Effects of Metabolic Inhibitors, Aeration, and Other Factors on Penetration and Translocation of Lindane in Pea Plants

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Factors affecting the penetration of lindane from a nutrient solution into pea roots and the translocation of the insecticide into pea greens were investigated. The selective transport of lindane into roots was dependent on energy supply systems of the root cells. The presence of metabolic inhibitors such as KCN (5×10^{-4} *M*) and DNP (10^{-5} *M*) resulted in an increased penetration of lindane and its decreased translocation into greens. Because of the inhibitory action of rutamycin and atractyloside on some phase of energy transformation, roots were exposed to these inhibitors at concentrations of 5×10^{-6} *M* and 2.5×10^{-6} *M*, respectively. This also resulted in an increased penetration of lindane into roots. Similar results were obtained under nonaerating conditions or aeration with nitrogen of the nutrient solutions. In general, a rapid accumulation of lindane was observed on or in roots within 1–2 hr after root exposure to the insecticide and a slower but constant increase in the translocation of lindane into greens occurred over a 6-day exposure period. The initial rapid "penetration" into roots was not dependent on the presence of pea greens, while after a 24-hr exposure period excised roots contained less insecticide than those of intact plants. The penetration of lindane into roots was proportional to the insecticide concentration up to 6 ppm, while its translocation into greens increased at a much slower rate.

Due to direct pesticide applications to soils or through "fallout" after crop treatment, a major portion of these chemicals ends up in the upper soil layers. Some of these residues can penetrate into roots of various crop plants and eventually can be translocated into the aerial plant parts. The extent of absorption and translocation of these biocides depends upon various environmental conditions such as soil type, temperature, rate and mode of application (Lichtenstein, 1959; Lichtenstein *et al.*, 1967), rate of transpiration (Craft, 1961; Hacskaylo *et al.*, 1961b; Tietz, 1954), and the nutritional status of crop plants (Casida *et al.*, 1952; Talekar and Lichtenstein, 1971; Yu and Morrison, 1969). Since many soils contain various residues of insecticides, contamination of plants growing in these soils is possible. It would, therefore, be very desirable to find a method which would make it possible to reduce the uptake of insecticidal residues by crops. To this end, more information is needed concerning the forces that govern penetration and translocation of insecticides in plants. Experiments were conducted at the University of Wisconsin with pea plants and lindane to study various factors that could affect the penetration and translocation of lindane in pea plants. Lindane (γ isomer of hexachlorocyclohexane) was selected because it is more persistent than organophosphorus and carbamate insecticides but less water soluble (6–10 ppm) than these compounds. In particular, four series of experiments were conducted to study the penetration and translocation of lindane into pea plants as affected by: (1) metabolic inhibitors and oxygen availability to roots; (2) the presence of the aerial parts of pea plants; (3) length of exposure to the insecticide; and (4) the insecticidal concentration in the root environment.

EXPERIMENTAL PROCEDURES

Plant Growth and Analyses. All experiments were conducted with pea plants (*Pisum sativum*, variety Perfection or Alaska Wilt Resistant) grown for 15 days in a growth chamber (12 hr of light at 28° and 12 hr of dark at 20°, relative humidity 65 ± 2%) within a complete nutrient solution as previously described (Talekar and Lichtenstein, 1971). Six plants each were grown in nutrient solution (Talekar and Lichtenstein, 1971) within round jars (500-ml capacity, 145 mm high and 70 mm wide) in experiments 1, 2, and 4, or 40 to 60 plants each in rectangular stainless steel tanks (31 cm × 25 cm × 18 cm) (Experiment 3). Unless indicated otherwise, nutrient solutions were continuously aerated by bubbling air through them.

After plants had been grown under the conditions particular for each of the four experimental series, they were harvested and roots and greens were separately extracted and analyzed by gas–liquid chromatography (glc) as previously described (Talekar and Lichtenstein, 1971). It is important to emphasize that before extraction all roots were thoroughly washed under running tap water and dried with blotting paper. The total fresh weight of each group was then recorded. This procedure did not differentiate between the residues within the roots and those that still could have adhered to the outside of the root epidermis. Analytical data referring to the residues "in roots" are therefore to be interpreted accordingly. In the following discussion residues of lindane found "in roots" are referred to as penetrated lindane, while those found in the aerial parts are referred to as "translocated."

SERIES 1. EFFECTS OF METABOLIC INHIBITORS AND AERATION ON THE PENETRATION AND TRANSLOCATION OF LINDANE INTO PEA PLANTS. To study the effect of metabolic inhibitors on the penetration and translocation of lindane in peas, plants were exposed to aerated nutrient solutions containing lindane only (control) or lindane plus either potassium cyanide (KCN), an inhibitor of cytochrome oxidase, or dinitrophenol (DNP), an uncoupler of oxidative phosphorylation. In addition, the antibiotics rutamycin or atractyloside were used because of their inhibitory action on some phase of energy transformation. If energy would be required for regulating the penetration of the insecticide into the roots and its further translocation into the aerial plant parts, the exposure of roots to these metabolic inhibitors should be noticeable. In the control series (no inhibitors) four groups of plants, each consisting of six pea seedlings, were grown for 16 days in insecticide-free aerated nutrient solutions. They were then transferred into a freshly

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Table I. Effects of Metabolic Inhibitors and Aeration on the Penetration and Translocation of Lindane in Peas. Results are Averages of Four Replicates (Six Plants Each)

Nutrient solution[a]	Lindane in plants after exposure to the insecticide during the last day of the growing period: Roots, ppm	Roots, % CK	Greens, ppm	Greens, % CK
Aerated plus				
None (control)[b]	44.22 ± 3.14	100	14.15 ± 1.89	100
Rutamycin, 5×10^{-6} *M*	51.16 ± 1.65	115.7[c]	13.59 ± 4.14	96.7
Atractyloside, 2.5×10^{-6} *M*	54.66 ± 2.43	123.6[d]	17.51 ± 2.73	124.6
None (control)[e]	41.62 ± 1.65	100	7.75 ± 0.51	100
KCN, 5×10^{-4} *M*	69.76 ± 3.40	167.6[d]	5.33 ± 0.59	68.8[d]
DNP, 10^{-5} *M*	50.83 ± 1.10	122.1[d]	6.12 ± 1.25	78.9[c]
Aerated with N_2	52.28 ± 4.96	125.6[c]	7.52 ± 0.52	97.0
Nonaerated throughout	52.62 ± 1.65	126.4[d]	4.58 ± 0.16	59.1[d]

[a] Inhibitors were added to nutrient solution during the last 2 days of growing period only. [b] Control: without rutamycin or atractyloside. [c,d] Differences in lindane concentrations between control plants and those grown under various experimental conditions were significant at the 1 %[d] and 5 %[c] levels. [e] Control: without KCN or DNP; served also as control for "Aeration with N_2" and "Nonaeration throughout."

prepared nutrient solution that had been treated with an acetone solution of lindane (1 ml of acetone per 1000 ml of nutrient solution), resulting in a concentration of the insecticide of 5 ppm. Plants were then grown for one additional day (day 17) in this solution before extraction and analyses.

Two identical tests were conducted (Table I), except that KCN was added for the last 2 days of the growing period to the nutrient solution on both day 16 and 17 at a concentration of 5×10^{-4} *M*, and DNP was added at a concentration of 10^{-5} *M*. The concentrations of metabolic inhibitors such as KCN and DNP were used because, in preliminary experiments with these compounds, no adverse effects on plant growth were noticeable. In additional tests, rutamycin and atractyloside were added to the nutrient solution on day 16 only at a concentration of 5×10^{-6} *M* and 2.5×10^{-6} *M*, respectively. These concentrations were used because of the limited water solubility of rutamycin. On day 17 lindane was added at a concentration of 5 ppm to the antibiotics containing nutrient solutions.

To study the effects of oxygen availability in the nutrient solutions, two additional tests were conducted. In one test nitrogen was bubbled through the nutrient solution during the last 2 days (16 and 17) of the plant growing period, while in the other test plants were held in a nonaerated nutrient solution during the entire 17-day growth period. Controls for these tests consisted of four groups of six plants each that were grown in nutrient solution through which air was bubbled throughout the entire growing period of 17 days.

The pH of the nutrient solutions at the beginning of the experiment was 5.5 but had increased to 7.2 at the end of the 15-day growth period. At that time the pH was adjusted in all nutrient solutions to 6.2. The pH of the freshly prepared and lindane-treated nutrient solution in which plants grew during day 17 was also adjusted to 6.2.

Analytical results obtained after analysis of plants by glc are presented in Table I. It is evident that in the presence of KCN, an inhibitor of cytochrome oxidase, more lindane penetrated into the roots and less was translocated into the greens in comparison to control plants. Similarly, significant differences were observed with DNP, which is an uncoupler of oxidative phosphorylation.

Rutamycin and atractyloside has been used because of their inhibitory action on some phase of energy transformation. Rutamycin, like oligomycin, is derived from a strain of streptomyces (Shaw, 1967). It blocks ATP-energized reactions and, like DNP, blocks the conversion of ADP to ATP. Rutamycin inhibits respiration when coupled to oxidative phosphorylation. "Atractyloside occurs in the rhizomes of *Atractylis gummifera*, a thistle growing in the southern Mediterranean area" (Heldt, 1969). It "inhibits the entry of ADP and ATP into mitochondria as well as the binding to intact particles." Its action is "specifically concerned with entry and exit of ADP/ATP into a mitochondrial compartment." Although used at concentrations lower than those of KCN and DNP in the nutrient solution, the effects of rutamycin and atractyloside were similar with roots, since significantly more lindane had penetrated due to their presence. However, no effect on translocation into greens was noticed at the inhibitor concentrations used.

It appears, therefore, that energy is required to regulate the penetration of lindane into the roots and for translocation of penetrated lindane from the roots into the greens. This energy is supplied through metabolic plant processes. Moreover, the reduction of oxygen pressure in the nutrient solution achieved through either aeration with nitrogen or nonaeration gave results similar to those achieved with the metabolic inhibitors. The selective transport of a nearly water-insoluble insecticide across root membranes appears to be dependent on energy supply systems in root cells.

Series 2. Effects of Aerial Parts of Pea Plants on the Penetration and Translocation of Lindane in Peas. It is assumed that the transpiration stream affects the uptake of pesticides by plants and that conditions favoring transpiration increase the uptake of pesticides by plant roots (Craft, 1961; Hacskaylo *et al.*, 1961a,b; Tietz, 1954). To study the importance of pea greens in processes of lindane penetration from a nutrient solution into pea roots, the following experiments were conducted. Six groups of pea plants, consisting of six pea seedlings each, were grown for 15 days in an insecticide-free nutrient solution as described. From three groups of plants, greens were then removed by cutting the stems at a point above the perforated plastic screw caps which held the plants in the glass jars. A freshly prepared nutrient solution was treated with lindane at 5 ppm and used for replacement of the solution in each of the six jars. Plants were then transferred and grown in the lindane-treated solution under aerating conditions for a period of 1, 2, or 24 hr. After that, the plants were removed and the roots were extracted for future analyses. Results obtained from these experiments are summarized in Table II.

It is evident that after 1 and 2 hr of exposure, concentrations of lindane in roots were eight and nine times greater than the initial concentration of the insecticide in the surrounding nutrient solution. The greens of pea plants, however, did not affect the penetration of lindane into the roots during the 1- or 2-hr exposure period since there was no significant differ-

Table II. Effects of Removal of Aerial Parts of Pea Plants on the Penetration of Lindane into Pea Roots. Results Are Averages of Three Replicates (Six Plants Each)

Hours of root exposure to lindane	Lindane (ppm) recovered from: Roots of intact plants		Excised roots	
	ppm	% CK	ppm	% CK
1	39.52 ± 1.60	100	40.04 ± 1.16	101.31
2	46.68 ± 1.61	100	44.82 ± 2.97	96.01
24	52.71 ± 3.58	100	40.61 ± 0.22	77.04[a]

[a] Differences between lindane concentrations in the excised roots and in the roots of intact plants were significant at the 1% level.

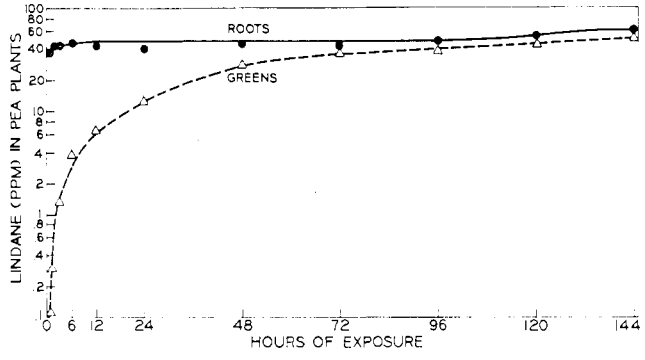

Figure 1. Penetration and translocation of lindane into pea plants during a 6-day period. Nutrient solution was treated initially with the insecticide at 5 ppm

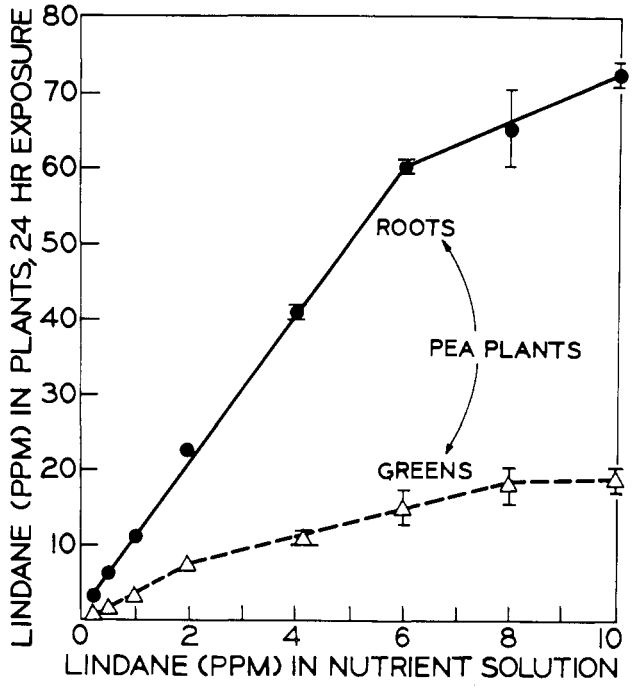

Figure 2. Effect of lindane concentration in nutrient solutions on the penetration and translocation of the insecticide into pea plants during a 24-hr exposure period

ence between the insecticide content in excised roots and in roots of intact plants. It appears that the penetration of lindane proceeded through rapid partitioning of the lipid-soluble lindane from the nutrient solution into the roots. When the exposure period was extended to 24 hr, however, the amount of lindane that had penetrated into the roots of intact plants was significantly larger than the amount found in or on excised roots (Table II). The level of lindane in the roots of intact plants increased steadily from 1 to 24 hr, while in the excised roots the amount of lindane after 24 hr of exposure was the same as that found after 1 hr of exposure. It is likely that after an initial rapid penetration of lindane into roots, a further penetration of the insecticide occurred only after some of the insecticide had been removed by translocation into the pea greens, possibly due to the transpiration stream (Tietz, 1954). This rapid penetration followed by a slower translocation was also demonstrated in the following experimental series.

Series 3. Effects of Exposure Time to Lindane on the Penetration and Translocation of the Insecticide into Peas. The previous studies had indicated a rapid accumulation of lindane on or in pea roots. To further elucidate the mechanism of penetration and translocation of the insecticide over an extended time period, 148 pea plants were grown within three stainless steel tanks for 15 days in a lindane-free nutrient solution. The plants were then transferred into freshly prepared nutrient solutions which had been treated with lindane at 5 ppm. After an exposure of the roots to the insecticide for 0.5, 0.75, 1.5, 3, 6, 12, 24, 48, 72, 96, 120, or 144 hr, four plants were removed from each of the tanks (total of 12 plants at each time) and were extracted and analyzed for lindane content in roots or in greens.

Results of these experiments are presented in Figure 1 and demonstrate the rapidity by which the insecticide accumulates in the root system, followed by a leveling off in lindane accumulation in the roots once a plateau of insecticide concentration (about 45 ppm) in roots was reached after 1.5 hr of exposure to the insecticide. This concentration in roots was 60 ppm after 6 days of exposure to the insecticide.

Within 30 min of exposure, a concentration of 30.51 ppm of lindane in the roots was obtained. This was six times the concentration of lindane in the nutrient solution. Tietz (1954) recorded a rapid accumulation of systox in the roots of *Vicia faba*, and Hacskaylo *et al.* (1961b) reported a similar rapid accumulation of phorate by roots of cotton plants from nutrient solutions. Lichtenstein *et al.* (1967) demonstrated that roots of pea plants grown for 26 days in lindane-treated sand (5 ppm) contained the insecticide at a concentration of 83 ppm, while the greens showed a concentration of 23 ppm. The rapid absorption and the magnification of lindane concentrations in roots of pea plants grown in treated nutrient solution seem to be associated with the inherent lipophilic nature of this chlorinated hydrocarbon insecticide, thus resulting in a rapid partitioning of lindane into the lipid components of the root epidermis. Beevers *et al.* (1952), Osborne *et al.* (1955), and van Overbeek *et al.* (1955) showed that the accumulation of organic compounds by cells increased as their polarity decreased. The translocation of insecticides into aerial plant parts, however, seems to increase with increasing polarity of the compounds (Reynolds and Metcalf, 1962). Although the major uptake of lindane by roots had occurred during the first 1–2 hr of exposure to the insecticide, a slower but constant increase in the translocation of lindane into the greens took place. Its concentration of 0.30 ppm in greens at the end of 1.5 hr had increased to 47.80 ppm at the end of 144 hr of growing in lindane-treated nutrient solution.

Series 4. The Effects of Lindane Concentrations in Nutrient Solutions on the Penetration and Translocation of the Insecticide into Peas. The previous experiment had been conducted with plants whose roots had been ex-

posed to lindane at a concentration of 5 ppm. To study the potential effects of increasing insecticide concentrations in the root environment, eight nutrient solutions were treated with lindane at 0.25, 0.5, 1, 2, 4, 6, 8, and 10 ppm. Fifteen-day-old plants (three groups of six plants each) were then grown for 24 hr in each of the nutrient solutions, when plants were harvested, extracted, and analyzed as described.

Results based on glc are presented in Figure 2. The penetration of lindane into roots appeared to be proportional to insecticidal concentrations up to 6 ppm, which comes close to the limit of the water solubility of lindane. The translocation of the insecticide into the greens, however, increased at a much slower rate when roots were exposed to increasing concentrations of the insecticide. The penetration and translocation of lindane, therefore, is to some extent also a function of the concentration of the insecticide in the root environment.

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Dissociation Constants of Succinic Acid 2,2-Dimethylhydrazide

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Dissociation constants were determined for succinic acid 2,2-dimethylhydrazide. The pK_1 and pK_2 were found to be 2.81 and 4.63, respectively.

Succinic acid 2,2-dimethylhydrazide (SADH) is an important plant growth retardant commercially available as Alar 85 and B-9. SADH is reported in the manufacturer's technical data sheet (Uniroyal, Inc., 1965) as a weak acid having an ionization constant of 1.12×10^{-5}. However, there is indication that SADH is bipolar in nature; namely, the solubility in water is enhanced by strong acids or bases, at acid pH SADH is retained by a strong acid cation exchange resin (Martin *et al.*, 1964), and hydrazides generally are monoacidic bases if the substituent does not contain a second nitrogen atom capable of being protonated (Gyenes, 1968). Further, preliminary studies indicated that the dissociation constant of the carboxyl group was considerably different from that reported.

Because of extensive studies underway on absorption of SADH requiring knowledge of the ionic nature of the molecule and the importance and extensive use of this chemical in research and commercial agriculture, a reassessment of the dissociation constant was essential.

EXPERIMENTAL SECTION

Apparatus. Dissociation constants were determined by potentiometric titration using a glass electrode (Schott & Gen., Mainz, Germany, S 30050-15 C) and a calomel reference electrode (Beckman 39402) in conjunction with a Beckman Century SS pH meter, calibrated in 0.01 pH units.

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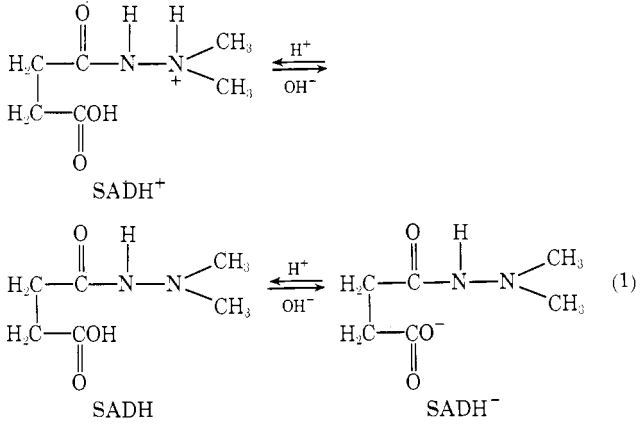
Chemicals. Technical grade SADH was Soxhlet extracted with acetone until a white solid precipitated. The precipitate was dissolved in hot isopropyl alcohol and cooled with magnetic stirring. The precipitate was filtered, air dried, and then vacuum dried at room temperature. Titration curves using this material coincided with those obtained with a reference sample (99.9% pure). C, H, and N analyses showed good purity. Theory: C, 44.99; H, 7.55; N, 17.49. Analysis: C, 44.82; H, 7.59; N, 16.38.

Procedure and Calculations. Titrations were carried out at 25 ± 1° in a nitrogen atmosphere. SADH concentration at half-neutralization was 0.01 *M*.

Dissociation equilibria may tentatively be written:



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