Translocation and Break-Down of Disodium Methanearsonate (DSMA) in Coastal Bermudagrass

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Tracer techniques with DSMA-^{14}C and chemical analyses for arsenic (As) were used to study the translocation and break-down of disodium methanearsonate (DSMA) as they affected As residues in Coastal bermudagrass [*Cynodon dactylon* (L.) Pers.]. DSMA was readily taken up from a nutrient solution by Coastal, but uptake from a soil was restricted. Radioautographs of foliar treated plants demonstrated acropetal and basipetal translocation of DSMA-^{14}C. Regardless of the stage of maturity at the time of application, approximately 25% of the As intercepted by the leaves was translocated to the roots and rhizomes within 5 days. Chemical and chromatographic analyses of extracts from DSMA-^{14}C-treated plants suggested that the carbon-arsenic bond remained largely intact. However, some ^{14}C activity was detected in respiratory CO_2 several days after treatment with DSMA-^{14}C.

Arsenic (As) residues present a problem to the use of arsenical herbicides for weed control in pastures and field crops. Frequent use of inorganic arsenicals such as calcium and lead arsenate has resulted in the accumulation of toxic levels of As in certain soils (Bishop, 1962). In contrast, organic arsenicals such as DSMA are less likely to reach toxic levels of As in the soil because of the low rates of application required for effective weed control (Ehman, 1965; Schweizer, 1967). Generally, high concentrations of As may be expected on Coastal bermudagrass forage harvested soon after application of organic arsenicals (McBee *et al.*, 1967). Since their use as herbicides in forage crops is permitted by regulating agencies on a no residue basis only, practices must be found to eliminate or reduce As residues if the materials are to be used for grassy weed control in forage production. However, arguments can be found in the literature as to the toxicity of As to livestock (Ehman, 1965; Grimmett, 1939; Peoples, 1964). Feeding trials have shown that the output of As in the urine balances the input in feed (Peoples, 1964).

McBee *et al.* (1967) suggested that the stage of plant development and the amount of foliage at the time of application may be factors affecting the high levels of As residues reached following foliar applications of arsenicals. Also, they suggested that the translocation of As out of the treated foliage into the roots and rhizomes might account for some of the loss of As residues with time. The stability of arsenicals within the plant may also affect the persistence of As residues. Uptake of As residues from the soil is also of some concern when using arsenical herbicides. Therefore, investigations were undertaken to determine the influence of these factors on As residues in Coastal bermudagrass.

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MATERIALS AND METHODS

Coastal bermudagrass was propagated from field collected rhizomes in flats filled with a Lufkin fine sandy loam at a pH of 6.4. The Coastal bermudagrass was maintained under greenhouse conditions, watered frequently, and fertilized at monthly intervals throughout the study. All treatments were replicated three times. The DSMA used in all studies was a 100% soluble powder formulation containing 25% As. A stock solution of DSMA-^{14}C containing 2.5 μg. of DSMA per μl. and an activity of 100 c.p.m. per μg. was used in all tracer studies. All foliar applications of DSMA included 0.1% surfactant, X-77 (Colloidal Products Corp.).

To determine the extent of translocation of DSMA from the point of application, tracer techniques with DSMA-^{14}C were employed. Radioautographs of entire plants were made 5 days after the application of DSMA-^{14}C to a single leaf. Since no information has been reported on the break-down of DSMA, the presence of ^{14}C activity does not establish the presence of As. Therefore, the fate of DSMA in the plant was studied to establish a relationship between ^{14}C activity and As content. Leaves from DSMA-^{14}C-treated plants were extracted overnight in a soxhlet with ten volumes of 80% ethyl alcohol. The extract was condensed *in vacuo* and analyzed by two-dimensional descending paper chromatography. The solvent systems consisted of butanol–acetic acid–water (3:1:2) in one direction and phenol saturated with water at room temperature in the other direction. An extract from untreated plants was spiked with DSMA-^{14}C and analyzed by the same procedure. Carbon-14 activity on the chromatogram was located with No-screen x-ray film (Eastman Kodak Co.) and the radioactive spots were analyzed for arsenic by the silver diethyldithiocarbamate procedure (Morrison, 1961). To obtain additional evidence concerning the fate of DSMA in the plant, respiratory CO_2 was analyzed for ^{14}C activity for several days following the application of DSMA-^{14}C. Respiratory CO_2 from leaf disks cut from Coastal leaves was trapped on

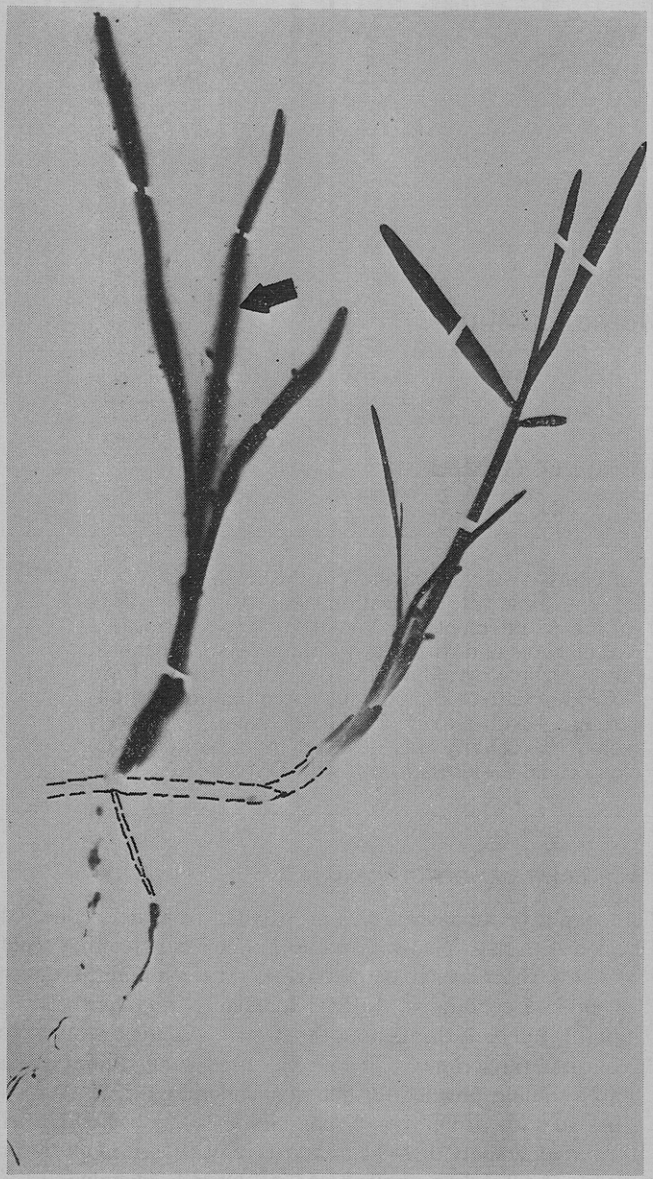

Figure 1. Radioautograph showing distribution of ^{14}C activity in Coastal bermudagrass 7 days after application of DSMA-^{14}C to a single leaf blade. Arrow indicates site of application of DSMA-^{14}C

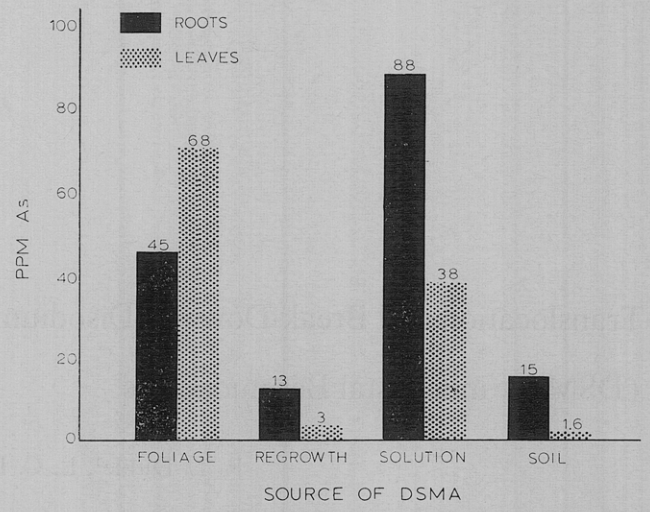

Figure 2. Distribution of As in Coastal bermudagrass 7 days after foliar, nutrient solution, and soil applications of DSMA

Regrowth from a foliar application represented 3 weeks growth after clipping to ground level 7 days after treatment.

alkali paper wicks held in Warburg flasks. Na_2CO_3 was recovered from the paper wicks, dried on planchets, and assayed for ^{14}C activity with a Beckman Lowbeta II counting system.

Uptake of DSMA from a nutrient solution (standard Hoagland's solution), a soil, and a foliar application was determined to gain information on soil fixation and both acropetal and basipetal movement of DSMA in Coastal. The soil application rate was 17.9 kg. DSMA per hectare while the foliar application was 4.5 kg. DSMA per hectare. The same concentration of DSMA used in the soil treatment (12 p.p.m.) was used in a nutrient solution to determine uptake from solution. A second set of foliar applications was clipped to ground level 7 days after treatment and allowed to produce new growth. This regrowth was harvested three weeks later and analyzed for As to study the redistribution of As. In all treatments, pH of the soil or the solution was adjusted to 6.4. The plants were harvested 7 days after treatment, and separated into roots (below ground parts) and leaves (above ground parts) prior to As analysis (Morrison, 1961).

The effects of stage of plant development and the amount of foliage present at the time of application were also determined. Treatments consisted of three stages of plant development: young growth, 3 weeks after planting; dense growth, 3 months after planting; and 5-cm. stubble, 3 months after planting and 7 days after clipping. Foliar applications equivalent to 4.5 kg. of DSMA per hectare were made with a DeVilbiss atomizer in a volume equivalent to 917 liters of water per hectare. Plants were harvested 5 days after treatment with DSMA and separated into roots and leaves. The samples were oven-dried, weighed, and ground to pass a 40-mesh screen. Duplicate As analyses were run on each sample.

Additional flats of plants clipped to a 5-cm. stubble were maintained to study the effect of subsequent growth on As residues. Samples from these flats were harvested 0, 5, 15, and 30 days after treatment and analyzed for elemental As.

RESULTS AND DISCUSSION

DSMA was found to be readily translocated in Coastal bermudagrass when applied to the foliage along with a surfactant. Carbon-14 activity was observed throughout the plant 5 days after a spot application of DSMA-^{14}C to a single leaf (Figure 1). The radioautographs showed that DSMA was moved in both acropetal and basipetal directions from the point of application; however, 76% of the DSMA recovered in the plant remained at the site of application. Close examination of the radioautographs revealed that ^{14}C activity was highest in the vascular bundles of leaves and suggested that DSMA was translocated through the vascular system.

Evidence obtained concerning the fate of DSMA in Coastal indicated that the molecule remained largely intact, but that it may have been complexed with some component of the plant. The chromatograms of extracts from DSMA-^{14}C treated plants showed only a single radioactive spot. Chemical analysis of the eluted radioactive spot established the presence of As at the site of the ^{14}C activity. The ratio of ^{14}C activity to As in the plant extract was essentially the same as in the DSMA-^{14}C solution applied to the plant. Since several radioactive spots would likely appear on the chromatogram if the carbon-arsenic bond was broken down, it may be assumed that DSMA remained intact. However, the radioactive spot from the DSMA-^{14}C treated plant extract had an R_f value of 0.43 in butanol–acetic acid–water compared to 0.56 for the plant

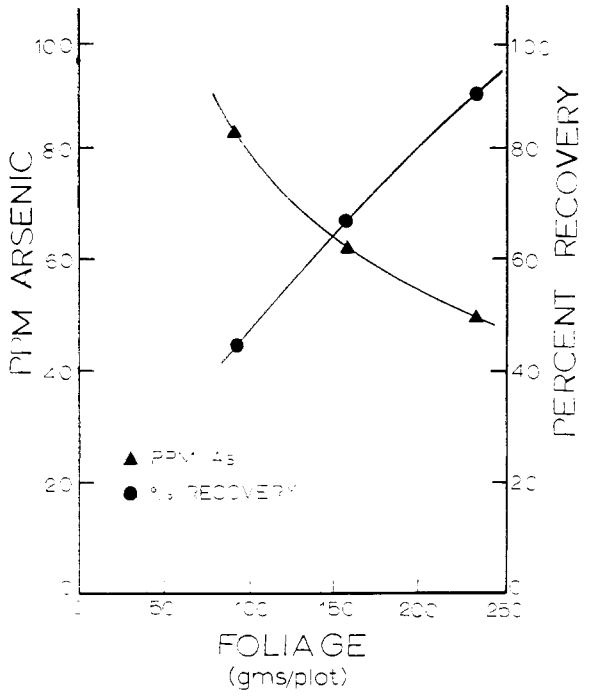

Figure 3. Relationships between p.p.m. As in foliage and percent of recovery of As in plant and amount of foliage present at time of application

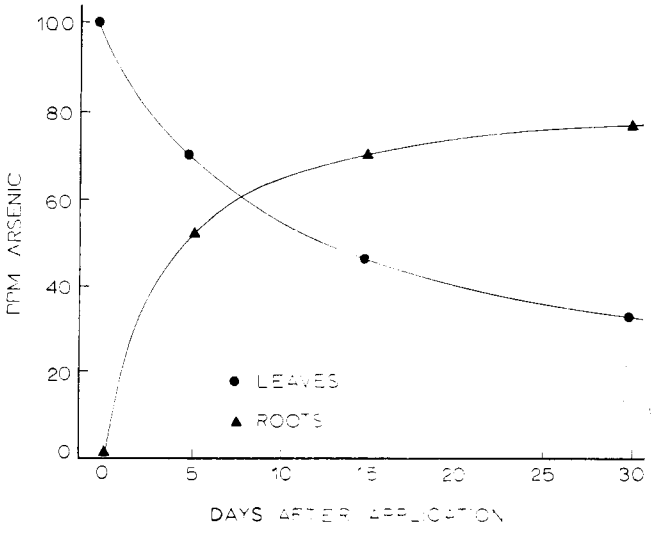

Figure 4. Effect of growth and translocation subsequent to date of application on As residues in Coastal bermudagrass

Table I. ^{14}C Activity in Respiratory CO_2 from Coastal Bermudagrass Leaf Sections Taken from DSMA-^{14}C Treatments

Days after Treatment	C.P.M./G.[a]
0 (before treatment)	0 a
2	17 b
4	15 b
6	23 b
8	19 b
10	21 b

[a] Means followed by the same letter designation do not differ significantly at the 5% level according to Duncan's multiple range test.

Table II. Arsenic Residues in Coastal Bermudagrass 5 Days after Application of 4.5 Kg. DSMA per Hectare to Foliage

Stage of Plant Development	Amount of Oven-Dry Foliage, G./Plot	As Residues,[a] Mg. As/Plot	
		Roots	Leaves
Young growth	158 b[b]	3.3 b	12.8 b
Dense growth	233 c	5.5 c	16.0 c
Stubble	110 a	1.9 a	10.3 a

[a] Value for As residues corrected for endogenous As in untreated plots.
[b] Means within columns followed by the same letter designation do not differ significantly at the 5% level according to Duncan's multiple range test.

extract spiked with DSMA-^{14}C. This suggested that DSMA-^{14}C was complexed with some component of the plant. A similar DSMA-plant extract-complex was reported in Johnsongrass (Sckerl and Frans, 1967,) and purple nutsedge (Duble *et al.*, 1968).

Further evidence on the stability of DSMA in Coastal was obtained by monitoring respiratory CO_2 from DSMA-^{14}C treated plants for ^{14}C activity. Activity was found in respiratory CO_2 two days after treatment, but the activity did not increase significantly up to 10 days after treatment (Table I). Carbon-14 activity lost in respiratory CO_2 10 days after treatment accounted for less than 0.1% of the DSMA-^{14}C applied. On the basis of chromatographic analysis, impurities in the formulation of DSMA-^{14}C were ruled out as a source of the ^{14}C activity obtained in respiratory CO_2; however, volatility of DSMA-^{14}C was not ruled out experimentally.

DSMA was readily taken up from foliar applications and from a nutrient solution, but not from a soil (Figure 2). The fact that Coastal roots accumulated arsenic much more readily from a nutrient solution than from a soil demonstrates fixation of arsenic by the soil. Foliar applications resulted in high As residues in both leaves and roots which was indicative of basipetal movement of DSMA. Acropetal movement of DSMA was also demonstrated by the high As residues found in the foliage after root absorption of DSMA. DSMA was also found to be redistributed to some extent following the removal of treated foliage. Thus, DSMA remained mobile in the plant in spite of the fact that it was apparently complexed with some component of the plant. The extent of this mobility supports the conclusion drawn from tracer studies that DSMA was readily translocated in Coastal bermudagrass.

Regrowth from Coastal clipped at ground level 7 days after a foliar application of DSMA showed relatively low As residues, indicating that little DSMA was moved into the new growth. However, the As content of the roots decreased from 45 to 13 p.p.m. during the 3 weeks of regrowth. Since the limited amount of As moved into new growth would not account for the decrease, As must have been released to the soil solution. This phenomenon has been demonstrated with 2,-4-D and other organic compounds (Foy and Yamaguchi, 1964), but has not been demonstrated with arsenicals.

The amount of foliage present at the time of application of DSMA was inversely related to the concentration of As in the foliage, and directly related to the "percent recovery" of As in Coastal (Figure 3). "Percent recovery" is defined here as the percent recovered from the plants as compared to the amount applied. Such relationships were expected since they reflect the dilution effect of excess foliage and the leaf surface area present to intercept the spray, respectively. Regardless of the amount of foliage present at the time of application, approximately 25% of the As intercepted by Coastal was translocated to the roots 5 days after treatment (Table II).

Arsenic residues in Coastal hay after application to a 5-cm. stubble were found to decrease with subsequent growth (Figure 4). Conversely, As residues in the roots increased up to 30

days after treatment, indicating that the complexed DSMA remained mobile within the plant for at least a month.

These results suggest that As residues in Coastal bermudagrass would be minimized if DSMA were applied only during establishment, or when a minimum leaf surface is present, and if the foliage was harvested and discarded 1 to 2 weeks after treatment. Some compromise would have to be made to obtain effective weed control in established grass stands. Generally, 7 to 10 days after clipping, weeds will have made sufficient regrowth for effective uptake of DSMA, and 1 to 2 weeks after treatment, weeds should be effectively controlled. Consequently, 2 to 3 weeks of Coastal growth would be discarded and, therefore, wasted in the process of weed control. The greatest potential use of DSMA would likely be during the establishment of Coastal bermudagrass when little forage is present to intercept the herbicide and subsequent growth and translocation would effectively reduce As residues. These practices would result in As residues which would likely be below As levels fed to cattle experimentally for 8 weeks which were found to have no detrimental effect on animals and to leave no As residue in the animal or its milk (Peoples, 1964). However, regrowth from treated areas, whether treatment is during or subsequent to establishment, will contain trace amounts of As for an undetermined length of time and would, therefore, be unacceptable for livestock consumption by present standards.

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