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Nature of the Residue in Soybean Plant after Treatment of Alloxydim-sodium

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The residue levels of apparent alloxydim-sodium (ADS), sodium salt of 2-(1-allyloxyaminobutylidene)-5,5-dimethyl-4-methoxycarbonylcyclohexane-1,3-dione, in soybean seeds were studied using ^{14}C -ADS compounds labeled at either C-7 or C-1,5 position. The ^{14}C -ADS was applied on the first trifoliate at the second trifoliate stage of the plant. One group of the treated plants was kept under the condition preventing from rainfall. Another group was showered artificially 3 or 7 days after treatment of ^{14}C -ADS and then kept outdoors for 9 or 10 weeks.

The residue levels equivalent to alloxydim-sodium in the seeds were 0.022–0.039 ppm at 9 weeks and 0.024–0.050 ppm at 10 weeks for all samples. So neither the difference in the condition such as artificial rain nor the difference in the position of ^{14}C -label affected the residue levels in the seeds. About 10% of the radioactivity in seeds harvested 9 weeks after treatment was found in lipid fraction. Most of the radioactivity was detected in the water soluble fraction after precipitating protein fraction, in which about 28% and 5% of the radioactivity were found in case of 7- ^{14}C and 1,5- ^{14}C , respectively. Thus the dimedone moiety of ADS was only a little incorporated into proteins.

INTRODUCTION

Alloxydim-sodium (ADS), sodium salt of 2-(1-allyloxyaminobutylidene)-5,5-dimethyl-4-methoxycarbonylcyclohexane-1,3-dione, has been found to be a potent herbicide for the control of graminaceous weeds in soybean, sugar beet and other tolerant broadleaf crops.¹⁾ In the previous paper,²⁾ the absorption, translocation and metabolic fate in soybean plants were studied when the herbicide was treated on the leaf surface. That study was carried out early spring time and under semi-controlled conditions, so the yield of the pods and seeds were poor and, consequently, the concentration of residual radioactivity in the seeds was not estimated correctly.

In the present study, suitable season and conditions for the growth of soybean plants were chosen to see the level and nature of the residue in soybeans. In addition to ADS [7- ^{14}C] as used in the previous paper,²⁾ ADS [1,5- ^{14}C] was used also in this study. Some

of the treated plants were showered artificially and compared with unshowered one on the residue in the plant parts. Showered plants were placed outdoors all day long for 10 weeks.

MATERIALS AND METHODS

1. Chemicals

Two kinds of ^{14}C -compounds were used. One was ADS [7- ^{14}C], i.e. butylidene-1- ^{14}C , which was synthesized at Fine Chemical Laboratory of Nippon Soda Co., Ltd.³⁾ The other was ADS [1,5- ^{14}C] which was synthesized at New England Nuclear Co.⁴⁾ The specific activity was 5.43 $\mu\text{Ci}/\text{mg}$ (1.87 mCi/mmol) and 30.67 $\mu\text{Ci}/\text{mg}$ (10.58 mCi/mmol) for 7- ^{14}C and 1,5- ^{14}C compounds, respectively. The radiochemical purity was 99% for 7- ^{14}C compound and 98% for 1,5- ^{14}C compound when they were checked by a thin layer chromatography (tlc) using a silica gel plate (Eastman Chromagram Sheet 6060®) and developing solvent of *n*-hexane : acetone (7 : 3).

2. Plant Treatment

Soybean (*Glycine max* MERRILL, variety; Toyosuzu) was grown in fertilized soil in a pot (22 cm depth and 28 cm diameter). The plants were maintained in a greenhouse until treatment. $7\text{-}^{14}\text{C}$ -ADS (17.3 mg) or $1,5\text{-}^{14}\text{C}$ -ADS (5.8 mg) plus non-labeled ADS (9.2 mg) was mixed with a surfactant (5 mg) and diluted with water (20 ml). The specific activity of the solution was 1.04×10^7 dpm/ml for $7\text{-}^{14}\text{C}$ compound and 2.03×10^7 dpm/ml for $1,5\text{-}^{14}\text{C}$ compound. Each solution (240 μl) was dotted with a microsyringe as uniformly as possible on three leaves of the first trifoliate at second trifoliate stage of the plants. The treated plants were divided into 3 groups for each ^{14}C -compound as follows. The group I was placed outdoors in the daytime (sunny; 33 days, cloudy; 19 days) and indoors at night and on rainy day (12 days) from July 13th to September 21st, 1976. The temperature was recorded as follows; 16.0–45.6°C in greenhouse and 15.9–31.8°C outdoors. The plants were watered as necessary. The group II was kept in a greenhouse for 3 days, then showered once at a rate of 20 mm or 40 mm within 2 hours by a artificial rain apparatus (Daiki Rika Kogyo Co., Ltd.) and placed outdoors for the same period as the group I. The group III was kept in a greenhouse for 7 days, then showered once at the same rate of artificial rain and placed outdoors for the same period as the group I.

3. Sampling and Analysis

Each one plant for a condition was harvested 9 and 10 weeks after treatment of ^{14}C -ADS. All of aerial part of the plant was sectioned into leaves, stem, pods and seeds. These plant parts were weighed and stored in a freezer until analysis. The stem and pods were separately lyophilized, converted to $^{14}\text{CO}_2$ by a sample oxidizer (Packard Tri-Carb 306®) and radioassayed by a liquid scintillation spectrophotometer (Packard Tri-Carb 3320®). The leaves and seeds were separately homogenized in 4-fold 50% aqueous methanol. The homogenate was filtered and the filtrate and the residue were radioassayed.

On the other hand, radioactivity in seeds was fractionated by modified Dekhuijzen and

Vonk methods⁵⁾ as shown in Fig. 1. The seeds were lyophilized and then pulverized with a mortar and pestle. The powder was extracted with ether or *n*-hexane in a Soxhlet apparatus. The residue was homogenized with a phosphate buffer (0.1 M, pH 8, 150 ml $\times$ 4 times) and the homogenate was centrifuged at 1,000 rpm for 5 min to separate the precipitate (cell wall residues) and supernatant. The supernatant was again centrifuged at $20,000 \times g$ for 20 min to separate the precipitate (plastid fraction) and supernatant (water soluble fraction A).

This fraction A was divided into 3 parts and the proteins were precipitated by different methods. (1) Trichloroacetic acid (TCA) was added to a final concentration of 5% (w/v). The precipitate was collected and washed three times with ice-cold 5% TCA solution and then ether. (2) Equal volume of aqueous solution of 6% cupric sulfate and 1.25% sodium hydroxide were successively added to the fraction A. The resulting precipitate was collected and washed with distilled water until sulfate ion disappeared. (3) Ethanol was added to reach a final concentration of 80%. The precipitate was collected. The radioactivity in each precipitate was counted after converting to $^{14}\text{CO}_2$.

RESULTS AND DISCUSSION

The aims of this experiment were to study the residue level of ADS equivalent in soybeans, the influence of rainfall to the residue level, and the difference in the behavior between two radioactive compounds, ADS [$7\text{-}^{14}\text{C}$] and [$1,5\text{-}^{14}\text{C}$].

The season was suitable for the plant to grow. During the growth of the plant an insecticide, methomyl, was sprayed to control insects on August 7th, 10th and September 2nd. The flowering time was around July 28th. All the treated leaves and the majority of the trifoliate fell down with natural senescence before 9 weeks after treatment of ^{14}C -ADS. The pods of 10 weeks sample were colored brownish and the seeds matured more than those of 9 weeks one.

The soybean plants were radioassayed 9 and 10 weeks after treatment. The fresh weights of 10 plants were measured at 9 weeks with results of leaves 292 g, stems 301 g, pods

Table 1 Residue levels (ppm) equivalent to alloxym-sodium in soybean plants.

¹⁴ C-Compound	Group*	Artificial rain		9 weeks				10 weeks
		Days**	mm/2 hr	Leaves	Stem	Pods	Seeds	Seeds
Alloxydim-sodium [7- ¹⁴ C]	I	Unshowered		0.15	0.06	0.02	0.030	0.041
	II	3	20	0.52	0.06	0.02	0.029	0.024
		3	40	0.12	0.08	0.02	0.026	0.026
	III	7	20	0.23	0.06	0.02	0.024	0.025
		7	40	0.26	0.08	0.03	0.038	0.032
Alloxydim-sodium[1,5- ¹⁴ C]	I	Unshowered		0.13	0.07	0.04	0.031	0.044
	II	3	20	0.44	0.08	0.07	0.032	0.024
		3	40	0.87	0.11	0.05	0.039	0.050
	III	7	20	0.15	0.08	0.06	0.039	0.044
		7	40	0.11	0.04	0.03	0.022	0.039

* See the text for details. ** Days after treatment of ¹⁴C-alloxydim-sodium.

Fig. 1 Fractionation of radioactivity in soybean seeds.

% is expressed as percentage of radioactivity present in seeds.

341 g and seeds 492 g. The residue levels of ADS equivalent in the plant parts are shown in part per million in Table 1. The order of the residue levels was leaves>stems>Pods≥seeds with both ^{14}C -compounds and minimum residue level was detected in the seeds. The residue levels in the seeds were found as 0.022–0.039 ppm at 9 weeks and 0.024–0.050 ppm at 10 weeks for all samples. So, the differences in the residue levels were not seen even if the showering condition was different. This result probably reflects that little radioactivity was washed away by rainfall from the leaf 3 or 7 days after treatment and also thereafter. In fact, by the analytical work done in this experiment, it was shown that only 11% of applied radioactivity was washed away from the treated leaves and 35% of the radioactivity was absorbed into the treated leaves and 8% and 4% of the radioactivity were detected in the 3rd and 4th trifoliate, respectively, 2 days after treatment. The difference in the residue levels was not observed also between $7\text{-}^{14}\text{C}$ and $1,5\text{-}^{14}\text{C}$ compounds except in the pods, in which about 2 times amount of the residues derived from $7\text{-}^{14}\text{C}$ compound was found in the residues derived from $1,5\text{-}^{14}\text{C}$ compound.

The radioactivity in the seeds was extracted and fractionated to investigate the nature of the residues. When the powder of 9 weeks seeds was extracted with 50% aqueous methanol, about 70% and 85% of the residual radioactivity were recovered in the extracts from $7\text{-}^{14}\text{C}$ and $1,5\text{-}^{14}\text{C}$ samples, respectively. On the other hand, the powder of the seeds was fractionated according to a flow sheet shown in Fig. 1. The results are also shown in the same Fig. 1. The lipids fraction contained about 10% of the initial radioactivity and also 10% of the fresh weight. Most of the radioactivity was recovered in the water soluble fraction A with about 80% of the initial radioactivity. So it was concluded that apparent ADS was not concentrated in soybean oil. Further fractionation of the fraction A revealed that about 28% of total radioactivity in seeds was incorporated in the protein fraction in case of $7\text{-}^{14}\text{C}$. But it was not clear whether radioactivity was incorporated into amino acids or not. Thus, the natures of radioactive compounds in the seeds were water soluble.

It seemed difficult to pursue the nature more than the present state because of the low radioactivity in the samples. Whereas, only 5% of total radioactivity in seeds was found in the protein fraction in case of $1,5\text{-}^{14}\text{C}$. Thus the dimedone moiety of ADS was scarcely incorporated into proteins.

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要 約

^{14}C -アロキシジン処理したダイズにおける残留性

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新規除草剤 Alloxidim-sodium (ADS) をダイズの第二葉期に葉面処理し, 収穫期における残留量と種子に取り込まれた微量の放射能の性質を調べた。種子に取り込まれた放射能は ADS 処理後 9 週目に ADS 換算で 0.022~0.039 ppm であり, 薬剤処理後 3 日または 7 日に人工降雨を与えた場合と無降雨区との差は見られなかった。また, $7\text{-}^{14}\text{C}$ と $1,5\text{-}^{14}\text{C}$ のラベル位の違いによる残留量の差も見られなかった。種子に取り込まれた微量の放射能は油脂区, 繊維素区への取り込みはほとんどなく, 多くは水溶性区に検出された。蛋白区への取り込みは ADS [$7\text{-}^{14}\text{C}$] 処理の場合, 種子中放射能の 28% に達したが ADS [$1,5\text{-}^{14}\text{C}$] 処理の場合は 5% で dimedone 骨格は大部分水溶性区に残存することが示された。