Fate of Amiben in Tomato Plants

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Residue analyses were made, using amiben-C^{14}, to determine how much of the herbicide could be detected in tomato plants and fruit after application to soil. Application of amiben early as compared to late in the season resulted in much lower levels of the herbicide in tomato plants and fruit. The level of amiben in prunings and fruit was low in relation to the level in the rest of the plant, indicating a lack of translocation into these organs. Unknown plant constituent(s) formed complexes with amiben. Acid or alkaline hydrolysis of these complexes released amiben. Poor translocation of amiben into fruit and side shoots may be related to the formation of complexes.

THE CHEMICAL, 3-amino-2,5-dichlorobenzoic acid (amiben), has given encouraging results as a selective herbicide for tomatoes. When a herbicide is applied to a crop, it is important to know how much is taken up and the ultimate fate in the crop, especially the edible portions. Sutherland (*5*) reported that a large amount of amiben was taken up from the soil by soybean plants, but that the herbicide disappeared rapidly in the plant. Most of the amiben remaining was in a conjugate and could be released by alkaline hydrolysis. Baker and Warren (*3*) found rapid loss of amiben-C^{14} in cucumber (susceptible) and squash (resistant), and found no difference in the metabolism of amiben-C^{14} by either species. Other radioactive components besides amiben were found in both species. The data indicate that more amiben was immobilized by squash roots than by cucumber roots.

The present studies were conducted to determine the residue of amiben in tomato plants and fruit and to determine in what form the amiben was present.

Experimental Procedure

Field grown Tecumseh tomato plants (*Lycopersicon esculentum*) were brought into the greenhouse and transplanted into number 10 tin cans on July 8, 1961. The experiment was conducted in a washed-air–cooled greenhouse. A methanol solution of carboxyl-labeled amiben-C^{14} (1.1 mc. per μmole) plus an appropriate amount of cold amiben to give the desired rate was pipetted onto the soil. Amiben treatments were applied on July 12 or on August 23 at 3 and 6 pounds per acre of total amiben (cold plus labeled). The amount of amiben-C^{14} was held constant at 1.5 pounds per acre in each treatment. The treatments were replicated three times.

All plants were pruned (axillary shoots removed) on August 4 and were harvested on September 15. At harvest, some plants had one ripe fruit and all plants had mature green fruit. Plant material was placed in the deep freeze for storage until analyses were made. Separate analyses were made on the above-ground portion minus prunings and fruit (hereafter referred to as the "whole plant"), the prunings, and the fruit. The roots were not analyzed.

The whole plant and prunings were dried in a forced-air oven for 1 week at 80° C., weighed, and ground in a Wiley mill. Ten-gram samples of the whole plant were extracted continuously for 3 days in a soxhlet apparatus with absolute methanol. The final volume of the methanol extract was made to 100 ml. in volumetric flasks. The prunings were handled similarly except that a 2-gram sample was extracted, and the final volume of the methanol extract was 25 ml. One-milliliter aliquots were pipetted into planchets, dried, and counted for radioactivity under a thin-window, gas-flow GM counter to 1000 counts or 15 minutes. Radioactivity counts were converted to micrograms of amiben by use of a standard curve obtained by adding appropriate amounts of plant extract from the controls to known amounts of amiben-C^{14}. The isotopic dilution of amiben-C^{14} was considered in the calculation of amiben residues so that the total residue of radioactive plus cold amiben is reported. A 25-mg. sample of extracted residue from the Soxhlet thimble from treatment 5 (highest activity) was digested by the method of Van Slyke *et al.* (*6*). No radioactivity was detected in the sodium carbonate, indicating complete extraction of radioactivity by methanol.

The following procedure was used for extracting radioactivity from fruits. Samples of fruit were allowed to thaw and were ground in a Waring Blendor with sodium hydroxide pellets at full speed for 5 minutes. Nine per cent of the fresh weight of the fruits was added as sodium hydroxide pellets without water. The homogenate was autoclaved for 1 hour at 4 to 5 pounds pressure (215° to 220° F). The autoclaved homogenate was cooled, adjusted to pH 2, and extracted three times with ethyl ether. The combined ether extract was filtered, evaporated to 10 to 15 ml., and made up to volume in 25-ml. volumetric flasks with methanol. One-milliliter aliquots were counted for radioactivity under an ultrathin window, gas-flow counter to 1000 counts. Approximately 100% of amiben-C^{14} added to control extracts was recovered by this method.

Methanol extracts of the whole plant and fruit were chromatographed to determine in what form amiben was present. Ascending paper chromatograms (Whatman No. 2 paper) were developed for 30 cm. with *n*-butanol, ethanol, ammonium hydroxide (2:1:1, v./v.). They were dried and scanned for radioactivity.

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Table I. Amiben Residue in Tomato Plants

Rate, Pounds per Acre	Amiben Residues: Whole Plant[a] P.P.M.	Whole Plant[a] Total µg. per plant	Fruit, p.p.m.[b]
PLANTS TREATED JULY 12			
3	6.2	247	0.06
6	13.3	568	0.15
PLANTS TREATED AUG. 23			
3	10.2	435	0.22
6	29.7	1367	0.52
L.S.D., 5% level	10.6	418	0.10

[a] Expressed on dry-weight basis.
[b] Expressed on fresh-weight basis.

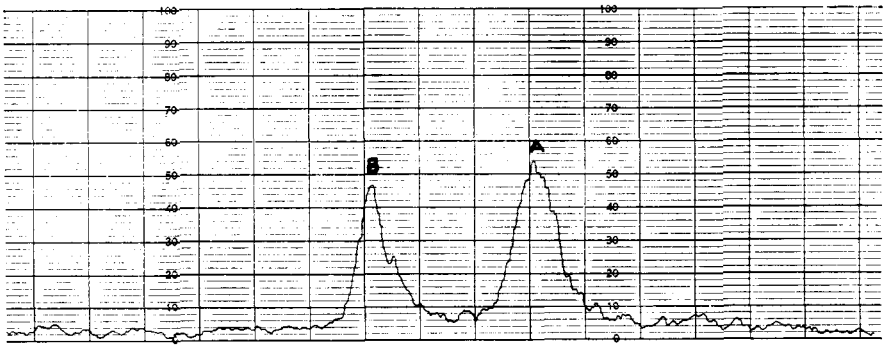

Figure 1. Chromatographic separation of radioactive components present in purified extracts from amiben-C^{14} treated tomato plants

Amiben (A); amiben-X (B)

An amiben-C^{14} complex (designated amiben-X) was observed on the paper chromatograms, and the following procedure was developed for its purification. Twenty grams of dried, whole plant was extracted with acetone in a Soxhlet for 24 hours. This did not remove all the radioactivity, but did remove large amounts of amiben-X. The extract was concentrated under reduced pressure and fractionated on a column of Celite (Johns Manville Hyflo Super Cel), charcoal, sodium sulfate (1 2/2, w./w.), which removed chlorophyll and other interfering substances. The column was eluted with methanol and concentrated ammonium hydroxide (25/1, v./v.). Three-milliliter fractions were collected and assayed for radioactivity. The eluate from tubes 8 to 13, which contained most of the activity, was combined, concentrated, and chromatographed.

Results

Chromatographic evidence indicated that radioactivity in tomato plants, prunings, and fruit treated with amiben-C^{14} was amiben or could be converted to amiben by acid or alkaline hydrolysis. Therefore, radioactivity will be referred to as amiben. Table I summarizes the amiben residues in the whole plant and fruit. No amiben was detected in the prunings within the limits of sensitivity of the method. Plants treated on August 23 contained a higher concentration of amiben than those treated on July 12. The total amount of amiben present was also higher in plants treated on August 23 compared to July 12. The concentration of amiben in the fruit was quite low in relation to the concentration in the whole plant. A more valid comparison of the concentration in fruits *vs.* the whole plant can be approximated by multiplying the p.p.m. in the fruit by 10 to convert these values to a dry-weight basis. On this basis, the concentration of amiben in the fruit was about one-tenth that in the whole plant.

Chromatogram scans of the radioactivity in concentrated whole plant extracts revealed two peaks. The major peak (about 75% of the activity) corresponded to amiben, R_f 0.66. A minor peak (amiben-X) was observed at about R_f 0.40. The minor peak was eluted, concentrated in the presence of several drops of concentrated HCl, and rechromatographed. The R_f then corresponded to amiben. All radioactivity in the fruit corresponded chromatographically to amiben. Any amiben-X present in the fruit was probably broken down by alkaline hydrolysis. Preliminary investigations, utilizing alkaline hydrolysis with whole plant extracts, also indicated that this method resulted in loss of amiben-X.

A scan of the chromatographed radioactivity after elution from the Celite–charcoal–sodium sulfate column indicates (Figure 1) the presence of both amiben and amiben-X. Amiben-X was chromatographed before and after acid hydrolysis, and the chromatograms were sprayed with ninhydrin. A different number and distribution of amino acids resulted.

Discussion

The lower level of amiben present in tomato plants treated early may indicate that amiben suffers breakdown in the soil or plant. Another possibility is that less amiben was taken up from the soil by younger tomato plants. A decline of amiben with time in plants has previously been reported (*3, 5*).

The lack of amiben in prunings and the low level in fruit may indicate that retranslocation into actively growing tissues is limited. Furthermore, the concentration of amiben in the fruit relative to the whole plant was lower for both rates of herbicide applied early as compared to late. Possibly amiben applied late had greater opportunity for direct translocation from roots to fruit. Perhaps the amiben taken up early by tomato plants was immobilized in the form of amiben-X and was not as easily retranslocated into the fruit. Lack of retranslocation of amiben (*2*) and immobilization of amiben (*3*) have been reported.

Formation of conjugates of 2,4-D, indoleacetic acid and benzoic acid with aspartic acid was reported by Andreae and Good (*1*). Auxin-phenol complexes have also been reported (*4*). The present study and the results of others (*3, 5*) indicate that amiben-complexes are formed in plants. These complexes may explain the poor mobility of amiben in plants.

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