COMMUNICATIONS

Translocation and Metabolism of Azinphos-Methyl in Bean Plants after Root and Leaf Absorption

During the course of studies concerned with the metabolism of [^{14}C]azinphos in bean plants, it became questionable whether the insecticide behaves systemically. Experiments carried out in this aspect merely partly confirmed results reported recently in the literature. Azinphos was absorbed by the leaf tissue only in a small amount and seems to move in the plant in the xylem rather than in both directions, xylem and phloem. Lasting systemic action of azinphos via root uptake appears to be limited since already some days after absorption a high amount of the radioactivity in the leaves was found to represent metabolites.

The term "systemic behavior" of a pesticide is difficult to define precisely. Since insecticides are mainly applied to growing crops the primary requisites of systemic action would be: the absorption of the insecticide into the plant, a lasting action, and the translocation within the plant thus rendering untreated areas insecticidal (Fest and Schmidt, 1973).

Azinphos-methyl (trade names Guthion and Gusathion) is described in the literature as a nonsystemic, broad-spectrum insecticide, acting by both contact and ingestion (Chemagro, 1974; FAO/WHO, 1969; Unterstenhöfer, 1965). However, Al-Adil et al. (1973) concluded from results of a recent study with ^{14}C-labeled azinphos that the compound should be classified as systemic since it was readily absorbed through roots or leaves and translocated unaltered throughout the plant system, thus rendering untreated areas insecticidal.

This conclusion seemed not to be supported by the results of a time sequence study on the metabolism of azinphos in beans (Steffens and Wieneke, 1975). Only a small amount (1.1–2.7%) of the radioactivity applied to the first trifoliate leaf was present in the organosoluble fraction after stripping the leaf surface to remove extracuticular residues. The main amount of radioactivity in the plant was always determined in the water fraction; however, no azinphos was ever detected in this fraction. The portion of radioactive compounds translocated from the treated leaf into the untreated plant parts increased with time but was relatively small and consisted mainly of water-soluble derivatives (88%). Other experiments (Wieneke and Steffens, 1975a) revealed that some of the azinphos applied to a leaf can penetrate into the tissue but most of it was found to be decomposed rapidly.

Therefore, to test the postulation of Al-Adil et al. (1973) that azinphos behaves as a systemic insecticide, part of their work with bean plants was repeated.

METHODS

The methods were principally similar to those described by Al-Adil et al. (1973); however, instead of ^{14}C-benzenoid-ring-labeled azinphos, ^{14}C-carbonyl-labeled azinphos (chemically pure) was used. Bean plants (variety Saxa) were grown in $^1/_2$ × Hoagland solution in a growth chamber (daytime, 25°C, 65% relative humidity; nighttime, 15°C, 90% relative humidity; 12 hr photoperiod). The plants were treated when the primary leaves were fully unfolded (three replicates, 700 ml/container).

At first, a preliminary experiment was carried out with a concentration of 8 ppm of [^{14}C]azinphos, dissolved in 40 ml of ethanol and then added to the nutrient solution as described by Al-Adil et al. (1973). However, after a few days of treatment the roots did not appear normal and the leaves were not fully turgid. Therefore, to find out whether this could be attributed to the addition of azinphos and/or ethanol to the nutrient medium, the following experiment (I) was designed: (A) nutrient solution without additives (control); (B) 8 ppm of azinphos dissolved, after evaporation of the solvent (benzene), in nutrient solution by shaking for 10 hr in a darkened bottle; (C) 8 ppm of azinphos dissolved in 40 ml of ethanol and then added to the nutrient solution, 150–200 ml of the solution evaporated and the solution refilled with H_2O before use; (D) same treatment as in C but 24 ppm of azinphos; (E) same treatment as in C but no evaporation; (F) same treatment as in C but no addition of azinphos; (G) same treatment as in F but no evaporation.

Experiment II was performed to repeat treatments B and C as described above for experiment I, using radioactive azinphos (2 μCi/plant per 250 ml of nutrient solution). An additional group H was set up with 8 ppm of azinphos previously dissolved with an emulsifier, instead of with ethanol (Steffens and Wieneke, 1975), and then added to the nutrient solution. The plants were harvested after 4 and 7 days, and with another set of plants, after 1 day. The roots were washed, first with water and then with benzene (2 min), to remove adsorbed azinphos. The plants were then separated into leaves, stems, and roots. The bean leaves were homogenized in an Ultra-Turrax blender with the following solvents: (1) acetone; (2) acetone–water (3:1, v/v); and (3) 2 vol of chloroform. The filtrates were pooled and the solvents evaporated. The concentrated extract was then subsequently separated into a hexane, chloroform, and water fraction. Details of the methods for clean-up, extraction, liquid scintillation counting as well as thin-layer chromatography are described elsewhere (Steffens and Wieneke, 1975; Wieneke and Steffens, 1975b). An aliquot of the labeled nutrient solution was taken before and at the end of the experiment and analyzed.

RESULTS AND DISCUSSION

The roots of all bean plants grown in nutrient solution with any addition of ethanol appeared abnormal even when probably most of the ethanol was evaporated before the plants were transferred to the solution. Already after 3 days the roots, and also the primary leaves, of those plants treated with the highest azinphos concentration or with azinphos but no vacuum evaporation of the solution

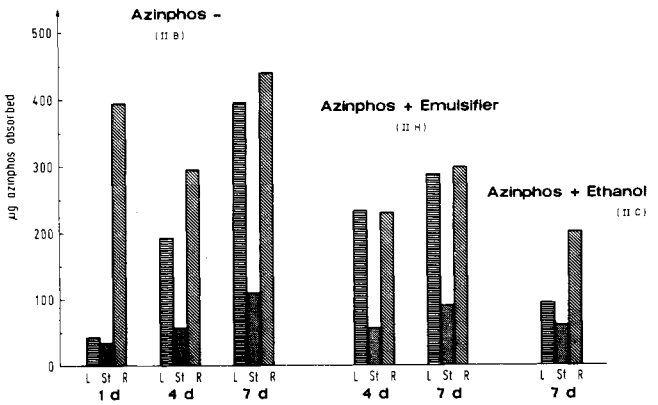

Figure 1. Distribution of azinphos equivalents in leaves (L), stems (St), and roots (R) of bean plants after absorption of [^{14}C]azinphos dissolved by different means in nutrient solution.

(experiment I, D and E) were not fully turgid and the solution started to foam. However, the plants were not completely dead 10 days after treatment but the growth was markedly reduced. In contrast, the solution of the control (group A) and of the group treated with azinphos not previously dissolved in ethanol (B) was not turbid and the plants and roots appeared normal. This was also the case when the plants absorbed azinphos previously dissolved in combination with an emulsifier (experiment II, H). Since it was visually demonstrated in experiments I and II that the addition of ethanol to the root medium was phytotoxic no further attempts were made to analyze histological details in this respect or the nutrient solution for bacterial growth.

The results of uptake and translocation of [^{14}C]azinphos (experiment II, radioactivity calculated as azinphos equivalents) by bean plants are depicted in Figure 1. Because of the laborious clean-up and analysis procedure only certain plants were analyzed as representative. The tendency is well pronounced. After 1 day and still after 4 days of uptake an accumulation of radioactivity, preferentially in the roots, can be noticed (B, azinphos -). In the leaves the amount of radioactivity increased continuously up to the 7th day of uptake, to a level comparable to that found in the roots. This tendency is underlined once again by the results of group H (azinphos + emulsifier).

The results of both treatments (experiment II, B and H) without the use of ethanol are in contrast with those reported by Al-Adil et al. (1973). They observed with two species, barley and bean plants, that the radioactive compound was taken up and translocated rapidly into the aerial parts within the first 24 hr, but then the translocation into the shoots slackened markedly.

The influence of ethanol in the root medium may be the reason why the amount of radioactivity determined in the leaves of group C (azinphos + ethanol, Figure 1) was small and the amount in the unextractable leaf residue was relatively high (Table I) as compared to the other groups (II, B and H).

The total uptake of azinphos 7 days after treatment (Figure 1, average of three plants) seems also to be much smaller for group IIC (469 µg) as compared with group IIB (946 µg) and group IIH (679 µg).

Whether the azinphos adsorbed at the roots was completely removed by the water washing and succeeding benzene strip is difficult to decide. The amount of radioactivity determined in the strip solution 7 days after treatment and expressed as a percentage of total (radioactivity in the plant + strip solution) was small: group B, 5%; group H, 1.8%, and group C, 0.8%. The recovery was, from the average of 12 plants, 86.7 ± 4.9%. Thus, the loss of radioactivity was relatively constant and may be due to a slight adsorption of radioactivity at the styrofoam lid of the nutrient vessel and to a certain extent to some precipitation in the nutrient solution. Also, some mineralization of azinphos in the nutrient solution and loss by plant respiration cannot be excluded.

Table I. Distribution of Radioactivity in Different Fractions Extracted from Bean Leaves after Root Uptake of [^{14}C]Azinphos from Nutrient Solution

Treatment	Days after labeling	Radioactivity, % of total[a] Hexane	Chloroform	Water	Unextractable residue	Remainder
Azinphos - (IIB)	1	24.3	1.2	63.7	9.0	1.8
	4	36.2	2.2	53.8	7.6	0.2
	7	7.2	2.6	78.7	11.0	0.5
Azinphos + emulsifier (IIH)	7	10.1	1.3	81.1	7.4	0.1
Azinphos + ethanol (IIC)	7	8.0	1.9	59.4	30.4	0.3

[a] ^{14}C in bean leaf = 100.

Table II. Distribution of Radioactivity in Different Fractions Extracted from Nutrient Solution Labeled with [^{14}C]Azinphos

Treatment	Days after labeling	Radioactivity, % of total[a] Hexane	Chloroform	Water
Azinphos - (IIB)	0	93.7	5.4	0.9
	1	33.8	11.1	55.0
	4	71.8	4.2	24.0
	7	43.0	9.7	47.3
Azinphos + emulsifier (IIH)	7	46.5	5.2	48.5
Azinphos + ethanol (IIC)	7	79.6	15.2	5.1

[a] ^{14}C in nutrient medium = 100.

In Table I the distribution of the radioactivity found in different fractions and extracted from the leaves is listed. Some deviation of the results between the first and fourth day after harvest (see also Table II) may be related to the use of two different plant sets but does not affect the trend. The radioactivity in the water and unextractable residue fractions increased as that in the organosoluble fractions decreased.

TLC (two directions in different systems) and cochromatography with reference substances (Wieneke and Steffens, 1975b) showed that nearly all the radioactivity in the hexane fraction and a small portion in the chloroform fraction still represented azinphos. This reveals that some azinphos must have been absorbed through the roots and was then translocated into the leaves. So far this conclusion is in agreement with the report of Al-Adil et al. (1973). However, in our experiments a high and increasing amount of radioactivity in the water fraction (Table I) indicates that a rapid degradation occurred in the plant. This observation is in agreement with results of other experiments when azinphos was injected into bean shoots (Wieneke and Steffens, 1975a).

In contrast, Al-Adil et al. (1973) claimed that 97.7% of the radioactivity in the total shoot represented azinphos

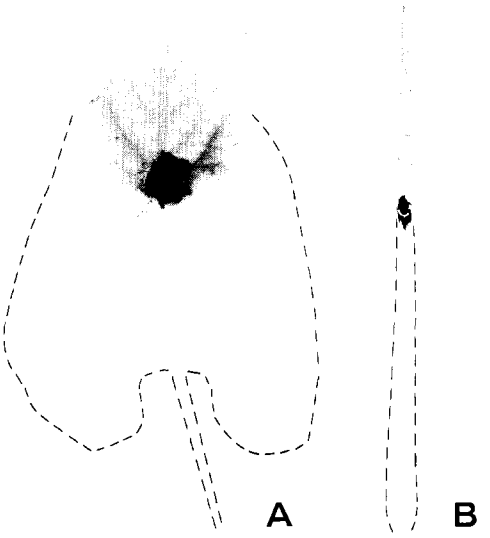

Figure 2. Autoradiograms of a primary bean leaf (A) and a barley leaf (B) 7 days after spot application of azinphos (specific activity, 0.4 ppm/μCi) dissolved with emulsifier in water.

which was identified by TLC, infrared, and mass spectrometry. In a more recent report, these findings were principally confirmed (Al-Adil et al., 1974). However, in both studies no effort seems to have been made to extract and fractionate for water-soluble metabolites. Furthermore, in the second study no indication is made as to how azinphos was dissolved and added to the nutrient solution and what specific activity was used. This impairs the evaluation of the translocation of azinphos into the upper plant parts.

To ascertain whether azinphos was possibly decomposed partly before root uptake, the nutrient solution was extracted and subjected to the same clean-up procedure as was used for the plant tissue analyses. The results are shown in Table II. Again, most of the radioactivity in the hexane fraction and part of the radioactivity in the chloroform fraction represented azinphos. A check made immediately after preparation of the labeled nutrient solution indicated that more than 95% of the radioactivity was present as azinphos (Table II, day 0). However, an apparent degradation occurred after the plants were transferred to the nutrient solution. This indicates that probably an increasing amount of radioactive compounds was absorbed by the plant roots as metabolites rather than as unaltered azinphos. Only in the nutrient solution of group C (azinphos + ethanol) was about two times the amount of radioactivity present in the hexane fraction 7 days after treatment, as compared to the groups without ethanol (II, B and H) in the medium.

Al-Adil et al. (1973) further stressed the systemic behavior of azinphos with the observation that it was readily absorbed and moved upward and downward in the plant after it was applied to the stem. Since in practice azinphos is sprayed in watery solution, we used spot application of emulsified instead of ethanol-dissolved azinphos to test the direction of movement. In Figure 2, two autoradiographs are shown demonstrating that, in barley and bean leaves, only slight absorption occurred, and labeled compounds were translocated predominantly in the acropetal direction. This is in agreement with results of other experiments which indicate that always only a small amount of organosoluble compounds was present in the leaf tissue (Wieneke and Steffens, 1975b). Again, it must be assumed that the use of 95% ethanol as a solvent for the application of azinphos to the plant (Al-Adil et al., 1973) influenced the permeability of the tissue.

The results of this investigation therefore underline that care has to be taken using ethanol as a solvent for chemicals in experiments with plants. It has been shown that after root uptake of azinphos the main amount of radioactivity in the bean leaves was present in the polar fraction (see Table I, e.g., group IIB; about 50 and 80% after 4 and 7 days of application). On the other hand, the total azinphos (including daughter products calculated as equivalents) translocated into the stem and leaves increased with time after application (see Figure 1, group IIB; 4.0, 12.6, and 25.3% of total applied after 1, 4, and 7 days, respectively).

However, leaf absorption was demonstrated to be small and the absorbed compounds were almost exclusively translocated in an acropetal direction. Furthermore, in other experiments azinphos was found to degrade readily inside the plant leaf and especially after shoot injection (Wieneke and Steffens, 1975a,b). In addition, Liang and Lichtenstein (1972) could not observe biological activity with organo- and water-soluble metabolites of azinphos.

In the soil, under field conditions, azinphos was decomposed rapidly enough so as not to allow a buildup of higher amounts (Chemagro, 1974; Schulz et al., 1970). Since the spray concentrations used in practice are relatively low, a marked root uptake may not occur.

Therefore, substantial systemic behavior of azinphos in bean plants should not be expected, although the systemic characteristics may differ with other plant species or be dependent on the mode and frequency of application and other factors. The conclusion that azinphos has no substantial systemic behavior is in general agreement with reports in the literature (Chemagro, 1974; FAO/WHO, 1969; Unterstenhöfer, 1965).

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