; Moustafa-Mahmoud and Sumner, 1992; Youssef *et al*., 1987; Sanogo *et al*., 2002). Application of fluchlovalin and alachlor to the soil reduced the effectiveness of fungicides in controlling the cowpea damping-off disease (Hans and Dodan, 1982). Application of nurflurazon, pendimethalin, fluometuron, prometryn, fomesafen and oxyfluorfen to the field soil significantly reduced the efficacy of fungicides, tolclofos-methyl, pencycuron, carboxin, flutonalit, metalaxyl and chloroneb against cotton seedling diseases (Moustafa-Mahmoud and Sumner, 1992). In contrast, the antifungal activity of fungicides, captan and mounsrin increased in the presence of herbicides, paraquat and simazine (Awadalla and El-Refaei, 1994).

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In a previous study we showed that application of pendimathalin and prometryn increased the incidence of *Rhizoctonia solani*-induced cotton seedling damping-off in the field (Heydari and Misaghi, 1998). Results of our *in vitro* studies also indicated that the above-mentioned herbicides did not affect the growth of *R. solani* significantly (Heydari and Misaghi, unpublished data). It was therefore thought that herbicide mediated increase in the diseases could be related to their interaction with fungicides.

To predict the efficacy of a fungicide against a target pathogenic fungus, it is important to determine the nature of its interaction with other pesticides including herbicides. The objective of this study was to investigate the impact of herbicides, pendimethalin, prometryn and trifluralin that are commonly used on cotton, on the efficacy of fungicides, metalaxyl, triadimenol and thiram for controlling *R. solani* a soil borne fungus causing cotton seedling damping-off disease in the field.

MATERIALS AND METHODS

Herbicide Used

Three herbicides, pendimethalin [N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine], prometryn [N,N-bis(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine] and trifluralin [2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-benzenamine] were tested in this study. These herbicides are currently registered for use as pre-emergence herbicides on many crops including cotton. Pendimethalin (EC-33%), prometryn (EC-40%) and trifluralin (EC-48%) were tested at the recommended field concentrations of 0.9, 1.3 and 0.7 kg active ingredient (a.i.) ha^{-1}, respectively (Heydari and Misaghi, 1998).

Field Experiments

Two field experiments with plots arranged in a Randomized Complete Block (RCB) experimental design were conducted at Safford and Tucson, Arizona. The soil at the Safford and Tucson sites were loam soils containing 14% clay, 36% silt, 50% sand and 15.1% clay, 33.0% silt and 51.9% sand, respectively. Each trial consisted of four blocks, one for each replicate. The fungal inoculum was prepared by wetting one kg of barley seeds with 500 mL of water, autoclaving at 15 psi for 60 min, infesting with *R. solani* anastomosis group (AG)-4, which was previously isolated from cotton seedling (Heydari and Misaghi, 1998) and incubating at 25°C for three weeks. The inoculum was air dried for 24 h, ground through a 3 mm sieve and stored in paper bags at 25-27°C in the laboratory until use. Eight g of the fungal inoculum were mixed with 200 g of field soil and the mixture was sprinkled by hand into planting furrow at 0.8 g fungal inoculum per linear m shortly before sowing.

Each of the four blocks at the Safford and Tucson field trials contained five randomly positioned 10-m-long plots (planting beds). Beds were 100 cm apart and the distance between plants were 40 cm. The treatments included: Control (no fungicide, no herbicide); fungicides; fungicides + pendimethalin; fungicides + prometryn; fungicides + trifluralin. Pendimethalin, prometryn or trifluralin was sprayed in a 60 cm band on the top of each plot at the recommended field concentration of 0.9, 1.3 and 0.7 kg a.i. ha^{-1}, respectively using a CO_2 pressurized back pack sprayer. Herbicides were incorporated into the top 5-7 cm of the soil with a rotomultcher shortly after application. An eight cm wide, 3 cm deep furrow at the crest of each 10-m-long plot was cut for the placement of *R. solani* inoculum and 300 seeds of cotton cv Deltapine 5415 treated or not treated with fungicides. metalaxyl, triadimenol and thiram. Fungicidal seed treatment was carried out by Gustafson Inc. (Dallas, TX) and we obtained the seeds directly from this company. It is notable that in Arizona usually cotton seeds are treated with the above mentioned fungicides against damping-off pathogens including *R. solani* and other possible soil born fungi such as *Pythium ultimum* which are very rarely present in Arizona field soils. Fields were irrigated the following days using furrow irrigation method. The impact of herbicides on the activity of fungicides against *Rhizoctonia solani* was determined 15, 25 and 50 days after sowing by counting the number of emerged cotton seedlings (stand). Fifteen days after sowing, about 10% of infected radicles and wilted seedlings in each plot were gently removed from the soil, brought to the laboratory and tested for the presence of *R. solani* ascertain that pre-and post-emergence damping-off was primarily due to *R. solani* infection. Seedlings were visually examined for *R. solani*-induced symptoms, tissues from the advancing edge of root and lower stem lesions were placed on Potato Dextrose Agar (PDA) medium and culture plates were examined for the presence of the fungus two days after incubation at 27°C.

Statistical Analysis

For each herbicide and each assessment time, the data for the stand count for different treatments in each field experiment was tested by analysis of variance (ANOVA) and means were compared by Duncan multiple range test, using COSTAT Statistical Software (Cohort Software, Berkeley, CA).

RESULTS AND DISCUSSION

Typical *R. solani*-induced damping-off symptoms were observed on all sampled, non-emerged and emerged seedlings. Radicles, roots and/or lower stems of all sampled seedlings yielded *R. solani* on PDA culture medium. Other cotton seedling pathogens, including *Thielaviopsis basicola* which is the second most prevalent cotton seedling pathogen in Arizona (Hine and Silvertooth, personal communications) were not recovered.

In both field experiments, seed treatment with fungicides caused a significant ($p<0.05$) decrease in the pathogenicity of *Rhizoctonia solani* based on the number of emerged seedlings (cotton stand), relative to the control (not treated with the fungicide) (Table 1 and 2). The increase in the stand caused by fungicides was most likely due to the ability of these products to reduce the incidence of pre- and post emergence cotton seedling damping-off caused by *R. solani* because, *R. solani* inoculum was incorporated into the soil in the field, cotton seedling diseases in Arizona are mainly caused by *R. solani* and *T. basicola* (Hine and Silvertooth, pers. comm.), *R. solani* was the only pathogen recovered from all sampled infected seedlings and typical *R. solani*-induced damping-off symptoms were observed on all sampled non-emerged infected seedlings and on emerged wilted seedlings.

In Tucson trail, only one of the test herbicides (pendimethalin) interfered with the ability of the fungicides to promote cotton stand by reducing the pathogenicity of the fungus (Table 1). In Safford trial, the ability of test fungicides to increase cotton stand was reduced in the presence of pendimethalin and prometryn, 15, 25 and 50 days after sowing (Table 2). Trifluralin had no effect on the activity of fungicides in Safford trial (Table 2).

The reported herbicide-mediated increase in the incidence of some soil borne plant diseases including cotton seedling damping-off (Altman and Rovira, 1989; El-Khadem and Papavizas, 1984; Harikrishnan and Yang, 2001, 2003; Heitefuss, 1988; Heydari and Misaghi, 1998;

Table 1: Stand count (the number of emerged seedlings) in plots treated or not treated with herbicides and fungicides 15, 25 and 50 days after sowing at the Tucson field trial

	Days after sowing		
Treatments	15	25	50
Control (no fungicide, no herbicide)	101^b	87^b	79^b
Fungicides	192^a	166^a	156^a
Fungicides + Pendimethalin	122^b	109^b	98^b
Fungicides + Prometryn	187^a	150^a	136^a
Fungicides + Trifluralin	207^a	160^a	145^a

Soils in all plots were infested with *Rhizoctonia solani*. Each value, representing the number of emerged seedlings in one plot, is an average of four values (from four replicates). Values followed by the same letter(s) in each vertical column are not significantly different ($p>0.05$) according to the Duncan multiple range test

Table 2: Stand count (the number of emerged seedlings) in plots treated or not treated with fungicides and herbicides 15, 25 and 50 days after sowing at the Safford field trial

	Days after sowing		
Treatments	15	25	50
Control (no fungicide, no herbicide)	153[b]	126[b]	91[b]
Fungicides	220[a]	191[a]	162[a]
Fungicides + Pendimethalin	166[b]	155[b]	117[b]
Fungicides + Prometryn	158[b]	146[b]	109[b]
Fungicides + Trifluralin	211[a]	196[a]	170[a]

Soils in all plots were infested with *Rhizoctonia solani*. Each value, representing the number of emerged seedlings in one plot, is an average of four values (from four replicates). Values followed by the same letter(s) in each vertical column are not significantly different (p>0.05) according to the Duncan multiple range test

Rovira and McDonald, 1986) was not observed in this study perhaps because of effectiveness of fungicides. The herbicide-mediated suppression of fungicidal activity in the Safford experiment occurred 15 and 25 days after sowing perhaps because herbicides concentrations in the soil were high shortly after application and diminished gradually due to inactivation (Heydari and Misaghi, 1998; Rovira and McDonald, 1986).

In the previous studies, herbicides have been reported to change fungicidal activity (El-Khadem and Papavizs, 1984; Hans and Dodan, 1982; Mahmoud and Sumner, 1992; Youssef *et al*., 1987; Sanogo *et al*., 2002) which are in agreement with the results of this study obtained in the Safford experiment The observed differences in the results of Tucson and Safford experiments may have been due to the presence of variable soil factors including texture, pH, temperature, moisture and organic matter contents. Since herbicides can interfere with fungicidal activity in certain plant pathogen combinations, it would be useful to determine the potential herbicide-fungicide interactions in any given plant-pathogen combinations.

CONCLUSION

Based on the results of this study it is concluded that some pre-emergence herbicides including pendimethalin and prometryn used to control parasitic weeds in the cotton fields may interfere with the efficacy of fungicides which are being used to manage cotton soil-born diseases such as damping-off. It is therefore important to take this possibility to the account, consider their possible interactions and carefully select these pesticides for controlling and managing cotton pests in the field.

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