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**Testing Laboratory Methods of Agricultural Chemicals against Injurious Insects in Soil.** Researches on the Wireworm, *Melanotus caudex* Lewis. XI. Masayoshi YOSHIDA & Yasunori SUZUKI (Laboratory of Applied Entomology, Faculty of Agriculture, Shizuoka University, Iwata, Shizuoka, Japan). Received June 30, 1958. *Botyu-Kagaku* **23**, 107, 1958.

**20. 土壌害虫に対する農薬の室内試験法.** ハリガネムシに関する研究 第11報 吉田正義・鈴木康徳（静岡大学農学部 応用昆虫学研究室）33. 6. 30 受理

木材粉を利用したハリガネムシの飼育装置に一定量の乳剤，ガス剤および粉剤を含有せしめ，相当長期間に亘る農薬の累積的殺虫効果を調べる土壌害虫に対する農薬の室内試験法を考案したのでこれを紹介すると共に若干農薬の特性について報告した.

## Introduction

Testings of insecticide against soil insects have been carried out with the end result of yield of crops, when applying or not applying. In this case, the result may be caused by two mingling factors, repellency and toxicity of the applied chemical. For the yield of crop differs in every year due to climatic factors, kind of soils and biological factors and so on, it is difficult to estimate the effectiveness of insecticide by this method. Since the large-scale field test has some faults as mentioned above, the small-scale testing method in a laboratory is urgently needed under same conditions as in soil.

Dusting and dipping methods are now generally used to test insecticides, but in the case of soil insects these method cannot be used, for the residual toxicity must be taken into consideration in great deal. The method is bound to put given quantity of insecticide into the soil where the insects live. But there are several difficulties in this method as following : the insects cannot live in the soil transferred to the laboratory due to the decrease of water content of soil and the changes of the microorganisms in soil.

In upland farm soil, moisture content is comparatively constant since its water maintenance is kept by capillarity, and this condition lets soil insects live in optimum life. Accordingly, to maintain water content of soil in the laboratory, water must be showered. But by this showering the soil turns solid and worms cannot survive. On the other hand, the low abilities of water maintenance of soil hinder to contain certain concentrations of insecticides. When the authors bred wireworms in the soil taken into the laboratory, microorganisms increased suddenly; its degree of increase was high when moisture contents of soil was high. Enormous multiplication of microorganisms in soil brings the worm to death; soil sterilization is necessary to test the agricultural chemicals in the soil. By this sterilizing procedure humus contained in soil burns and its water maintenance becomes difficult. To solve these difficulties mentioned above, the authors used sawdust as a substitute of soil, of which water-holding capacity is large and which is easily sterilized in autoclave. As for soil insecticides, in particular, the stability of their chemical composition and the residual effectiveness are important. By the usual methods mentioned above it is difficult to appraise exactly effectiveness of the chemicals as soil insecticide. To find out effective soil insecticides, the authors applied dusts, emulsions and gas agents now on the market for the method concerned.

## Materials and Methods

The test insects, larvae of *Melanotus caudex* Lewis (size : 1.5—2.0 cm), were collected at the experiment farm (Shinbara & Iwata, Shizuoka Pref.) in June and October 1957, in which seasons the worm is living near the soil surface.

The insecticides used were as follows:

(1) emulsions: Lindes (Lindane 10 %)

(2) gas agents: Nemafume 20, Dowfume 40, Dowfume W 85, Fumazone, Telone and Dorlone

(3) dusts: Heptachlor (2.5%), Ardrin (4%)

The breeding of wieworm:

The authors investigated the reason why wireworms cannot live in the air, exposing them in several conditions of humidity, and found that their body water evaporates through their body wall in such a way as water vapour from the water surface does. Stigmata and wounds on integument have generally been believed as evaporation positions, while the authors believe that another part is operative for this role, for the evaporation speed is constant and great at different humidities.

The authors, after dipping some kinds of wireworm (*Melanotus caudex, Melanotus senilis, Lacon fulinosus*) and *Anomala rufocuprea* into ammoniacal silver solution, found that the deoxidated parts on their integument turned to black. Two reducing layers clearly stained with black were found at sterna of abdomen longitudinally along the basal line, and also at the base of legs in sterna of thorax. The staining speed and the size of stained places are in accord with the evaporation speed and the degree of evaporation; those parts concerned, which are presumably polyphenol layers, are considered to be connected with the evaporation site and the respiration of wireworms.

The average mortality under the rearing conditions of different combinations of temperature and moisture content of sawdust were examined, and it was found that the rearing in the sawdust (50 g) in a large-mouthed bottle (500 cc) with sterile water (100 cc) at 20° was optimum for wireworm. The sawdust used in this test was boiled for several hours after washing out impurities contained therein, dried by wind and sieved with 1 mm mesh.

As regards the water capacities of sawdust and Kuroboku soil, the comparison was given in Table 1. The water capacity of the former was septuple as that of the latter.

Test of insecticides:

(A) Emulsions

In this experiment the large-mouthed bottles (500 cc), containing sawdust (50g), were plugged with cotton, and sterilized. Sterile water (100 cc) with insecticide of certain concentrations were sprayed into sawdust bottles uniformly. After the content of bottle was agitated with a glass rod and tamped to the definite height, five wireworms were put in each of them.

(B) Dusts

After mixing chemical dusts with sawdusts, sterile water was added. Successive manipulations were made as mentioned above.

(C) Gas agents

After damping and tamping sawdust in a bottle as in the case of emulsion, a certain gas agent was injected by a pipette into a hole previously made at the center of sawdust. After levelling its surface, test worms were put in.

These experiments were carried out in the incubator regulated at 20°, optimum for wireworm living and unsuitable for microorganism multiplication, thus the sterilization of sawdust may be meaningless, under this experimental condition.

Discrimination of living or death of test wireworms was made by Yoshida-Mino method (1954), examining their digging abilities into soil.

Table 1. Water capacities of soil and sawdust.

| Division of water capacity | | Kuroboku soil | Sawdust |
|---|---|---|---|
| Weight (%) | Rough | 71.18 | 496.64 |
| | Dense | 60.94 | 417.17 |
| Volume (%) | Rough | 58.64 | 92.86 |
| | Dense | 58.04 | 93.53 |

### The Mortalities of Wireworm under Different Concentrations of Lindes Emulsion

According to the preliminary experiments, 100% and 0% mortalities were obtained at 1/500—1/70000 dilution of Lindes, and the authors carried out experiments within these limits two or four times repeatedly, to estimate the mortalities at each concentration.

On the 1st, 5th, 10th, 20th and 40th day after the treatment, the average mortalities of wireworms at each concentration were given in Table 2. Taking the logarithms of concentration on X axis, worm's mortalities on Y axis, sigmoid curves were obtained as shown in Fig. 1. LD-50 was calculated by the probit method of Bliss (1935) to compare the cumulative effectiveness shown by each curve. The value of LD-50 increased in a straight line from the 1st day till the 20th after the treatment, while on and after the 20th day this value did not change much. The dosage-mortality curve on the 20th day after the treatment is very like that on the 40th day: in other words, living worm and dead one were able to be discriminated clearly on and after the 20th day after the treatment.

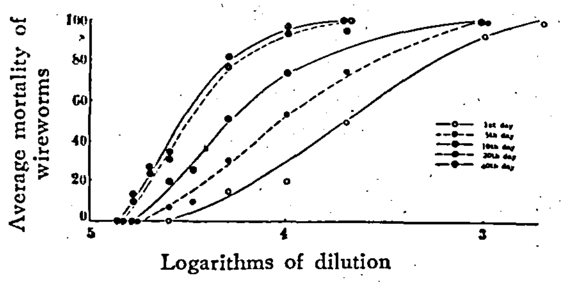


Fig. 1. Relations between the average mortality of wireworms treated with Lindes solution and the logarithms of dilution at 20°.

### Comparison of Effectiveness of Lindes Solution in the Case of Present Method and the Dipping Method

In order to compare the results obtained by the both methods, usual method of dipping into certain solutions of Lindes for 5 min. was made, and the mortalities of wireworms on the 4th day after the treatment were examined, results are shown in Table 3. According to Table 3, LD-50 of Lindes by the dipping method was 1/1220 dilution. But, by the present method which breed wireworms in the sawdust mixed with Lindes emulsion, LD-50 was about 1/30000 dilution, namely 1/24 time as that of dipping. Expecting the residual effectiveness of insecticides, the

Table 2. Average mortalities of wireworms at each Lindes dilution.

| Days after treatment \ Dilution | 1/500 | 1/1000 | 1/5000 | 1/10000 | 1/20000 | 1/30000 | 1/40000 | 1/50000 | 1/60000 | LD-50 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 100% | 93.3% | 50.0% | 20.0% | 15.0% | —% | 0 % | % | % | 1/4902 |
| 5 | | 100 | 75.0 | 53.3 | 30.5 | 10.0 | 6.7 | — | 0 | 1/10352 |
| 10 | | 100 | 95.0 | 73.8 | 51.3 | 25.8 | 20.0 | — | 0 | 1/19048 |
| 20 | | | 100 | 93.3 | 77.5 | — | 31.5 | 27.3 | 10.5 | 1/29155 |
| 40 | | | 100 | 96.7 | 81.8 | — | 35.0 | 25.5 | 13.3 | 1/32787 |

Table 3. Average mortalities of wireworms caused by dipping in each dilution of Lindes.

| Dilution | 1/100 | 1/200 | 1/400 | 1/800 | 1/1600 | 1/3200 | 1/6400 | 1/12800 | 1/25600 | LD-50 |
|---|---|---|---|---|---|---|---|---|---|---|
| Mortality | 100% | 80% | 65% | 65% | 45% | 40% | 30% | 5% | 0% | 1/1220 |

Table 4. Results (Lindes emulsion, at 20°) compared in the case of sawdust and Kuroboku soil on the 10th day after the treatment.

| Dilution | 1/1000 | 1/5000 | 1/10000 | 1/20000 | 1/30000 | 1/40000 | 1/50000 | LD-50 |
|---|---|---|---|---|---|---|---|---|
| Kuroboku soil | 100% | 100 % | 100 % | 90 % | 73.3% | 53.3% | 33.3% | 1/40552 |
| Sawdust | 100 | 95.0 | 73.8 | 51.3 | 25.8 | 20.0 | 0 | 1/19055 |

diluted concentration was enough for the present purpose.

### Comparison of the Effectiveness of Lindes on Sawdust and Kuroboku Soil

To breed the worm under 20°, it was necessary to sterilize sawdust or soil for prevention of microorganisms. The diminution of soil water was remarkable in the case of Kuroboku soil, the soil could not be used when more than ten days had passed after the treatment. This fact was remarkable as temperature ascended.

Both results (Lindes emulsion, at 20°) in the case of sawdust and Kuroboku soil on the 10th day after the treatment were shown in Table 4. LD-50 in the cases of Kuroboku soil breeding and sawdust one were 1/40552 and 1/19055 dilution, respectively; the wireworm's resistivity in the case of the soil was half as that of sawdust. Because the water-containing capacity of sawdust is greater than that of soil, Lindes is more in amount in the former than in the latter; but wireworm's resistivity was weak in the latter case than in the former breeding. It was considered that the reason is due to the diminution of soil water.

### Tests of Gas Agents against Wireworms

Using the present method, relations between the concentration of each gas agent and the average mortality were examined; the results in the case of corked or uncorked bottles on the 8th day after the treatment were shown in Table 5. According to Table 5, the ranking in the effectiveness of gas agents agreed with the orders of respective quantities of ethylene dibromide and dichloropropene in both cases. At the corked division the toxicity of dichloropropene slightly exceeded that of ethylene dibromide, but the ranking of the effectiveness against wireworm reversed at the uncorked one. In the case of field application of insecticides, Dowfume W 85 seems to be more efficient than Telone.

### Tests of Chemical Dusts against Wireworms

For an example of dust testing, average mortalities of wireworms against dusts of Ardrin and Heptachlor on the 25th day after the treatment at 20° were given in Table 6. Taking the logarithms of concentrations on X axis, the mortalities on Y axis, the wireworm's resistivity to certain chemical dusts were considered to be shown by a cumulative curve of normal distribution, as in the case of Emulsion. The values (LD-50) of Heptachlor and Ardrin showed 0.0062 g and 0.0060 g respectively: the toxicities (cumulative effectiveness) of both chemical dusts were similar.


### Summary

(1) In order to test the cumulative effectiveness of insecticides against soil insects, the authors made breeding equipments of wireworms (*Mela-*


Table 5. Comparative effectiveness of gas agents in LD-50, either uncorked or corked.

| Material and rate | LD-50 (uncorked) | Ranking | LD-50 (corked) | Ranking |
|---|---|---|---|---|
| Dowfume W 85 (ethylene dibromide 82.7%) | 0.0159cc | 1 | 0.00442cc | 3 |
| Dowfume 40 (ethylene dibromide 41.0%) | 0.0252 | 3 | 0.00750 | 4 |
| Nemafume 20 (ethylene dibromide 20.7%) | 0.0832 | 5 | 0.02120 | 5 |
| Fumazone (1.2 dibromo-3-chloropropene 47.6%) | 0.7500 | 6 | 0.09620 | 6 |
| Telone (1.3 dichloropropene 100%) | 0.0182 | 2 | 0.00333 | 1 |
| Dorlone (Mixture of D. f. W 85 :Telone=1 : 5) | 0.0315 | 4 | 0.00376 | 2 |

Table 6. Average mortalites of wireworms treated with certain dusts on the 25th day after the treatment at 20°.

| Concentration | 0.001g | 0.003g | 0.005g | 0.008g | 0.010g | 0.015g | 0.020g | LD-50 |
|---|---|---|---|---|---|---|---|---|
| Heptachlor | 0% | 10.0% | 40.0% | 60.0% | 80.0% | 90.0% | 100.0% | 0.0062g |
| Ardrin | 0 | 6.7 | 33.3 | 70.0 | 86.7 | 100.0 | 100.0 | 0.0060 |

*notus caudex*) using sawdusts, as a substitute for soils. Sawdust has high water-holding capacity, easily sterilized, and it is useful for testing methods of insecticide of various modes, emulsions, gas agents and dusts.

(2) Emulsions : the large-mouthed bottles (500 cc), containing sawdust (50 g), were plugged with cotton and sterilized. Sterile water (100 cc) was poured for keeping certain moisture content and added insecticides of the solutions of certain concentrations. After the bottles were agitated and tamped to the same height, five wireworms were put into each of them.

(3) Dusts : after mixing chemical dust with sawdusts, sterile water was added and the same manipulations were made as mentioned above. Gas agents : after damping and tamping sawdust in a bottle as in the case of emulsion, a certain gas agents was put by a pipette into a hole previously made at the center of the bed of sawdust; after levelling its surface, test worms were put in.

(4) These experiments were carried out in the incubator at 20°, optimum for wireworm and unsuitable for microorganism, thus sterilization of sawdust is meaningless at this condition.

(5) LD-50 of Lindes increased in a straight line from the 1st day till the 20th after the treatment, while on and after the 20th day these values did not change much. The sharp distinction between living worm and dead one was seen on and after the 20th day after the treatment.

(6) LD-50 of Lindes by the dipping method was 1/1220 dilution. When wireworms were bred in the sawdust mixed with Lindes emulsions, its LD-50 was about 1/30000 dilution, namely 1/24 time as in the case of dipping. Expecting the residual effectiveness of insecticides, the diluted concentration was enough for the purpose.

(7) The wireworms' resistivity (Lindes, at 20°, on the 10th day after the treatment) in the soil was half as that in sawdust; it was considered that the reason is due to the diminution of soil water.

(8) The ranking of gas agents effectiveness agreed with the orders of respective quantities of ethylene dibromide and dichloropropene in both cases. At the corked division the toxicity of dichloropene slightly exceed that of ethylene dibromide, but the ranking of the effectiveness against wireworms reversed at the uncorked one.

(9) LD-50 of Heptachlor and Ardrin on the 25th day after the treatment showed 0.0062 and 0.0060 g respectively : the toxicities (cumulative effectiveness) of both chemical dusts were the same.

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**On the Several Ecological Problems of the Breeding of Larvae of the Common Housefly, *Musca domestica vicina*.** Analysis of Ecological Factors in Biological Assay of Insecticide. II. Haruhisa UENO (Wakayama Prefectural Institute of Sanitation). Received July 3, 1958. *Botyu-Kagaku* **23**, 113, 1958 (with English résumé 115).

## 21. 実験用イエバエ *Musca domestica vicina* 幼虫の飼育についての二三の知見 殺蛆剤の効力試験における生物学的要因の解析 第2報

上野晴久（和歌山県衛生研究所） 33. 7. 3 受理

実験用に最適なイエバエ幼虫の飼育条件，浸漬法を用いて効力試験をする場合の浸漬処理そのものの影響，異常蛹の蛹期間中の体重の変化等イエバエを用いて薬剤の効力試験をする時に生ずる 2, 3 の問題について，実験考察を加えた。

### ま え が き

種々の殺蛆剤の効力試験としてイエバエ（*Musca domestica vicina*）の幼虫はかなり広く使用されており，その飼育法については Basden[1]，Hafez[2]，