SECTION III. SOLVENT EFFECTS ON PLANT RESPONSE TO PREEMERGENCE HERBICIDES IN AN ARTIFICIAL ENVIRONMENT

SOLVENT EFFECTS ON PLANT RESPONSE TO PREEMERGENCE HEBICIDES IN AN ARTIFICIAL ENVIRONMENT

Z.J. Reicher and N.E. Christians

ABSTRACT

The objectives of this experiment were to determine the effect on rooting of ethanol and dimethyl sulfoxide (DMSO) as solvents for preemergence herbicides and to observe the effect of preemergence herbicides on rooting of Kentucky bluegrass (*Poa pratensis* L.) under aseptic conditions on tissue culture media. Bensulide was dissolved in water, ethanol, and DMSO whereas DCPA, prodiamine, and pendimethalin were dissolved only in ethanol and DMSO. Oat (*Avena sativa* L. 'Lang') seedlings were placed on a Murashige and Skoog (MS) medium containing the herbicides. Rooting was measured every six hours in a 30 hour experiment. Bensulide dissolved in 0.1% (v/v) DMSO produced less root growth after 12 h than bensulide dissolved in water or in 0.1% ethanol. Bensulide dissolved in water produced more root growth than bensulide dissolved in DMSO or in ethanol from the 18th to 30th hour of the experiment. DCPA dissolved in DMSO restricted root growth between 12 to 30 h when compared to DCPA dissolved in ethanol. There was no difference in rooting response to the two solvents with prodiamine or pendimethalin.

Four preemergence herbicides were dissolved in 0.05% (v/v) ethanol. 'Glade' Kentucky bluegrass was germinated aseptically and placed on MS medium containing the herbicides. Root length was measured after 12 days. Bensulide at 3.8×10^{-7}, 7.5×10^{-7}, and 1.1×10^{-6} M, DCPA at 5.0×10^{-7}, 1.0×10^{-6}, and 1.5×10^{-6} M, pendimethalin at 7.5×10^{-8}, 1.5×10^{-7}, and 2.3×10^{-7} M, and prodiamine at 2.5×10^{-8}, 5.0×10^{-8}, and 7.5×10^{-8} M inhibited rooting when compared to the control.

Chemical names used: O,O-bis(1-methylethyl) S-[2-{(phenylsulfonyl)amino}ethyl] phosphorodithioate (bensulide);dimethyl tetrachloroterephthalate (DCPA); N-(1-ethylpropyl)-3,4-dimethyl-2,6-dintrobenzenamine (pendimethalin); N^3,N^3-di-n-propyl-2,4-dinitro-6-(triflouromethyl)-m-phenylenediamine (prodiamine).

INTRODUCTION

Preemergence herbicides have very low water solubilities which can limit their study in water based media. To increase the water solubility, they can be dissolved in a nontoxic solvent before being added to the media. Dimethyl sulfoxide (DMSO) has been used to increase the solubility of DCPA in a Hoagland's solution for studing rooting of oats (2). DMSO has exellent solvent properties and can improve the transport of various materials in plants (1). Various concentrations of ethanol have also been used to increase the solubility of preemergence herbicides in plant studies (4,5). However, little is known about the relative effects of these two solvents on plant responses to herbicides.

The objective of this experiment were to determine the effect on rooting of ethanol and DMSO as solvents for preemergence herbicides under aseptic tissue culture conditions. The effect of preemergence herbicides on rooting of Kentucky bluegrass was also investigated.

MATERIALS AND METHODS

Dehulled 'Lang' oat seeds were sterilized in 1.25% NaOCl (25% v/v commercial laundry bleach) for 15 minutes and washed six times with sterile distilled water. All following steps were performed aseptically in a laminar flow hood. The seeds were allowed to imbibe sterile distilled water for six hours before placing on agar medium (8g Difco-Bacto agar/liter). The seeds were germinated in darkness at 25^{o}C for 50 h. Technical grade herbicides were allowed to dissolve in either sterile distilled water, 95% ethanol, or undiluted DMSO for four hours. Full strength Murashige and Skoog medium (3) was adjusted to pH 5.8 before adding 8 g Difco Bacto-agar/liter. The medium was autoclaved at 1.1 kg cm^{-2} and 121^{o}C for 15 min and the herbicides added when the medium was still warm. Additional ethanol or DMSO was added to bring their final concentration to 0.1% (v/v). The media were poured into 110 X 15 mm round petri dishes and allowed to cool. Three oat seedlings with primary seminal roots 5-15 mm long were placed on the media and their roots gently pressed into the media. The petri dishes were covered and sealed with parafilm. The original position of each primary root was marked on the petri dish covers. To force the roots to grow through the media, the dishes were angled 30^{o} from the vertical. The new positions were marked every six hours during incubation in the dark at 25^{o}C. At the end of the 30 hour experiment, distances between successsive marks were measured to the nearest millimeter. Each treatment was replicated three times and the experiment was repeated.

Four preemergence herbicides, bensulide, DCPA, pendimethalin, and prodiamine, were investigated in independent studies. Bensulide was examined in a three by four factorial with water, ethanol, and DMSO as solvents and four concentrations of bensulide, 0.0, 1.5 X 10^{-6}, 3.0 X 10^{-6}, and 4.4 X 10^{-6} M. The other herbicides were tested in two by four factorials with only ethanol and DMSO as solvents. Water was not used as a solvent because of the very low water solubility of these herbicides. DCPA was used at 0.0, 5.0 X 10^{-7}, 1.0 X 10^{-6}, and 1.5 X 10^{-6} M whereas pendimethalin and prodiamine were used at 0.0, 5.0 X 10^{-8}, 1.0 X 10^{-7}, and 1.4 X 10^{-7} M. The solvents were present at 0.1%(v/v) in the control treatments in all studies.

The findings in the oat studies revealed that ethanol inhibited rooting less and was more suitable as a solvent than DMSO. The next step was to evaluate the effect of preemergence herbicides on rooting of Kentucky bluegrass.

'Glade' Kentucky bluegrass seeds were sterilized in 2.5% NaOCl for 20 min and washed six times with sterile distilled water. The seeds were germinated for eight days at 25°C with a 16 hour photoperiod. The preparation of the media and petri dishes were identical to the oat studies except that 95% ethanol was the only solubility agent used. Bensulide was used at 3.8 X 10^{-7}, 7.5 X 10^{-7}, and 1.1 X 10^{-6} M, DCPA at 5.0 X 10^{-7}, 1.0 X 10^{-6}, and 1.5 X 10^{-6} M, pendimethalin at 7.5 X 10^{-8}, 1.5 X 10^{-7}, and 2.3 X 10^{-7} M, and prodiamine 2.5 X 10^{-8}, 5.0 X 10^{-8}, and 7.5 X 10^{-8} M. The herbicides concentrations were determined on molar ratios of their respective recommended field rates and the final ethanol concentration was 0.05% (v/v). This concentration of ethanol was also present in the controls. Three seedlings with 5-10 mm long primary seminal roots were used per petri dish. The growth of the the roots from their original position was measured to the nearest millimeter after 12 days of incubation at 25°C with a 16 hour photoperiod. Data was collected on two seedlings per dish. Three seedlngs were placed in each dish to compensate for occasional seedling damage that occurred when pressing the roots into the media. Each treatment was replicated four times and the experiment was repeated.

RESULTS

A difference in response to the solvents was seen with bensulide (Table 1). After 12 h, the DMSO treatments reduced rooting 33% compared to water whereas the rooting of the ethanol treatments did not differ from the water (Table 2). Throughout the rest of the experiment, ethanol reduced rooting up to 21% and DMSO reduced rooting up to 29% compared to the treatments with water as a solvent. The response over concentration to water remained linear throughout the experiment whereas ethanol and DMSO induced quadratic responses after 24 h (Table 3).

DMSO and ethanol produced different responses in rooting with DCPA from 12 thru 30 h. DMSO restricted growth an average of 20% compared to ethanol. There was an increase in root growth from 1.0 to 1.5 uM DCPA in ethanol throughout the experiment.

There was not a significant difference in rooting response to the two solvents with prodiamine or pendimethalin although there was a numerical trend where ethanol consistently had more root growth than DMSO.

Following completion of the oats studies, the Kentucky bluegrass experiment was performed. An analysis of variance revealed no difference in response between the repeated studies so the bluegrass experiment was treated as a simple randomized complete block design with eight replications. The high rates of DCPA, pendimethalin, prodiamine, and bensulide decreased root length 36%, 48%, 60%, and 71%, respectively when compared to the control (Table 4). Root growth was inhibited more with an increasing rate of bensulide, pendimethalin, and prodiamine.

This was not seen with DCPA treatments where root growth actually increased with the higher concentrations.

DISCUSSION

The difference in response to the solvents with bensulide is likely due to a difference in absorption of the herbicide. DMSO is an excellent penetrant which could explain the decrease in rooting as early as 12 h. The response with ethanol surfaced six hours later suggesting the herbicide was slower in penetrating the root surface with ethanol than with DMSO.

The bensulide study revealed that preemergence herbicides dissolved in ethanol and DMSO inhibited rooting more than when dissolved in water. Even though the use of solvents are often the only alternative to dissolving insoluble compounds in massive amounts of water, this study suggests that ethanol and DMSO used as solvents for insoluble compounds may alter the effect of the compound. The effect of the solvents may have been magnified under the conditions of this study. The solvents may have different effects under a different set of conditions. The effects of the solvents should be evaluated for use under different conditions than those used in this study.

DCPA dissolved in DMSO inhibited rooting more compared to the DCPA dissolved in ethanol. Previous papers reported that DMSO, when used as a solvent for DCPA, had no effect on root growth of oats in a sand medium (2). But these data suggests that DMSO may compound an inhibiting effect of DCPA on rooting.

The rooting responses to DMSO and ethanol varied among the preemergence herbicides in the oat study. The concentrations used in this investigation were based on water solubility and also responses seen in earlier literature (1,3). There was no attempt to use equal concentrations of each herbicide or concentrations based on field recommendations. The solvent response for each herbicide may not be the same over a different range of concentrations. If the response of a given herbicide over the concentrations used in the oat study was linear, a different range of concentrations may induce a quadratic response. The different responses to the herbicides do indicate that the response to the solvents is herbicide specific.

Herbicides dissolved in DMSO were more inhibitory to rooting than when dissolved in ethanol. DMSO is an excellent penetrant and it is very likely compounds or elements attached to it would move with it (1). The chemical compositions of the herbicides could dictate the attachment to DMSO. It is very likely that the herbicides that could attach to DMSO moved into the roots faster and/or in higher concentrations with DMSO causing more damage. The response to DMSO and ethanol did not differ with the dinitroanilines (pendimethalin and prodiamine) but did differ largely with DCPA and bensulide. This suggests responses may vary among the herbicide families.

Ethanol was used as the solvent with the Kentucky bluegrass because DMSO consistently inhibited rooting more compared to ethanol. The bensulide study suggested that 0.1% (v/v) ethanol may also be inhibitory so the concentration was lowered to 0.05% (v/v) for the bluegrass study. Dissolving the herbicides in water would probably have been the safest but this was not feasible with their low water solubilities.

DCPA is the safest of the preemergence herbicides used under these conditions. Even though the herbicides were used in molar ratios of their respective field recommendations, the results do not directly relate to field conditions. The concentrations of herbicides used in this study were often at a maximum based on their water solubilities and considerably higher than expected under field conditions. This does demonstrate though that these preemergence herbicides do have the capacity to inhibit rooting of Kentucky bluegrass.

The use of aseptic conditions to study preemergence herbicides was very effective. This method may be difficult to relate to field conditions but is still useful for comparison of various herbicides. One can rapidly screen a large number of herbicide treatments in a relatively small area. This technique could also be used for observing environmental effects such as light intensity, photoperiod, and temperature on plant growth.

LITERATURE CITED

1. Cutter, E. G., F. M. Ashton, and D. Huffstutter. 1968. The effects of bensulide on the growth, morphology, and anatomy of oat roots. Weed Res. 8:346-352.

2. Garren, R. 1967. Uptake and distribution of labeled dimethyl sulfoxide and its influence on nutritive element transport in plants. Ann.New York Acad. Science 141:127-130.

3. Holmsen, J. D. and F. D. Hess. 1984 Growth inhibition and disruption of mitosis in oat (Avena sativa) roots. Weed Sci. 32:732-738.

4. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:743-749.

5. Nishimoto, R. K. and G. F. Warren. 1971. Site of uptake, movement, amd activity of DCPA. Weed Sci. 19(2):152-155.

6. Nishimoto, R. K. and G. F. Warren. 1971. Shoot zone uptake and translocation of soil-applied herbicides. Weed Sci. 19(2):156-161.

Table 1: Significance of F tests of treatment effects on rooting of oats under aseptic conditions

	bensulide					DCPA					prodiamine					pendimethalin				
	6 h	12 h	18 h	24 h	30 h	6 h	12 h	18 h	24 h	30 h	6 h	12 h	18 h	24 h	30 h	6 h	12 h	18 h	24 h	30 h
Treatment	NS	**	**	**	**	NS	**	**	**	**	NS	**	**	**	**	NS	**	**	**	**
Solvent(S)	NS	**	**	**	**	NS	*	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Concentration(C)	*	**	**	**	**	*	**	**	**	**	NS	**	**	**	**	NS	**	**	**	**
Linear	**	**	**	**	**	*	**	**	**	**	*	**	**	**	**	**	*	**	**	**
Quad.	NS	NS	NS	*	**	NS	NS	*	**	**	NS	*	**	**	**	NS	*	**	**	**
C*S	NS	NS	NS	NS	NS	NS	*	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C(linear)*S	NS	NS	NS	NS	NS	NS	NS	*	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C(Quad)*S	NS	NS	NS	NS	NS	*	**	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, *, ** Nonsignificant(NS) or significant at 5% or 1%, respectively.

Table 2: Mean oat root growth(mm) for solvents combined over four concentrations of each herbicide

		Hours				
Herbicide	Solvent	6	12	18	24	30
bensulide	H_2O	1.9	3.7	5.1	6.6	7.6
bensulide	ETOH	1.7	3.3	4.3	5.3	6.0
bensulide	DMSO	1.5	2.5	3.7	4.7	5.4
DCPA	ETOH	1.9	3.8	5.8	7.4	8.8
DCPA	DMSO	1.7	3.2	4.6	5.7	6.6
prodiamine	ETOH	1.5	2.4	2.7	3.0	3.7
prodiamine	DMSO	2.7	2.1	2.4	2.8	3.0
pendimethalin	ETOH	1.4	2.6	3.4	3.9	4.4
pendimethalin	DMSO	1.5	2.6	3.3	3.8	4.0

[a] The $LSD_{(0.05)}$ of the bensulide means for the respective hours are NS, 0.5, 0.6, 0.8, and 0.9. The separation of means for the other herbicides are found in Table 1.

Table 3: Regression equation over herbicide concentration for growth of oat roots treated with bensulide dissolved in either water, ethanol, or DMSO

	Water	R^2	Ethanol	R^2	DMSO	R^2
6h	y=2.16-0.62x	8	y=2.22-0.06x	14	y=1.94+0.13x	8
12h	y=4.38-0.10x	19	y=4.72-0.19x	36	y=3.83-1.15x	23
18h	y=6.76-1.35x	26	y=6.56-1.35x	46	y=5.83-1.87x	44
24h	y=9.39-0.72x	41	$y=8.22-1.83x-0.09x^2$	38	$y=7.78-2.97x+0.65x^2$	52
30h	y=11.50-0.85x	46	$y=9.83-3.71x+0.73x^2$	40	$y=9.06-3.36x+0.68x^2$	51

Table 4: Mean primary seminal root length of Kentucky bluegrass after 12 days

Herbicide	Rate(Moles/L)	root length(mm)
Control	-	6.9
DCPA	5.0×10^{-7}	4.6
DCPA	1.0×10^{-6}	4.8
DCPA	1.5×10^{-6}	5.1
Bensulide	3.8×10^{-7}	4.4
Bensulide	7.5×10^{-7}	4.4
Bensulide	1.1×10^{-6}	2.0
Pendimethalin	7.5×10^{-8}	4.9
Pendimethalin	1.5×10^{-7}	4.2
Pendimethalin	2.3×10^{-7}	3.6
Prodiamine	2.5×10^{-8}	4.7
Prodiamine	5.0×10^{-8}	3.7
Prodiamine	7.5×10^{-8}	2.8
$LSD_{(0.05)}$		1.2