Absorption and Translocation of γ-BHC by Rice Plants*. Yutaka TSUKANO and Terumaro SUZUKI (National Institute of Agricultural Sciences, Nishigahara, Kita-ku, Tokyo). Received Feb. 1, 1962. *Botyu-Kagaku*, 27, 12, 1962

3. 稲による γ-BHC の吸収移行について 農薬の製剤に関する研究 II. 塚野 豊・鈴木照麿 (農業技術研究所) 37. 2. 1 受理

γ-BHC を含む水耕液を用い，この薬剤が稲の根を通じて内部へ吸収されるか否かを調べた．苗では 6.9p.p.m. の液で10日間の処理により，根部に 39p.p.m.，地上部に 12 p.p.m. の γ-BHC が見出され，幼穂形成期のものでは 4.0p.p.m. の液を用いて14日間処理したところ，茎・葉鞘部で 34p.p.m. 葉身部で 4.0p.p.m. の γ-BHC が検出された．水溶液の濃度をあげると吸収速度は上昇したが，溶媒，界面活性剤を用いて濃度を高めた液からの吸収は却って低かった．処理後，γ-BHC を含まない液で培養を続けると，葉鞘部の γ-BHC 量は減ったが，葉身部では増加した．以上の結果からみて，γ-BHC は稲の根を通じて茎・葉部へ吸収移行され得るものと考えられる．

Introduction

During recent years, a number of studies have been made on the absorption and translocation of γ-BHC (γ-hexachlorocyclohexane) by plants. In 1950, Starnes[1] reported that both aqueous suspensions and benzene extracts of the potato foliage and tubers were highly toxic to *Aedes* larva when the plants were grown in soil containing the γ-isomer of benzene hexachloride. Similar effects were found in various plants against different insects by Kozlova and Dvortzova[2], Ehrenhardt[3], Koehler and Gyrisco[4], and others. Haines[5] observed the presence of lindane in the guttated liquid as well as in the plant tissues of corn plants which had been cultured in a nutrient solution containing this insecticide, while Lilly and Fahey[6] found BHC in the seeds of various plants grown in the soil treated with this chemical. Quantitative studies were carried out by Bradbury and Whitaker[7] and Lichtenstein[8]. The former group showed that wheat seedlings removed up to 100μg./g. (fresh weight) of γ-BHC in seven days from the aqueous solution bathing their roots.

In relation to the application of γ-BHC for the control of the rice stem borer, *Chilo suppressalis* Walker, much attention has been paid in Japan to the behavior of this insecticide in and on the rice plant. Koshihara and Okamoto[9] reported that the treatment of paddy soil with γ-BHC at the time of puddling before the transplantation of rice seedlings was effective for the control of the insect at the first brood. They claimed that this effect had resulted from the absorption and translocation of the toxicant through the roots. Horiguchi[10] observed a similar effect, but he attributed the effect mainly to the insecticide-containing water between the leaf sheaths which had been brought up there as the capillary effect and was concentrated due to the evaporation of water. Ishii and his coworkers[11] reported that radioactive materials were found only on and near the roots of a rice seedling when the roots were exposed to C^{14}-labeled γ-BHC emulsion. They, therefore, concluded that this compound was not easily translocated into other parts of the rice plant.

During the course of studies on the spray formulation of γ-BHC for the control of the rice stem borer, it was suggested that the absorption and translocation of this insecticide may take place in the rice plant, resulting in prolonged persistence and increased effectiveness. While a number of workers had already proved the uptake of γ-BHC by various kinds of plants, positive evidence had not yet been shown of the absorption and translocation of this chemical by the rice plant. It seemed, to the authors, that the different results and explanations mentioned above should be attributed to the difference in the experimental conditions.

The present paper deals with the studies which were undertaken to examine the uptake of γ-BHC by the rice plant. The root application in

*) Formulation of Pesticides. II.

water culture was chosen, as the first step, because of the simplicity of experimental techniques, though the leaf surface seemed to be more important as a possible pathway of the sprayed insecticide.

Materials and Methods

(1) γ-BHC Solutions.

Several kinds of γ-BHC solutions were prepared and used. Mineral nutrients were added to all the solutions. Solutions II and III were prepared by vigorously agitating the suspension of the insecticide in water followed by filtration, while Solution IV contained organic supplements. The concentrations were determined chemically.

(a) Solution I. This solution was prepared by diluting Solution III with an equal volume of water reducing the concentration to 4.0μg. of γ-BHC per ml.

(b) Solution II. An aqueous solution containing 6.9μg. of γ-BHC per ml.

(c) Solution III. An aqueous solution containing 8.0μg. of γ-BHC per ml.

(d) Solution IV. This was prepared by pouring a solution of the following composition under vigorous agitation into 20 l. of water: 200 mg. of lindane, 2.0 g. of Emasol 1130 (a commercial surfactant), and 20 ml. of acetone.

(2) The Determination of γ-BHC Content.

The determination of γ-BHC content was carried out by the Schechter-Hornstein method[12].

(3) Uptake of γ-BHC by Rice Seedlings.

Rice seedlings were grown in a nutrient solution and kept in a greenhouse. When they were 36 days old, the nutrient solution was replaced by Solution II. Precautions were taken to avoid contamination of the aerial part with the solution. Each of 30 seedlings, some after 4 days and others after 10 days, were removed from the solution, washed with water, and divided into two parts, i. e., the aerial part and the root. Each part was weighed, macerated in a blendor with anhydrous sodium sulfate and chloroform, and extracted in a Soxhlet apparatus. The extract was then treated with fuming sulfuric acid to remove interfering substances. The volume of the resultant solution was then recorded and aliquots were taken for analysis.

(4) Uptake of γ-BHC by Rice Plants at the Head Forming Stage.

Rice seedlings which had been grown in a seedling bed were transferred to pots filled with a nutrient solution. Three seedlings were placed in a pot, where they were held in a pebble-packed bamboo basket. Seventy days after transplantation, the nutrient solution was replaced by Solution I. The pots were covered with polyethylene tubes to reduce the evaporation of water, and were kept from rain. The solution was renewed every two days during the treatment (Figure 1).

Figure 1. Rice plants at the head forming stage which were under treatment with γ-BHC solution.

At two day intervals, each of three plants were removed from the solution and washed thoroughly with water. After being dried in the air, the plants were sampled leaving only the roots and the lowest 5 cm. portions of the aerial parts. Each sample was then divided into two parts, i. e., the leaf blade and the remainder. The latter will be referred to as the stem and sheath hereinafter. Each part was weighed, cut into pieces, macerated with methylene chloride and anhydrous sodium sulfate and extracted in a Soxhlet apparatus. The elimination of interfering substances and the determination of γ-BHC content were carried out in the same way as described in the preceding section.

The average recovery from the stems and sheaths was 90 percent, while that from the blades was 84 percent.

(5) Effect of Differences in the γ-BHC Concentration.

. Rice plants at the head forming stage were treated similarly, some with Solution III and others with Solution IV. Samples were taken after 2 and 4 days. The extraction, the elimination of interfering substances, and the determination of γ-BHC content were carried out in the same way as described above.

(6) Changes in γ-BHC Content after the Treatment.

After being treated for 4 days with the solutions, rice plants at the head forming stage were cultured for an additional 10 days in a nutrient solution without the insecticide. The extraction, the elimination of interfering substances, and the determination of γ-BHC content were carried out in the same way as used previously.

Table 1. Uptake of γ-BHC by rice seedlings from the aqueous solution, 6.9 μg. per ml.

Exposure	Aerial parts			Roots		
	Fresh weights*	Total amounts*	Amounts per unit weight	Fresh weights*	Total amounts*	Amounts per unit weight
Days	g.	μg.	p. p. m.	g.	μg.	p. p. m.
4	0.38	1.7	4.5	0.24	2.9	12
10	0.35	4.3	12.5	0.22	8.5	39

*) These figures are the average per seedling of the results obtained.

Table 2. Uptake of γ-BHC by rice plants at the head forming stage from the aqueous solution, 4.0 μg. per ml.

Exposure	Stems and sheaths			Blades		
	Fresh weights*	Total amounts*	Amounts per unit weight	Fresh weights*	Total amounts*	Amounts per unit weight
Days	g.	μg.	p. p. m.	g.	μg.	p. p. m.
1	48	60	1.3	37	9	0.2
2	51	380	7.5	35	14	0.4
4	56	780	14.0	42	31	0.9
6	62	1250	19	36	37	1.0
10	66	1750	26	34	66	2.0
14	58	2070	34	32	126	4.0

*) These figures are the average per plant of the results obtained.

Table 3. Effect of differences in the concentration.

Solution	Concentration	Exposure	Stems and sheaths			Blades		
			Fresh weights*	Total amounts*	Amounts per unit weight	Fresh weights*	Total amounts*	Amounts per unit weight
	p. p. m.	Days	g.	μg.	p. p. m.	g.	μg.	p. p. m.
I	4.0	2	51	380	7.5	35	14	0.4
I	4.0	4	56	780	14.0	42	31	0.9
III	8.0	2	51	660	13.0	—	—	—
III	8.0	4	54	1100	20	40	28	0.7
IV	20	2	48	460	9.7	—	—	—
IV	20	4	50	630	19	36	30	0.3

*) These figures are the average per plant of the results obtained.

Results

The results of the treatment of rice seedlings are recorded in Table 1.

The uptake of γ-BHC by rice plants at the head forming stage is shown in Table 2.

The effect of differences in the γ-BHC concentration are presented in Table 3.

The changes in γ-BHC content after the treatment are given in Figure 2.

In all cases, two series of tests were conducted, and duplicate analyses were carried out for each sample. The mean standard deviation was 11 percent.

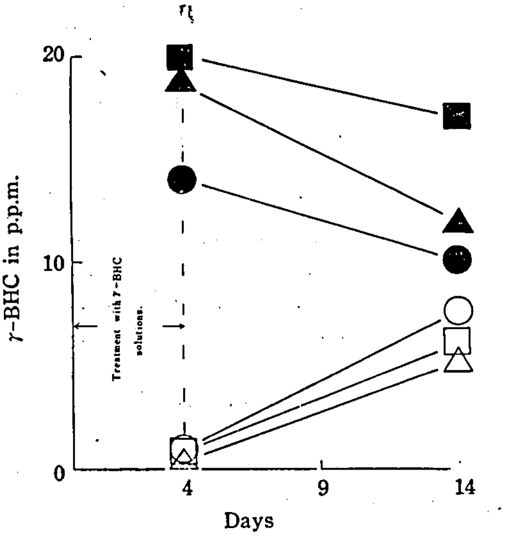

Figure 2. Changes in γ-BHC content after the treatment.

After 4 days' treatment with γ-BHC solution, rice plants were cultured for an additional 10 days in a nutrient solution without the insecticide.

Solid symbols represent the content in the stems and sheaths, and open symbols show those in the blades. Circles represent results from the treatment with Solution I, squares with Solution III, and triangles with Solution IV.

Discussion

The results given in Table 1 show that rice seedling roots absorbed γ-BHC when they were exposed to the aqueous solution. It is also evident from Tables 1, 2 and 3 that the insecticide was transferred from the aqueous solution to the aerial parts of both the seedlings and the plants at the head forming stage grown in the culture solution containing the insecticide. These results seem to give a positive answer to the question whether or not γ-BHC is actually absorbed and translocated by the rice plant.

In these experiments, precautions were taken to keep the sheaths above the water surface, so that only the roots were exposed to the solution. It is, therefore, unlikely that the insecticide found in the aerial parts was derived by direct contact from the culture solution. It would also be difficult to attribute the presence of the insecticide in these parts to the volatilization and subsequent adsorption by the plant surface. Except for the experiment with the seedlings, the plants were kept outdoors under well-aerated conditions. Under these circumstances, only a small amount of the toxicant would have been adsorbed, even if a considerable amount had evaporated from the solution.

The absorption by the roots and subsequent translocation probably by the transpiration stream, therefore, seems to be the major course, if not the only one, which accounts consistently for the existence of the insecticide in the aerial parts.

It can be seen from Table 1 that rice seedlings, weighing 0.57 g., contained 12.8 μg. of γ-BHC after 10 days' exposure to the aqueous solution of 6.9 μg. per ml. This value corresponds to the concentration of 22.5 μg. per g. of fresh weight. It is also seen from Table 2 that the insecticide content reached 18 μg. per g. of fresh weight in the aerial parts of the rice plants after 10 days' treatment of the roots in the aqueous solution of 4.0 μg. per ml.

It has been pointed out that the rate of uptake varies with different crops, at different stages, and under different treatment conditions. Though there is no great significance in comparing the content with those in other plants, those values calculated are found to be somewhat lower than those obtained with 10 day old wheat seedlings by Bradbury and Whitaker[7], who showed that the γ-BHC content in the seedlings was

66 μg. per g. of fresh weight after 9 days' exposure.

As shown in Tables 2 and 3, the insecticide content increased rapidly in the stems and sheaths, while it increased at much slower rates in the blades. These facts suggest that some of the absorbed insecticide was held during translocation. Volatilization from the blade surface might have been partly responsible.

Table 3 shows the effect of differences in the concentration. The insecticide was taken up more rapidly from the aqueous solution of higher concentration, but the treatment with Solution IV resulted in slower uptake, though the insecticide concentration was higher in this solution than those in the others. Solution IV contained the insecticide beyond the solubility in water with the aid of supplements. The presence of these supplements may have lowered the efficiency of the uptake because of the phytotoxic effect.

It is shown in Figure 2 that the γ-BHC content decreased in the stems and sheaths but increased in the blades during 10 day period of additional culture with the nutrient solution, which followed the treatment with the insecticide solution. These facts may well be explained by assuming that the insecticide already present in the stems and sheaths at the end of the treatment moved into the blades during the 10 day period, while the translocation from the roots was much less.

From these results, it may be concluded that the rice plant can absorb and translocate γ-BHC, the amount depending upon various conditions.

Methods are now being developed in Japan for applying γ-BHC in the soil and on the water surface of paddy fields for the control of the rice stem borer. Further studies seem to be necessary on the uptake of this insecticide through the roots, since this process seems to be involved when these application methods are used.

Summary

Studies were conducted on the absorption and translocation of γ-BHC by the rice plant through the root. The roots of rice seedlings contained 39 p.p.m. of γ-BHC after they had been exposed to the aqueous solution of 6.9 p.p.m. for 10 days, while 12 p.p.m. of the insecticide was found in the aerial part of the seedlings. Rice plants at the head forming stage took up the insecticide steadily from the aqueous solution through their roots, the concentrationsbeing 34 p.p.m. in the stems and sheaths and 4.0 p.p.m. in the blades after 10 days' exposure of their roots to the aqueous solution of 4.0 p.p.m. The insecticide was absorbed in higher rates from an aqueous solution of higher concentration, but the presence of acetone and a surfactant in the solution lowered the uptake. The γ-BHC content in the stems and sheaths decreased during the 10 days after the exposure, while much increase was observed in the content in the blades. From these results, it was concluded that the rice plant can absorb and translocate γ-BHC through the root system.

Literature Cited

1) Starnes, O. : *J. Econ. Entomol.* **43**, 338(1950).
2) Kozlova, E. N. and Dvortzova, E. I. : *Doklady Vsesoyuz. Akad. Sel'skokhoz. Nauk im V. I. Lenina* **17**, 41 (1952); cf. *Rev. Appl. Entomol.* **41**, 293 (1953).
3) Ehrenhardt, H. : *Anz. Schädlingskunde* **27**, 1 (1954).
4) Koehler, C. S. and Gyrisco, G. G. : *J. Econ. Entomol.* **50**, 346 (1957).
5) Haines, R. G. : *ibid.* **49**, 563 (1956).
6) Lilly, J. H. and Fahey, J. E. : *ibid.* **49**, 815 (1956).
7) Bradbury, F. R. and Whitaker, W. C. : *J. Sci. Food Agr.* **7**, 248 (1956).
8) Lichtenstein, E. P. : *J. Agr. Food Chem.* **7**, 430 (1959).
9) Koshihara, T. and Okamoto, D. : *Nippon Oyo Dobutsu-Konchugaku Kaishi* **1**, 32 (1957); Okamoto, D. and Koshihara, T. : *Shokubutsu Boeki* **13**, 243 (1959).
10) Horiguchi, H. : *Shokubutsu Boeki* **14**, 165 (1960).
11) Ishii, S., Enjoji, S. and Sekiguchi, K. : *Botyu-Kagaku* **24**, 184 (1959).
12) Schechter, M. S. and Hornstein, I. : *Anal. Chem.* **24**, 544 (1952).