DERRIS DEPOSITS
Fate of Deposits on Young Bean Plants

C. PAGÁN and M. P. MORRIS
Federal Experiment Station, U. S. Department of Agriculture, Mayaguez, Puerto Rico

The stability of derris deposits was investigated to determine the duration of protection from insect infestation of plants treated with derris preparations. It was previously considered that absorption and translocation of the rotenoids gave protection from insect infestation up to 30 days. The study showed that residues of latex emulsions, derris powders, and water suspensions of derris powders lose their toxicity almost completely within 10 days. This loss in toxicity is due to decomposition or other chemical changes on the surface of the treated plants, and not to absorption and translocation. The absence of absorption and translocation should be of interest to food processors who are concerned with the off-flavors often encountered after the use of certain types of insecticides.

THE ADVENT AND EXTENSIVE COMMERCIAL UTILIZATION of the new systemic insecticides introduced new problems for food technologists. Formerly, foodstuffs could be processed after a simple washing, which removed any adhering insecticidal residue. Where systemic insecticides have been used, the usual washing process does not remove the material absorbed by the plant. This problem is of special interest to processors of canned foods, who consider the presence of mere traces of insecticides as undesirable. This high regard for the safety of the consumer has limited the use of some organic phosphorus compounds, particularly in vegetable production for the processing industry. Any insecticide which has "systemic properties" must therefore be given special consideration by both the entomologist and the food technologist.

During recent investigations on the commercial possibilities of derris latex it was necessary to determine the extent to which the rotenoids (the toxic principles of derris) were absorbed and translocated. Measurable quantities were anticipated, as several investigators have stated that the rotenoids are absorbed and translocated (*1, 3, 4*).

Toxicity of Derris Residues to Guppies

Guppies (*Lebistes reticulatus* Peters) were used in the biological assays of this investigation. These readily available and easily maintained fish are sensitive to a few micrograms of the rotenoids per liter of test solution. Their use permitted the detection of minute quantities of rotenoids which could not be detected by the common insect-feeding tests. The data obtained with the guppies were highly reproducible, and have been correlated with those obtained on houseflies and Mexican bean beetle larvae (*6*).

Seeds of a native variety of *Phaseolus vulgaris* L. were planted in fertile soil in 4-inch clay pots, and 10 days after germination 100 of the most uniform seedlings were selected for treatment. The upper surfaces of the two primary leaves and of the first set of trifoliate leaves of 50 plants were painted with the expressed, undiluted juice of fresh derris roots (*Derris elliptica* var. Changi III), using 3.6 ml. of latex per 10 plants. The remaining 50 plants were kept as controls. Treated and control plants were kept in a greenhouse during the experiment.

Immediately after the rotenone-bearing latex had dried on the leaves, 10 treated and 10 control plants were selected for analysis. The leaves were detached, weighed, and then ground in a Waring blender with 150 ml. of distilled water. The suspensions of ground material were filtered through a plug of cotton and diluted to 200 ml. One milliliter of each solution, representing 160 mg. of fresh bean tissue, was dispersed in 1 liter of water and tested for toxicity. Each test solution was replicated three times using 10 fish per replication. Pure rotenone was used as a standard of comparison in all tests. An average of 1.5 mg. of rotenone per gram of fresh bean tissue was recovered from the treated plants, indicating that 48 mg. of rotenone or its equivalent had been applied to the leaves of each set of 10 plants.

Five days after treatment another set of control and treated plants was harvested for analysis. The new leaf growth that had developed during that period was analyzed separately from the treated portion. The samples were washed carefully with water before extracting as described above. The washings from the treated leaves were highly toxic to guppies. However, both the washed, treated leaves and the new growth from treated plants failed to show toxicity to guppies. This suggested that the toxic components could at this time be washed completely off the plants and indicated that any fraction absorbed by the leaves was necessarily less than 0.002% of the plant material.

Ten days after treatment the leaves from another group of control and treated plants were sampled and analyzed in the same way. This time the concentration in the aliquot was increased 30 times. The treated leaves, after thorough washing, showed 2.0% of their original toxicity. This toxicity was probably due to strongly adsorbed or

possibly absorbed rotenoids, as it could not be removed by washing with water. The new growth of the treated set showed no toxicity. Since the lower limit of sensitivity of the fish was approximately 0.05 p.p.m. of rotenone, about 0.001% of rotenone in the plant material would have been detected under the conditions of this test. This indicated that any translocation of toxic materials to the new leaves was negligible. (Table I.)

In a second experiment 10 grams of dried, powdered derris roots was suspended in 100 ml. of water. The stems, primary leaves, and the first trifoliate leaves of bean seedlings selected for uniformity were painted with 4.5 ml. of this dispersion per set of 10 plants. Biological assay showed 45 mg. of rotenone or its equivalent in 4.5 ml. of derris suspension. The plants were harvested and sampled as in the previous experiment, dried at 50° C., ground to pass an 80-mesh screen, and extracted with acetone. Analyses of the acetone extracts showed that the treated portions possessed approximately 6% of their original toxicity. Again the new growths showed no toxicity. Chemical analysis for rotenone and related compounds with a modified red color test (*5*) gave positive results only on the treated leaves (Table II).

A third experiment was carried out using pure rotenone dispersed in water at a concentration of 0.2%. No toxicity could be detected either in the treated leaves or new growth of the bean plants 5 days after application of the material. Negative results were also obtained from analyses of bean plants which were watered on alternate days with a suspension of either derris powder or rotenone. When suspensions were applied to the underside of the primary and first trifoliate leaves, the new growth was likewise found to be nontoxic.

Theoretically, the loss of toxicity of leaves treated with derris root extracts may be due to the decomposition of the toxic components, the absorption and translocation of toxic material, or a combination of both processes. Rotenone and related compounds may also be absorbed into the plant and metabolized into nontoxic constituents. However, the very low water solubility of these compounds makes their absorption and translocation in large amounts improbable. Even in plant genera like *Derris* and *Lonchocarpus* which synthesize rotenone, this compound is found exclusively in the lower stem and roots and then only in separate specialized cells (*7*).

Table II. Toxicity of Acetone Extracts of Leaves from Young Bean Plants Treated with a Water Suspension of Powdered Derris Root[a]

Sample Analyzed	Weight of Dry Sample, G. In 10 plants	Weight of Dry Sample, G. Tested[b]	Mortality of Guppies, %	Rotenone Recovered from 10 Plants, %
Five days after treatment				
Control plant leaves	3.73	0.06	0	0
Treated plants				
Treated leaves	1.67	0.06	46.7	6.3
New growth, leaves	2.42	0.12	0	0
Ten days after treatment				
Control plant leaves	8.34	0.16	0	...
Treated plants				
Treated leaves	2.06	0.16	80.0	5.6
New growth, leaves	6.75	0.32	0	0
Rotenone standards				
0.05 p.p.m.	...	...	12.0	...
0.10 p.p.m.	...	...	43.2	...
0.20 p.p.m.	...	...	80.6	...

[a] Average of 45 mg. of rotenone or its equivalent applied per 10 treated plants.
[b] Weight dry bean tissue represented in 1 liter of test solution with 10 fish. Each test solution replicated 3 times.

Table I. Toxicity of Water Extracts of Leaves for Young Bean Plants Treated with Fresh Derris Root Latex[a]

Sample Analyzed	Weight of Fresh Sample, G. In 10 plants	Weight of Fresh Sample, G. Tested[b]	Mortality of Guppies, %	Rotenone Recovered from 10 Plants, %
One hour after treatment				
Control plant leaves	32.8	0.16	0	...
Treated plant leaves	31.0	0.16	80.0	100.0
Five days after treatment				
Control plant leaves	56.6	2.3	0	...
Treated plants				
Treated leaves, washed	54.2	2.2	0	0
Washings of treated leaves	...	...	100.0	...
New growth leaves	27.8	2.2	0	0
Ten days after treatment				
Control plant leaves	49.2	4.6	0	...
Treated plants				
Treated leaves, washed	55.0	4.4	32.1	2.0
Treated leaves, unwashed	60.0	4.8	50.0	3.3
New growth leaves	58.7	4.7	0	0
Rotenone standards[c]				
0.05 p.p.m.	...	...	17.0	...
0.10 p.p.m.	...	...	39.6	...
0.20 p.p.m.	...	...	72.0	...

[a] An average of 48 mg. of rotenone or its equivalent applied to each set of 10 plants.
[b] Weight of fresh bean tissue represented in 1 liter of test solution. Each test solution was replicated three times with 10 fish per replicate.
[c] Rotenone standards were run with each set of samples and showed no statistically significant variations.

Conclusions

The possibility exists that the rotenoids may be absorbed and metabolized into compounds which are insecticidal in character, but are not detectable by guppy bioassay or by chemical tests. Hundreds of alterations have been made in the complex structure of rotenone in the search for new insecticides, and almost invariably, these have led to less toxic compounds. Each basic structural unit of the rotenone molecule has been exhaustively studied in searching for the secret of this powerful insecticide. These studies indicate that any simple metabolite resulting from the breakdown of rotenone is not likely to retain the insecticidal character of the rotenoids.

The guppy method of bioassay showed no measurable absorption and translocation of the toxic components. Therefore, it seems highly probable that the loss of toxicity of the treated areas was due to decomposition rather than to absorption and translocation. This agrees with the work of Chisholm and Goodhue (*2*), who showed that deposits from sprays prepared from powdered derris root lost 90% of the toxicity within 10 days after deposition on glass plates.

Literature Cited

(1) Casida, J. E., and Allen, T. C., *Agr. Chemicals*, **7**, (6) 41 (1952).
(2) Chisholm, R. D., and Goodhue, T. D., *Soap*, **14**, (12) 117 (1938).
(3) Fulton, R. A., and Mason, H. C., *J. Agr. Research*, **55**, 903 (1937).
(4) Fulton, R. A., and Mason, H. C., *Science*, **85**, 264 (1937).
(5) Jones, M. A., *J. Assoc. Offic. Agr. Chemists*, **29**, 127 (1946).
(6) Pagan, C., *J. Econ. Entomol.*, **41**, (6) 942 (1949).
(7) Worsley, R. R. Le G., and Nutman, F. J., *Ann. Appl. Biol.*, **24**, 696 (1937).

Received for review June 5, 1953. Accepted June 30, 1953.