Plant Pathology Journal

ISSN 1812-5387

science alert

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Plant Pathology Journal 7 (1): 104-108, 2008
ISSN 1812-5387

Effect of Surfactants on Bioherbicidal Activity of *Alternaria helianthi* on Multiple-Seeded Cocklebur

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Abstract: Multiple-seeded cocklebur (*Xanthium strumarium* L.) is a biotype which has different morphology and higher seedling production ability than common cocklebur. Greenhouse studies were conducted to investigate the bioherbicidal activity of *Alternaria helianthi* (Hansf.) Tubaki and Nishih. on multiple-seeded cocklebur as affected by various rates of Tenkoz COC® (crop oil concentrate), Activator 90 ®(non-ionic surfactant), BAS 9050 0 S® (methylated oil), Silwet L-77® (organosilicone surfactant) and Top film® (natural based surfactant). Taking X as the recommended rate for each surfactant, 0-X, ¼-X, ½-X, X and 2-X rates were used for each of the surfactants. Surfactants were added to the conidial suspension of *A. helianthi*. Each surfactant rate was also applied with sterile water without any *A. helianthi* spore. Treated plants were kept in the dew chamber for 6 h before transferring to the greenhouse. At the end of the experiment (two weeks after treatment), plant shoots were clipped at the soil surface and the fresh weights were determined. *Alternaria helianthi* resulted in significant reduction in fresh weight of multiple-seeded cocklebur as compared to the plants treated without the fungus. Among five surfactants, Activator 90® and Silwet L-77® had significant effects on the bioherbicidal activity of *A. helianthi* in reducing fresh weight of multiple-seeded cocklebur. Fresh weight decreased with increase in surfactant rates. Our data demonstrate that *A. helianthi* control multiple-seeded cocklebur more with higher rates of Activator 90® and Silwet L-77®.

Key words: *Alternaria helianthi*, biological control, surfactant, multiple-seeded cocklebur, weed, *Xanthium strumarium*

INTRODUCTION

Cocklebur (*Xanthium strumarium* L.) is an economically important weed of soybean [*Glycine max* (L.) Merr.], cotton (*Gossypium hirsutum* L.) and peanut (*Arachis hypogaea* L.) (Byrd and Coble, 1991; Royal *et al*., 1997; Rushing and Oliver, 1998). Bloomberg *et al*. (1982) showed that heavy infestation of cocklebur resulted in yield reduction of 50 to 75% in soybean. In addition to the substantial yield reduction, this weed is becoming a concern because several biotypes are resistant to some conventional herbicides. Abbas *et al*. (1996) showed 12 different biotypes of cocklebur, in which the biotypes from Bolivar county, Mississippi, are resistant to imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid) and the biotypes from Duck Hill county, Mississippi, are resistant to MSMA (monosodium methylarsonate). A common cocklebur biotype resistant to imidazolinone herbicides was found by Barrentine (1994). Haighler *et al*. (1988) identified a biotype resistant to the organic arsenical herbicides.

Multiple-seeded cocklebur is a particular biotype of cocklebur, which has a different leaf and stem morphology as compared to the common cocklebur. The burs of the multiple-seeded cocklebur are large, round, covered with hairy spines or prickles, with each seed terminated by a beak (Abbas *et al*., 1999). This biotype has up to 25 seeds per bur, usually producing up to nine seedlings, whereas common cocklebur has two seeds per bur and usually produces only one seedling (Abbas *et al*., 1999). Higher seedling production ability increases the weediness of this particular biotype (Abbas *et al*., 1999; Barrentine, 1974; Bloomberg *et al*., 1982; Buchanan and Burns, 1971; Snipes *et al*., 1982).

As some biotypes of cocklebur have become resistant to the conventional herbicides (Barrentine, 1994; Haighler *et al*., 1988, 1994), the need for an alternative to the chemical weed control methods like biological control has become obvious. *Alternaria helianthi* (Hansf.)

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Tubaki and Nishih. has been well documented as a potential bio-control agent for cockleburs (Abbas and Egley, 1996; Abbas and Barrentine, 1995; Quimby, 1989). This is a pathogen of sunflower (*Helianthus annus* L.) and can infect other plants in compositae family (Allen *et al*., 1983).

The role of surfactants in increasing the activity of plant pathogens in biological weed control has been well documented (Boyette *et al*., 1996; Connick *et al*., 1990; Daigle and Connick, 1990; Watson and Wymore, 1990). Surfactants may improve leaf wettability, improve spore deposition and retention and prolong water retention to overcome dew period requirements (Green *et al*., 1998). However, there are no guidelines for selecting the compatible surfactants for bio-control agents. Thus, there is a need to investigate the compatibility of surfactants for different pathogen-weed systems.

In the present study, the objective was to determine the effect of five surfactants at various rates on *A. helianthi* activity on multiple-seeded cocklebur.

MATERIALS AND METHODS

General: Seeds of multiple-seeded cocklebur were collected from plants growing at experimental plots at the Southern Weed Science Research Nursery, Stoneville, Mississippi, established from the seeds from Bell County, Temple, Texas, Collected in 1995. The burs were soaked in water for a week before planting in a 1:1 potting mix of jiffy mix and soil, (Jiffy Mix, Jiffy Products of America, Inc., Batavia, IL 60510). Germinated seeds were transplanted to 10 cm^2 pots and grown in the greenhouse (28 to 33° C, 40 to 78% relative humidity and 12 h day length) until 6 to 8 leaf stage.

Preparation of fungal culture: Fungal cultures were taken from a stock culture of *A. helianthi* and inoculated in fresh sunflower leaf-agar medium prepared by the procedure described by Abbas and Barrentine (1995). Dried sunflower leaves (25 g) were homogenized with 1 L of distilled water in a blender at high speed for 5 min. The homogenate was centrifuged for 10 min at 10,000 x g and the supernatant was filtered through a double-layer cheesecloth. The filtrate was combined with 20 g of agar, autoclaved and poured into 9 cm petriplates, 20 mL each. The plates were inoculated with *A. helianthi* and incubated at 18°C in alternating regimes of 14 h of fluorescent light at 165 E m^{-2} sec^{-1} and 10 h of dark period. After 10 to 14 days of incubation, 5 mL of autoclaved distilled water was added to each plate and the conidia of *A. helianthi* were scraped from each plate and collected in autoclaved distilled water. Conidial suspensions were homogenized by a polytron PT3000 (Brinkmann Instruments, Inc., Westbury, NY) and counted in a haemocytometer. The concentration of the conidial suspensions were adjusted to 1×10^5 conidia per mL.

Effect of surfactants on bioherbicidal activity: Effect of five surfactants on the bioherbicidal activity of *A. helianthi* were studied on multiple-seeded cocklebur. The surfactants used in the present study were Tenkoz COC® (crop oil concentrate), Activator 90® (non-ionic surfactant), BAS 9050 O S® (methylated oil), Silwet L-77® (organosilicone surfactant) and Top film® (natural based). Taking X as the recommended rate for each surfactant, we used 0-X, ¼-X, ½-X, X and 2-X rates for each of the surfactants. The actual rates used were 0, 0.25, 0.5, 1 and 2% (v/v) for Tenkoz COC®; 0, 0.06, 0.125, 0.25 and 0.5% (v/v) for Activator 90®; 0, 0.25, 0.5, 1 and 2% (v/v) for BAS 9050 O S®; 0, 0.05, 0.1, 0.2 and 0.4% (v/v) for Silwet L-77®; and 0, 0.06, 0.125, 0.25 and 0.5% (v/v) for Top film®. Each surfactant rate was applied without and with *A. helianthi*. Surfactants were added to the conidial suspension before spraying. The six to eight-leaf seedlings were sprayed using a Spra-Tool (Crown Industrial Products Co., Hebron, IL, USA). The treated plants were subjected to 6 h of dew periods before transferring to the greenhouse. Plants were watered and fertilized (with N:P:K, 20:20:20) as needed. At the end of the experiment (two weeks after treatment), plant shoots were clipped at the soil surface and the fresh weights were determined. Experimental designs were completely randomized for all experiments and each treatment had three replications. Data were subjected to analysis of variance (ANOVA) using the general linear model procedures of the Statistical Analysis System (SAS, 1999).

RESULTS AND DISCUSSION

In all experiments, *A. helianthi* resulted in severe damage and fresh weight reduction of multiple-seeded cocklebur plants as compared to the treatments without the fungus and the fresh weight reduction was significant at 99% level of significance as indicated by the *P* values in the ANOVA (Table 1). The disease symptoms appeared on infected plant leaves and stems as soft necrotic lesions within 24 h of treatment. Necrotic lesions became dry and larger with time, resulting in death of some plants within 1 week depending on severity of symptoms. These data confirm the effectiveness of *A. helianthi* as a bioherbicidal pathogen for multiple-seeded cockleburs. This agrees with the findings of Abbas *et al*. (2004) where *A. helianthi* caused severe disease infestation and growth reduction of multiple-seeded cockleburs.

Table 1: ANOVA on the effect of various surfactants and *Alternaria helianthi* on fresh weight of multiple-seeded cocklebur

	p-values				
Silwet	Silwet COC®	Tenkoz Activator 90®	9050®	BAS L-77®	Silwet Top Film®
A	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S	0.6569	0.0091	0.1418	<0.0001	0.4008
A×S	0.0945	0.0002	0.0830	<0.0001	0.0512

[a]A = *A. helianthi* (with and without); S = Surfactant rates

p-values in the ANOVA show that among five surfactants, Activator 90® and Silwet L-77® had significant effects on the bioherbicidal activity of *A. helianthi* in reducing fresh weight of multiple-seeded cocklebur (Table 1). Tenkoz COC®, BAS 9050® and Top Film® did not have any effect on fresh weight. *Alternaria helianthi* reduced the fresh weight of cocklebur plants more at higher rates (0.5%) of Activator 90® as compared to treatments with lower rates (0.06%) or without surfactant (Fig. 1). With Silwet L-77®, *A. helianthi* resulted in lower fresh weight of multiple-seeded cocklebur plants with higher surfactant rates as well (Fig. 2). Fresh weights were significantly lower at 0.2 and 0.4% rate of Silwet L-77®, as compared to 0.05% rates. At 0.4% rate of Silwet L-77® phytotoxicity was observed on control plants (plants treated with 0.4% Silwet L-77 in sterilized water without any fungal conidia) within 24 h of treatment (Fig. 3). Walker and Tilley (1997) observed similar phytotoxicity due to 0.4% rate of Silwet L-77® on sicklepopd. Previous research also showed that *A. helianthi* when applied with Silwet L-77® may cause up to 100% mortality of normal and multiple-seeded cockleburs (Abbas *et al*., 2004).

Overall, *A. helianthi* resulted in better control of multiple-seeded cockleburs in terms of fresh weight when applied with Activator 90® or Silwet L-77® as compared to Tenkoz COC®, BAS 9050® and Top Film®. Our data demonstrate that with 6 h dew period, *A. helianthi* reduced the fresh weight of multiple-seeded cocklebur more with higher rates of Activator 90® and Silwet L-77®. Enhanced bioherbicidal activity by specific adjuvants was previously reported by Babu *et al*. (2003). They showed that the application of *Alternaria alternata* in an oil emulsion enhanced disease incidence on waterhyacinth (*Eichhornia crassipes*) as compared to a 0.1% solution of Tween 80®.

Activator 90® and Silwet L-77® have shown potential for achieving effective biological control of multiple-seeded cockleburs by *A. helianthi*. The precise mode by which these surfactants enhance the activity of *A. helianthi* is yet to be determined. Surfactants may modify leaf wettability causing fungal conidia to adhere more closely to leaf tissues than fungal conidia in water. Specific surfactants may prolong water retention to overcome dew period requirements as suggested by

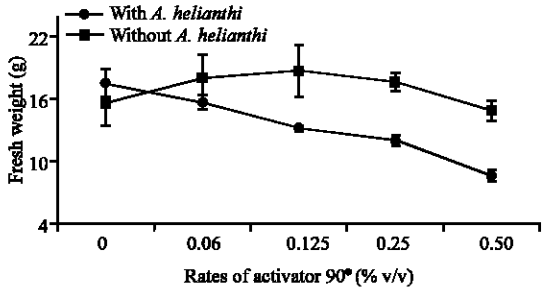

Fig. 1: Effect of various rates of Activator 90® (% v/v) on fresh weight of multiple-seeded cocklebur treated with and without *Alternaria helianthi* two weeks after treatments. Vertical bars represent standard error of means

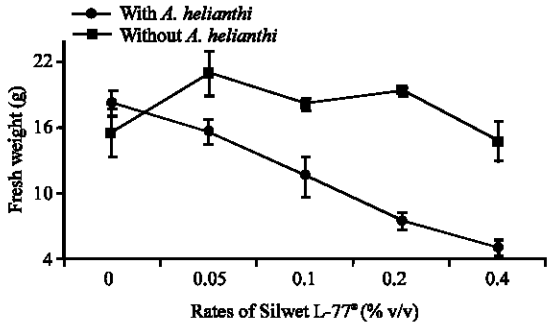

Fig. 2: Effect of various rates of Silwet L-77® (% v/v) on fresh weight of multiple-seeded cocklebur treated with and without *Alternaria helianthi* two weeks after treatments. Vertical bars represent standard error of means

Green *et al*. (1998). Silwet L-77® may stimulate spore germination of *A. helianthi*, allowing conidia to produce multiple germ tubes to penetrate stomata or possibly wounds caused by Silwet L-77® as well. Abbas and Egley (1996) showed that germination and germ tube production of *A. helianthi* on cocklebur leaves increased with addition of Silwet L-77® or corn oil. Previous research demonstrated that Silwet L-77® promoted the activity of fungi and bacteria on the leaves of their host kudzu (*Pueraria lobata*) (Boyette *et al*., 2002; Zidak *et al*., 1992). Multiple-seeded cocklebur has characteristics that suggest that it might be difficult to control, particularly the fact that it produces numerous seedlings per bur, giving the possibility of rapid population growth. However, the results of the current study demonstrate that multiple-seeded cocklebur is highly susceptible to *A. helianthi* and its effectiveness can be enhanced by use of appropriate surfactants in proper rates with a minimum dew period. As these experiments were tested in the greenhouse, more research is needed in field situation for

Fig. 3: Effect of Silwet L-77® at 0.4% (left) and 0.2% (right) rates (v/v) on multiple-seeded cocklebur 24 h after treatment

better understanding of the feasibility of this approach. Research to enhance the activity of *A. helianthi* against other biotypes of cocklebur is also needed.

ACKNOWLEDGMENTS

We thank B.J. Johnson for valuable technical assistance. This research was partially supported by the Massachusetts Agricultural Experiment Station, Manuscript No. 3413.

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