Ovicidal effect of certain organic thiocyanates on the common red spider

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UMI®
OVICIDAL EFFECT OF CERTAIN ORGANIC THIOCYANATES ON
THE COMMON RED SPIDER

by

James Harvey Cochran

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Entomology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College
1946
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I. INTRODUCTION

During the past 15 years, a wide variety of new compounds has been recommended for the control of the common red spider, or spider mite as it is sometimes called. Most of these compounds, however, have enjoyed only a short period of popularity, which may be ascribed in part to their lack of ovicidal properties. Furthermore, a majority of the compounds which possessed ovicidal properties were injurious to cultivated plants, especially those grown in the greenhouse.

A review of the literature indicates that in the past few carefully controlled laboratory experiments have been conducted on ovicides for this mite. A reference by Jary and Austin (1938) was, in fact, the only one that mentioned ovicides for the common red spider in the title, and very few of the other references relating to acaricides contained definite mortality data on the eggs. It seemed appropriate, therefore, that ovicidal experiments on this important pest be conducted.

The primary purpose of these investigations was to determine the ovicidal effects of certain organic thiocyanates. In addition, however, preliminary tests were made with hexachlorocyclohexane (gamma isomer), 1-trichloro-2,2-bis (p-chlorophenyl) ethane, 2'-hydroxy-2,4,4,7,4'-pentamethylflavan and a number of commercial acaricides.
II. REVIEW OF LITERATURE

Many references were found relating to the control of the common red spider; but many of the early references and a few of the recent publications were confusing, because some investigators used the terms red spider and common red spider indiscriminately when referring to a group of closely related mites, e.g., European red mite (Paratetranychus polius C. and F.), citrus red mite (Paratetranychus citri McG.), avocado red mite (Paratetranychus yothersi McG.) and many other distinctly separate species. The name, common red spider, however, has been most generally associated with the mites belonging to the genus Tetranychus, but today some of the leading authorities do not agree on the classification of these mites. According to McGregor (1942), the references in the early literature pertaining to the genus Tetranychus carry little weight as to the species concerned. He stated that no method has yet been discovered for distinguishing between the females of the various species. He has demonstrated that the species could be identified by use of certain male structures, but these have been known and employed only in recent years. McGregor states that he has never identified mites in America which belonged to the species Tetranychus telarius L., but that two closely related species commonly
found in this country are *Tetanychus bimaculatus* Harv. and *Tetanychus althaeae* Von Hanst.

The following references indicate the confusion which exists with respect to the identity of red spiders. Ewing (1914) in Oregon made one of the earliest and most thorough studies of the common red spider. He identified this mite as *Tetanychus telarius* L. and claimed that the term *bimaculatus* was synonymous with *telarius*. He described the life history, control, and made a physiological examination of the pigments in the variously colored mites. This mite was stated to be parthenogenetic and eggs from fertilized females produced principally females; but those from unfertilized females produced only males. This investigator found that nicotine and white mineral oil emulsions were superior as acaricides to sulfur and lime sulfur sprays. He claimed that spraying with the above compounds would kill a large percentage of the mites if properly done, but that it would not give results as satisfactory as those with most insects.

Wilson (1931) studied the life history and control of a mite attacking *Asparagus plumosus* in Florida, which he called the two-spotted mite, *Tetanychus telarius* L. The incubation period of this mite was found to vary greatly depending on the prevailing temperature. At temperatures ranging from 50° to 55° F., 15 days were required for the eggs to hatch, but in mid-July the eggs hatched in 2 days. He found that
nicotine sulfate and pyrethrum sprays gave ineffective control of this mite. The addition of sulfonated oils to these compounds increased the kill, but they were not as effective as rotenone. Soaps gave effective control of the active stages when used at high concentrations, but they did not kill the eggs. McGregor and McDonough (1917) investigated the life history and control of the red spider attacking cotton. The species was identified as Tetramychus bimaculatus Harv. These investigators tested 74 compounds or mixtures; however, even with the better materials applications at weekly intervals were necessary for effective control, since none of the materials killed the eggs. On the basis of the descriptions of the mites and life histories contained in these references it is indicated that all of these investigators were working with the same species. The life history of the common red spider, Tetramychus telarius L., in England was reported (Entomological Investigations 1926) to be similar to that of the common red spider in America. Eggs from fertilized females produced approximately 80 per cent females and 20 per cent males and all of the eggs from unfertilized females produced males. A higher percentage of the unfertilized eggs hatched than of the fertilized ones. Some preliminary toxicity experiments with naphthalene were conducted on the two types of eggs, but the concentrations used killed 100 per cent of both types.
Almost all of the compounds used in these experiments have been previously evaluated against red spiders. However, they were tested principally on the active stages. The insecticidal and acaricidal references relating to these products form an extensive bibliography from which the more pertinent ones have been selected and are discussed below.

Organic thiocyanates have been tested on a large number of different species of insects and mites, and in almost every case they have been highly toxic. Bousquet, Salzberg, and Dietz (1935) tested a homologous series of alkyl thiocyanates on aphids, thrips and red spiders. They found that the methyl, ethyl, and butyl thiocyanates were relatively non-toxic, but beginning with the compound containing six-carbon atoms there was a definite rise in toxicity which reached a peak with the twelve-carbon compound and then dropped markedly with the fourteen and sixteen-carbon derivatives. Jary and Austin (1938) showed that dodecyl (lauryl) thiocyanate was more effective against the eggs of the red spider *Tetramyces telsarius* L. than beta-butoxy-beta-thiocyanodiethyl ether, secondary alkyl thiocyanate, and monochloronaphthalene. On the other hand, Callaway and Musgrave (1940) found that beta-butoxy-beta-thiocyanodiethyl ether was more toxic to the eggs of the bedbug than lauryl thiocyanate.

The following are additional acaricidal references relating to the commercially prepared compositions used in this investi-
gation, and to various other compounds which have been recom-
mended for the control of the red spider. Darley (1931) found
that rotenone was more toxic to red spiders than nicotine or
pyrethrum. Compton (1931) obtained effective control of both
the active stages and the eggs with a derris extractive spray
("Derrisol") plus sulfonated oxidation products of petroleum
hydrocarbons ("Penetrol"). Compton and Kearns (1937) tested
a composition containing 8 per cent potassium ammonium-seleno
sulfides ("Selocide"), sulfur, hydrogen sulfide, cyclohexylamine
derivatives, lauryl thiocyanate ("Loro"), beta-butoxyethyl-beta-
thiocyanodiethyl ether ("Lethane") and other materials in
conjunction with sodium oleyl sulfate and a resinous sticker
("Grasselli" spreader-sticker). All of these materials gave
satisfactory control, and specific claims were made that the
selenium composition, hydrogen sulfide, cyclohexylamine deri-
vatives and thiocyanates killed the eggs. These investigators
reported that a concentration of "Selocide" which killed 100
per cent of red spiders from snapdragon killed only 2 per cent
of the mites from rose. Kearns and Compton (1938) showed that
\( \text{n,n-amylobenzyloxylohexylamine} \) was an effective ovicide for this
mite. They found that the degree of embryological develop-
ment of the egg, at the time the spray (\( \text{n,n-amylobenzyloxyloxylo-
hexylamine} \) was applied, was a factor contributing to its
susceptibility. In the cases of an incomplete kill of the
eggs, the majority of the unaffected individuals emerged
either the day following the application of the spray or
5 days later. Roth and Pyenson (1941) killed red spiders on
roses in the greenhouse with mannan monolaurate plus derris
extractives ("NNOR"). Hackett (1943) reported that rotenone
bearing powders (Cubé) were of little value for controlling
this mite on lima beans, but that 2,4-dinitro-6-cyclohexyl-
phenol dusts were very satisfactory. Morrison and Mote (1940)
obtained very high kills of all stages with mechanically
diluted dusts containing 2,4-dinitro-6-cyclohexylphenol.
These investigators found that lauryl thiocyanate, rotenone,
pyrethrum and nicotine sulfate were compatible with this com-
 pound, but they did not enhance the toxicity. Ark and
Tompkins (1941) killed the adults and eggs with a 2 per cent
aqueous solution of phthalic glyceryl alkyd resin. Weigel and
Johnson (1940) developed a tartar emetic spray containing
glycerol instead of brown sugar for the control of red spiders.
The recent work of Neiswander and Morris (1940) showed that
plants grown in nutrient solutions or soil containing selenium
remained free of red spiders. Speyer (1944) found that
petroleum oil emulsions killed a higher percentage of the eggs
and resting stages than of the active stages. (He claimed
that the leaf absorption of the oil was the cause of the low
toxicity of the sprays to the active stages.) The toxicity
of these compounds to the active stages was increased, by
adding 0.25 per cent of calcium chloride to the spray water
without lowering the kill of the eggs. Haring (1946) killed 98.9 per cent of the mites on carnations with an azobenzene-
bentonite dust containing 20 per cent azobenzene. He also
reported kills averaging from 90 to 99.75 per cent of all
stages including the eggs by painting the steam pipes with a
composition containing 70 per cent azobenzene and 30 per cent
"Celite 209", using one pound to 40,000 cubic feet of green-
house space. King and Frear (1943) determined the relation
of chemical constitution of some \( p \)-heterocyclic compounds
to their toxicity to red spiders, *Tetranychus telarius* L.
They found that an increase in toxicity was gained by in-
creasing the number of benzene rings in the compound.

Very few of the references were related to the use of
1-trichloro-2,2-bis (\( p \)-chlorophenyl) ethane ("DDT") on the
common red spider. Some investigators, however, claimed
that the use of "DDT" on apple trees caused an increase in
the European red mite population. Dean (1945) stated that
no outbreaks of this mite were observed in New York State
in orchards sprayed with "DDT", but that the only mite
infestation that was observed proved to be that of the common
red spider.

No references were found relating to the use of hexa-
chlorocyclohexane and 2-hydroxy-2,4,4,7,4'-pentamethylflavan
for the control of the common red spider.
III. MATERIALS

Biological: Eggs from the common red spider, which was identified by McGregor (1946) as *Tetranychus bimaculatus* Harv., inhabiting beans and two varieties (Better Times and Briarcliff) of rose plants were used in these experiments.

Chemicals: Chemically pure octyl, decyl, lauryl, myristyl, and cetyl thiocyanates, beta-butoxy-beta-thiocyanodiethyl ether, hexachlorocyclohexane (gamma isomer), xylene, and several commercially prepared compositions were used in these experiments.

The alkyl thiocyanates and beta-butoxy-beta-thiocyanodiethyl ether were formulated with a long chain alcohol sulfate emulsifying agent, ("Duponol O. S.") (Guples, 1940) in a ratio of 25 parts by weight of the chemical compound with 75 parts of "Duponol O. S." Hexachlorocyclohexane was formulated by mixing 10 parts of the compound with 90 parts of a 50-50 mixture of xylene and "Duponol O. S." All of these compositions produced a stable emulsion when added to water with slight agitation.

The chemical composition of the commercially prepared insecticides, acaricides and emulsifying or wetting agents, which were employed in these tests, are listed below:
"Loro"

Active ingredients

<table>
<thead>
<tr>
<th>Description</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliphatic thiocyanates (principally lauryl) not less than</td>
<td>90%</td>
</tr>
<tr>
<td>(thiocyanate sulfur 5.75%)</td>
<td>50%</td>
</tr>
<tr>
<td>Aliphatic sulfonates not less than</td>
<td>40%</td>
</tr>
<tr>
<td>(combined sulfur 0.4%)</td>
<td></td>
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</tbody>
</table>

Insert ingredients

Manufacturer — — — — — — — — E. I. du Pont de Nemours & Co. Inc.

"Lethane 440"

Active ingredients

<table>
<thead>
<tr>
<th>Description</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliphatic thiocyanate</td>
<td>100%</td>
</tr>
<tr>
<td>Phthalic anhydride glycerol alkyd resins</td>
<td>24.0% by weight</td>
</tr>
<tr>
<td>Steam distilled pine oil</td>
<td>16.3%</td>
</tr>
<tr>
<td>Petroleum oil</td>
<td>20.3%</td>
</tr>
<tr>
<td>(California classification of petroleum distillates:)</td>
<td></td>
</tr>
<tr>
<td>(Unclassified, minimum)</td>
<td>39.4%</td>
</tr>
<tr>
<td>(Unsulfonated residue)</td>
<td></td>
</tr>
</tbody>
</table>

Manufacturer — — — — — — — — Röhm & Haas Co.

"MNOR"

Active ingredients

<table>
<thead>
<tr>
<th>Description</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technical Mannitan Monolaurate</td>
<td>100%</td>
</tr>
<tr>
<td>Rotenone</td>
<td>97.2 - 98.4%</td>
</tr>
<tr>
<td>Other derris extractives</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

Manufacturer — — — — — — — — Atlas Powder Co.
"CycloneX"

Active ingredients

Alkylated cyclohexylamine derivatives 70%
(salicylbenzylcyclohexylamine)

Inert ingredients 30%

Manufacturer - - - - Niagara Sprayer & Chemical Co. Inc.

"IN-2018"

Active ingredients 3.6%

Rotenone 0.95%

Other derris extractives 2.85%

Inert ingredients 96.4%
(Includes solvents, emulsifying and stabilizing agents)

Manufacturer - - - - E. I. du Pont de Nemours & Co. Inc.

"Multicide"

Inert ingredients

Water 14%

Pyrethrum extractives not more than 16%

Manufacturer - - - - McLaughlin Gormley King Co.

"Black Leaf 40" (Nicotine sulfate)

Active ingredients - nicotine 40%

Inert ingredients 60%

Manufacturer - - - - Tobacco By-Products & Chemical Corp.

"Grasselli Spread-Stopper"

Sodium oleyl sulfate and a synthetic resinous sticker.
(Cuples 1940)

Manufacturer - - - - E. I. du Pont de Nemours & Co. Inc.
"Duonol.  O.  E."

Long-chain alcohol sulfate - a light brown mobile liquid with wetting, emulsifying and detergent properties.

Manufacturer - - - - - E. I. du Pont de Nemours & Co. Inc.

Hexachlorocyclohexane (containing 12 per cent of the gamma isomer) and 1-trichloro-2,2-bis (p-chlorophenyl) ethane were used as dispersible powders containing 50 per cent of the active ingredients. The 2'-hydroxy-2,4,4,7,4'-penta-methylflavan was formulated as a 30 per cent dispersible powder.

Manufacturer - - - - - E. I. du Pont de Nemours & Co. Inc.
after the plants had been sprayed. All of the leaves and a definite number (ten) of eggs with ten feet of entrance to the interference of the interference were counted. It was concluded that the first method tried was a slight modification of the interference and reported previously, but they were not entirely satisfactory. The first two were tried, the first two of these showed some improvement. There was a large number of tests in attempt to develop a method which would give consistent results and at the same time would enable one to mature and withstand frost. They were not necessary to remove any that were included in the plants, and, in addition, the plants had to be removed daily to remove all of the leaves from the plants before the eggs. Furthermore, this method was the only one for it was necessary to displace the sprayer on the leaf and to differentials in the leaves. The results obtained were entirely predictable, probably the leaves of the interference were not entirely covered by the leaves with the method employed by previous investigators. The interference caused by spraying interference potato plants, but a few planters for the common red sprayer com-

II. METHODS

-15-
up eggs were then removed from the enclosed area. This procedure proved unsatisfactory because the tanglefoot was toxic to the leaves and caused them to curl or fall off before the final record could be made. Also, these plants had to be inspected and the mites removed as in the original method, although less time was required to inspect the plant since only a limited area had to be examined. Furthermore, the slightest break in the tanglefoot barrier permitted mites from other sections of the plant to enter the restricted area and oviposit fresh eggs.

The second method consisted of the following procedure: Small sections of the leaf containing ten eggs were removed from infested plants, and pasted on a piece of filter paper, by means of a thin starch paste. (The eggs were sprayed by the turntable technique which will be described later.) This procedure eliminated the daily examination of the plants since the young mites crawled away as soon as they hatched, but the small sections of the leaf curled and twisted to such an extent that it was almost impossible to count the unhatched eggs.

The disadvantages of the foregoing methods indicated that the ideal method would be one in which the eggs could be collected and sprayed on a uniformly flat surface, such as paper, cloth, or glass. Finally, the third method was developed in which the eggs were collected on filter paper. The
The technique employed to collect the eggs was essentially as follows: A circle about one inch in diameter was drawn on a sheet of filter paper (nine cm); the filter paper was dipped in water for a few seconds; then placed on a flat glass surface (an inverted beaker or petri dish). By use of a wet camel's hair brush, approximately thirty adult female mites were quickly transferred from an infested plant to the center of the circle. The filter paper containing the mites was then placed on a sheet of glass, and the mites were covered with a wide mouth bottle (approximately 1 inch in diameter), which contained a band of tanglefoot inside of the neck just above the lip. After the desired number of lots had been transferred, they were placed under a light. The filter paper dried rapidly and the mites began to crawl about and oviposit their eggs at random on the portion of the paper covered by the bottle. The bottles were removed after four hours, and most of the mites on the paper crawled off immediately. The eggs on the filter paper were counted under a binocular microscope and any mites dead or living and any damaged eggs were removed. There were several factors which had to be considered if satisfactory results were to be obtained with this technique. First, care had to be used in selecting the females to insure that only the older, larger and darker colored mites were transferred, because the adult female does not normally begin oviposition until she is approximately three
days old. (With experience it is possible to select a high proportion of egg laying females and thereby insure a rather uniform number of eggs from each lot.) Second, the filter paper had to be kept wet while transferring the females. Third, the light had to be uniformly distributed around the bottle to prevent the mites from congregating and ovipositing their eggs in a mass. Fourth, if the mites were confined too long, they formed a heavy web over the eggs which trapped the young larvae and prevented their escape.

This procedure possessed the following advantages: The eggs were of a known age; the time and expense of growing a large number of plants were eliminated; the eggs were held on a uniformly flat surface which eliminated the differences caused by the variation in contour and texture of the leaf's surface; more tests could be conducted in a given time, and the results obtained were reasonably uniform.

In most of the tests, the eggs were sprayed according to the following procedure: The filter paper containing a known number of eggs was placed in a petri dish on a revolving turntable and sprayed by passing it, three times, through a settling mist spray. The spray was delivered under a uniform compressed air pressure of twenty pounds per square inch, by an ordinary nasal atomizer stationed fourteen inches above the turntable. After the eggs were sprayed, the paper was removed from the petri dish and placed on a clean glass tray.
Mortality counts were made on the seventh day, and the data were recorded as follows: Number of eggs that failed to hatch and the number of mites that died after hatching. Any dead mites found within the circle were placed in the latter category. The eggs in a few of the experiments were sprayed by means of an apparatus similar to that described by Tattersfield and Morris (1924), which consisted of spraying the eggs under a bell jar.
V. RESULTS

The results obtained with the homologous series (octyl through the cetyl derivatives) of alkyl thiocyanates using four different dosages are given in table I. Six subsamples were tested at each dosage on eggs approximately 24 hours old which were kept at a constant temperature of 28°C and a relative humidity from 60 to 70 per cent before and after spraying. The sprays were applied by the turntable method.

The myristyl and cetyl derivatives, were retested at greater dosages; at the same time, beta-butoxy-beta-thiocyanodiethyl ether was tested, and lauryl thiocyanate was retested at 0.008 per cent and one lower concentration (0.007 per cent). The results of these experiments are given in table II. Six subsamples were tested at each dosage of lauryl thiocyanate, but only four replicate tests were used for the other compounds. The age of the eggs, the method of applying the sprays, and the temperature and humidity conditions were the same as those listed above.

Dosage mortality curves were calculated for octyl, decyl, lauryl, and myristyl thiocyanates and beta-butoxy-beta-thiocyanodiethyl ether from the data presented in tables I and II by the Bliss (1935) method. In using this method, the logarithm of the dosage is plotted against the net percentage
of mortality expressed in probits. The net mortality represents the observed mortality after adjustment for the mortality in the water-treated controls (Abbott 1925). The dosage mortality-curve was not calculated for oetyl thiocyanate, because the highest dosage (0.2 per cent) gave a net mortality less than 10 per cent. The data for the 0.008 and 0.013 per cent dosages were omitted in the calculation of the curve for myristyl thiocyanate. The chi-square test was used to determine how well the data agreed with the straight line relationship. A comparison of the dosages expressed in per cent W/V (ratio of weight to volume) of thiocyanates required to give 50 per cent (LD50) net mortality, together with the confidence limits for chances of 19 in 20 are given in table III. The slopes and chi-squares for each of the calculated lines are also given.

The five alkyl thiocyanates were retested several weeks later at a single dosage of 0.017 per cent. Each treatment was replicated five times from one large group of eggs. The age of the eggs, the temperature and humidity conditions, and the method of applying the sprays were the same as those described for table I. The results are shown in table IV.

A few preliminary experiments were conducted with hexa-chlorocyclohexane, 2-hydroxy-2,4,4,7,4'-pentamethylflavan, and 1-trichloro-2,2-bis(p-chlorophenyl) ethane. These compounds were tested as dispersable powders at a dosage of one per
cent of the active ingredient. Decyl thiocyanate and beta-butoxy-beta-thiocyanodisteryl ether at 0.017 per cent were used as standards for comparison. Eggs 48 hours old, which were kept at a constant temperature of 26° C., were sprayed by the bell jar method; only two replicate tests were made with each material. The data are shown in table V.

Hexachlorocyclohexane was also tested using the gamma isomer formulated with xylene and "DuPontol O. S." A dosage of one per cent hexachlorocyclohexane (in a spray containing 4.5 per cent xylene and 4.5 per cent "DuPontol O. S.") gave a net mortality of 72.8 per cent; decyl thiocyanate at a dosage of 0.013 per cent killed 37.5 per cent. A nine per cent solution of the 50-50-mixture of xylene and "DuPontol" (without the hexachlorocyclohexane) killed 28.0 per cent of the eggs. Eggs 24 hours old, which were kept at 26° C., were sprayed by the bell jar method.

Table VI gives comparative data on the ovicidal efficiency of commercial sprays containing lauryl thiocyanate, beta-butoxy-beta-thiocyanodisteryl ether, rotenone, pyrethrum, nicotine sulfate, mannitan monolaurate, and n,n-amylbenzylcyclohexylamine, all of which have been recommended for the control of the common red spider. (These compounds were tested at the concentration ordinarily recommended for the control of the active stages.) Eggs 24 and 48 hours old were used in these tests; however, an equal number of replicate tests
were made for each compound from any one group of eggs. These tests were conducted under room conditions in which the temperature and humidity were not controlled. The sprays were applied by the turntable method.

Table VII shows the results obtained with eggs oviposited by mites inhabiting rose plants and those laid by mites feeding on bean. Decyl thiocyanate was used at a dosage of 0.013 per cent and eight subsamples for each host plant were sprayed by the turntable method. Eggs 24 hours old, which were kept at 26° C. and a relative humidity of 60 to 70 per cent, were used in these experiments.

The results obtained on eggs of different ages are given in table VIII. These data were obtained from four separate tests. In the first test, decyl thiocyanate plus "Duponol" and "Duponol" alone were tested on eggs 4, 24, 48 and 72 hours old which were kept at a temperature of 26° C. The second test consisted of spraying eggs 4, 24, 48 and 72 hours old with beta-butoxy-beta-thiocyanodiethyl ether ("Lethane 440") at a temperature of 26° C. In the third test, eggs 4, 24, and 48 hours old were sprayed with beta-butoxy-beta-thiocyanodiethyl ether at a temperature of 30° C. (In these three experiments the eggs were laid on consecutive days; and were all sprayed at the same time, by the turntable method.) The fourth experiment consisted of spraying eggs 4, 24, and 48 hours old with decyl thiocyanate plus "Duponol" and
"Duponol" alone at a temperature of 30°C. In this test the eggs were all laid on the same day; but were sprayed on different days, by the bell jar method. Four subsamples ranging from 30 to 40 eggs each were treated for each age group in all of the tests.
### Table I. Relative Efficiency of Alkyl Thiocyanates Against the Eggs of the Common Red Spider

<table>
<thead>
<tr>
<th>Thiocyanate</th>
<th>Dosage (W/V)*</th>
<th>Total No. Eggs</th>
<th>Number Dead</th>
<th>Mean Mortality Per cent</th>
<th>Net Mortality Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Eggs</td>
<td>Mites</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Octyl</td>
<td>0.025</td>
<td>204</td>
<td>143</td>
<td>11</td>
<td>75.49</td>
</tr>
<tr>
<td></td>
<td>0.017</td>
<td>208</td>
<td>67</td>
<td>10</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>0.015</td>
<td>217</td>
<td>27</td>
<td>4</td>
<td>84</td>
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<td>208</td>
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<td>208</td>
</tr>
<tr>
<td></td>
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<td>222</td>
<td>198</td>
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<td>201</td>
</tr>
<tr>
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<td>216</td>
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<td>127</td>
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<tr>
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<td>0.008</td>
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<td>243</td>
<td>236</td>
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<td>30</td>
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<tr>
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<td>218</td>
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<td>16</td>
<td>21</td>
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<tr>
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<td>0.015</td>
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<td>27</td>
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<td>0.008</td>
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<tr>
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<td>230</td>
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<td></td>
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<td>229</td>
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<td>5</td>
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<td>222</td>
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<td>227</td>
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<td>9</td>
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<td>0.075</td>
<td>868</td>
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</table>

*Ratio of weight to volume
<table>
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<tr>
<th>Compound</th>
<th>Dosage Per cent W/V</th>
<th>Total No. Eggs</th>
<th>Number Dead</th>
<th>Mean Mortality Per cent</th>
<th>Net Mortality Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Eggs</td>
<td>Mites</td>
<td>Total</td>
</tr>
<tr>
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<td>0.008</td>
<td>214</td>
<td>112</td>
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<td>114</td>
</tr>
<tr>
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<td>0.007</td>
<td>218</td>
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<td>52</td>
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<td>9</td>
<td>98</td>
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<td>150</td>
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<td>&quot;Duponol C. S.&quot;</td>
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<td>238</td>
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<td>2</td>
<td>6</td>
</tr>
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<td>Compound (IP20, per cent)</td>
<td>CHI-square Probability</td>
<td>CHI-square (W/V)</td>
<td>Slope</td>
<td>Median Lethal Dose (mg)</td>
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<tr>
<td>---------------------------</td>
<td>------------------------</td>
<td>----------------</td>
<td>-------</td>
<td>------------------------</td>
<td></td>
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<tr>
<td>Tetrahydroxyzatine</td>
<td>7.412</td>
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<td></td>
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<tr>
<td>Dextrohydroxyzatine</td>
<td>7.167</td>
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<td>beta-xyloxy-beta</td>
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<td>6.458</td>
<td>6.370</td>
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</tr>
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<td></td>
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<td></td>
</tr>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<tr>
<td>0.004 0.0005</td>
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<td>0.000 0.0001</td>
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Table IX. Comparison of Median Lethal Doses and Slopes
Table IV. Relative Efficiency of Alkyl Thio cyanates Against the Eggs of the Common Red Spider

<table>
<thead>
<tr>
<th>Thiocyanate*</th>
<th>Total No. Eggs</th>
<th>Number Dead</th>
<th>Mean Mortality Per cent</th>
<th>Chi-Square</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Eggs</td>
<td>Mites</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Octyl</td>
<td>190</td>
<td>42</td>
<td>0</td>
<td>42</td>
<td>21.31</td>
</tr>
<tr>
<td>Decyl</td>
<td>202</td>
<td>197</td>
<td>0</td>
<td>197</td>
<td>97.30</td>
</tr>
<tr>
<td>Lauryl</td>
<td>196</td>
<td>195</td>
<td>1</td>
<td>196</td>
<td>100.00</td>
</tr>
<tr>
<td>Myristyl</td>
<td>187</td>
<td>10</td>
<td>11</td>
<td>21</td>
<td>10.92</td>
</tr>
<tr>
<td>Cetyl</td>
<td>174</td>
<td>8</td>
<td>1</td>
<td>9</td>
<td>5.12</td>
</tr>
<tr>
<td>&quot;Duponol O. S.**</td>
<td>132</td>
<td>6</td>
<td>4</td>
<td>9</td>
<td>4.69</td>
</tr>
</tbody>
</table>

* All thiocyanates used at a dosage of 0.017 per cent.

** "Duponol O. S." used at a dosage of 0.053 per cent.
Table V. A Comparison of the Ovicidal Effects of Hexachlorocyclohexane, 1-trichloro-2,2-bis (p-chlorophenyl) Ethane and 2'-hydroxy-2,4,4,7,4'-pentamethylflavan

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dosage Per cent</th>
<th>Total No. Eggs</th>
<th>Number Dead</th>
<th>Mean Mortality Per cent</th>
<th>Net Mortality Per cent</th>
</tr>
</thead>
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<tr>
<td>Hexachlorocyclohexane</td>
<td>1.00</td>
<td>76</td>
<td>17</td>
<td>28</td>
<td>45</td>
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<tr>
<td>1-trichloro-2,2-bis (p-chlorophenyl) ethane</td>
<td>1.00</td>
<td>80</td>
<td>22</td>
<td>12</td>
<td>34</td>
</tr>
<tr>
<td>2-hydroxy-2,4,4,7,4'-pentamethylflavan</td>
<td>1.00</td>
<td>96</td>
<td>22</td>
<td>57</td>
<td>79</td>
</tr>
<tr>
<td>Beta-butoxy-beta-thiocyanodiethyl ether</td>
<td>0.017</td>
<td>92</td>
<td>40</td>
<td>9</td>
<td>49</td>
</tr>
<tr>
<td>Decyl thiocyanate</td>
<td>0.017</td>
<td>82</td>
<td>81</td>
<td>1</td>
<td>82</td>
</tr>
<tr>
<td>&quot;Dupontol 0.5.&quot;</td>
<td>0.053</td>
<td>80</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Check (water)</td>
<td>---</td>
<td>105</td>
<td>5</td>
<td>5</td>
<td>10</td>
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</tbody>
</table>
Table VI. The Ovicidal Effects of Certain Commercial Organic Acaricides on the Common Red Spider

<table>
<thead>
<tr>
<th>Principal Toxic Ingredient</th>
<th>Commercial Name of Spray</th>
<th>Dosage V/V</th>
<th>Total No. Eggs</th>
<th>Total No. Dead</th>
<th>Mean Mortality Per cent</th>
<th>Net Mortality Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrethrins</td>
<td>&quot;Multicide&quot;</td>
<td>1-250</td>
<td>344</td>
<td>47</td>
<td>15.29</td>
<td>6.91</td>
</tr>
<tr>
<td>Nicotine Sulfate</td>
<td>&quot;Black Leaf 40&quot;</td>
<td>1-250</td>
<td>367</td>
<td>56</td>
<td>15.70</td>
<td>7.36</td>
</tr>
<tr>
<td>Derris resin plus Mannite monolaurate</td>
<td>&quot;NNOR&quot;</td>
<td>1-400</td>
<td>402</td>
<td>141</td>
<td>35.36</td>
<td>28.89</td>
</tr>
<tr>
<td>Rothenone</td>
<td>&quot;IN-201B&quot;</td>
<td>1-400</td>
<td>382</td>
<td>38</td>
<td>10.36</td>
<td>1.82</td>
</tr>
<tr>
<td>N-M-Amylbenzylcyclohexylamine</td>
<td>&quot;Cyclonox&quot;</td>
<td>1-600</td>
<td>390</td>
<td>296</td>
<td>76.74</td>
<td>73.62</td>
</tr>
<tr>
<td>Beta-butoxy-beta-Thiocyanodisthyl ether</td>
<td>&quot;Lethane 440&quot;</td>
<td>1-400</td>
<td>410</td>
<td>543</td>
<td>94.69</td>
<td>83.15</td>
</tr>
<tr>
<td>Lauryl thiocyanate</td>
<td>&quot;Loro&quot;</td>
<td>1-1000</td>
<td>417</td>
<td>414</td>
<td>99.11</td>
<td>99.02</td>
</tr>
<tr>
<td>Check (water)</td>
<td>---</td>
<td>---</td>
<td>445</td>
<td>47</td>
<td>9.02</td>
<td>---</td>
</tr>
</tbody>
</table>

* Ratio of Volume to Volume.
Table VII. The Effect of Host Plant on the Resistance of the Eggs of the Common Red Spider to Decyl Thiocyanate

<table>
<thead>
<tr>
<th>Host Plant</th>
<th>Total No.</th>
<th>Number Dead</th>
<th>Mean Mortality Per cent</th>
<th>Net Mortality Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eggs</td>
<td>Eggs</td>
<td>Mites</td>
<td></td>
</tr>
<tr>
<td>Bean</td>
<td>243</td>
<td>212</td>
<td>6</td>
<td>218</td>
</tr>
<tr>
<td>Rose</td>
<td>229</td>
<td>122</td>
<td>8</td>
<td>130</td>
</tr>
</tbody>
</table>

* Decyl Thiocyanate was used at a dosage of 0.013 per cent plus
  "DuPontol O. S." 0.039 per cent.
<table>
<thead>
<tr>
<th>Test No.</th>
<th>Compound</th>
<th>Dosage Per Cent W/V</th>
<th>Temp. °C</th>
<th>Eggs 4 hours old</th>
<th>Eggs 24 hours old</th>
<th>Eggs 48 hours old</th>
<th>Eggs 72 hours old</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Decyl thiocyanate</td>
<td>0.013</td>
<td>26</td>
<td>44.23</td>
<td>62.58</td>
<td>63.03</td>
<td>81.66</td>
</tr>
<tr>
<td></td>
<td>Plus &quot;Duponol O.S.&quot;</td>
<td>0.039</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;Duponol O.S.&quot;</td>
<td>0.039</td>
<td>26</td>
<td>0.00</td>
<td>0.00</td>
<td>2.23</td>
<td>46.73</td>
</tr>
<tr>
<td>2</td>
<td>Beta-butoxy-beta-thiocyanodiethyl ether***</td>
<td>0.032</td>
<td>26</td>
<td>41.91</td>
<td>46.27</td>
<td>33.33</td>
<td>74.33</td>
</tr>
<tr>
<td>3</td>
<td>Beta-butoxy-beta-thiocyanodiethyl ether***</td>
<td>0.032</td>
<td>30</td>
<td>49.34</td>
<td>43.43</td>
<td>81.44</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Decyl thiocyanate</td>
<td>0.013</td>
<td>30</td>
<td>11.33</td>
<td>31.91</td>
<td>62.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plus &quot;Duponol O.S.&quot;</td>
<td>0.039</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;Duponol O.S.&quot;</td>
<td>0.039</td>
<td>30</td>
<td>0.00</td>
<td>1.30</td>
<td>55.50</td>
<td></td>
</tr>
</tbody>
</table>

* Does not include the 4 hour period during which eggs were oviposited.
** Obtained from 4 subsamples ranging from 30 to 40 eggs each for each age group.
*** "Lethane 440" was used at a dosage of 1 gm. to 749 cc. water
VI. DISCUSSION OF RESULTS

The data in tables I, II, III and IV indicate that the order of toxicity of the thiocyanates and beta-butoxy-beta-thiocyanodiethyl ether to eggs of the red spider is as follows:

Lauryl > decyl > beta-butoxy-beta-thiocyanodiethyl ether > octyl > myristyl > cetyl.

The order of toxicity of the homologous series of alkyl thiocyanates is the same as that found by Siegler and Popenee (1925) and Tattersfield and Cimingham (1927) with a homologous series of fatty acids. Bousquet, et al, (1935) reported a similar relationship in the order of toxicity of these thiocyanates to the active stages of the red spider. These investigators claimed that the unusually high efficiency of the twelve-carbon compound can probably be explained on the basis of favorable physical properties leading to good spreading and penetrating properties. However, they state that no one physical property will explain this peak in toxic action at the twelve-carbon product, and that it is probably the resultant of several factors. The physical properties generally used to explain toxicity are found to increase or decrease progressively with molecular weight without showing a maximum or minimum.

The results obtained with lauryl thiocyanate and beta-butoxy-beta-thiocyanodiethyl ether are similar to those
reported by Jary and Austin (1938).

The results with hexachlorocyclohexane, 1-trichloro-
2,2-bis (p-chlorophenyl) ethane and 2'-hydroxy-2,4,4,7,4'-
 pentamethylflavan, decyl thiocyanate and beta-butoxy-beta
thiocyanodiol ethyl ether give only an indication of their
relative ovicidal effects, because the first three were tested
as dispersible powders and the thiocyanates were used as
miscible oils. However, it is apparent from the results
that the first three compounds mentioned were not highly
ovicidal. The dispersible powders formed a thick, hard film
over the eggs which probably trapped the young larvae and pre-
vented their escape, and in addition, they appeared to pro-
duce a desiccative action on the eggs. It was noted that
many of the mites from eggs treated with the flavan compound
had crawled a short distance before they died. Therefore,
this compound is probably more toxic to the active stages
than to the ova. A few observations were made in one of the
College greenhouses in which a bed of carnations was dusted
with "DDT" for the control of thrips. An increase in the
mite population was noted after a week, but this was probably
due to the fact that the plants were not syringed during this
period. It was apparent, however, that "DDT" had little
effect on the mites or their eggs.

The data in table VI were analyzed for homogeneity by
means of the chi-square test, using Snedecor's (1938) method
development proceeds they change from a pale yellow to a
white in period of 4 hours (peanut white in color) and as
instances of the process in color were noted some of the
eggs died. When first laid the eggs are usually
the eggs in color during the various stages of development,
since the eggs of the common red spider under certain
acting
and 1-naphthylacetetothylene showed considerable change
Novato, DuPont-de-Puerto-Rico-Pharmaceutical Co.
secreted non-toxic to the eggs, while the spores containing
metastate, pyrethrum, kerosene, and naphthenic monomers were

warrant the conclusion that the composition of the
various spore compositions are sufficiently large to
between subseparate, the difference in the mean mortalities
1.31. In spite of the variation in the results
time the spores were applied, a high humidity appeared to
in part to the differences in the relative humidity at the
the mortality increased from day to day can probably be ascribed
The variation in
of the various weather conditions remained the same. The variation
conducted on different days; however, the order of toxicity
same day, but considerable variation occurred between these
variation was observed between replicates applied on the
was that the population was effectively heterogeneous. little
for unrepeatable sample sizes. The chi-square for these data indi-

-35-
yellowish-orange color. A few hours before hatching two carmine spots are visible. (These are the eye-spots of the young larva which are never visible in the freshly laid eggs.) It was apparent in most cases that the higher dosages of the thiocyanates and beta-butoxy-beta-thiocyanodiyethyl ether stopped the embryological development immediately, but almost all of the eggs sprayed with the other compounds developed through the eye-spot stage. (In one instance in which eggs four hours old were exposed to the vapor of azobenzene, all of the eggs developed through the eye-spot stage; but only 11 per cent hatched.)

The difference in the resistance of the eggs laid by mites inhabiting rose and those oviposited by mites feeding on bean plants is difficult to explain, because the mites from both host plants were identified as the same species. However, this difference might possibly be related to the fact that the mites inhabiting beans were taken from a stock culture which had not been subjected to any toxic sprays for many generations; and those feeding on rose were collected from plants which had been sprayed several times with various insecticides and fungicides.

The results in table VIII indicate only slight differences in the resistance of the eggs from the time they were laid to the time the eye-spots appeared, but eggs sprayed after the eye-spots appeared (eggs 72 hours old at 26⁰ C. and eggs
48 hours old at 30° C.) were considerably more susceptible than the younger eggs.
VII. SUMMARY

1. A satisfactory laboratory method for conducting ovicidal tests for the common red spider was developed. With this method, the eggs are collected and sprayed on filter paper rather than on the plants.

2. The order of toxicity to red spider eggs of octyl, decyl, lauryl, myristyl and cetyl thiocyanates and beta-butoxy-beta-thioctianodiethyl ether was as follows:

   \[ \text{Lauryl} > \text{decel} > \text{beta-butoxy-beta-thioctianodiethyl ether} \]
   \[ \geq \text{octyl} > \text{myristyl} > \text{cetyl}. \]

3. The following median lethal (LD50) dosages for the thiocyanates and beta-butoxy-beta-thioctianodiethyl were obtained:

   Lauryl 0.008 per cent, decyl 0.014 per cent, beta-butoxy-beta-thioctianodiethyl ether 0.022 per cent, octyl 0.020 per cent, myristyl 0.045 per cent and cetyl > 0.20 per cent. (The slope of the line for beta-butoxy-beta-thioctianodiethyl ether was greater than that for octyl thiocyanate.)

4. Contact sprays containing lauryl thiocyanate, beta-butoxy-beta-thioctianodiethyl ether, and \( \text{n,n'-amylbenzylcylohexylamine} \) were more ovicidal than sprays containing nicotine sulfate, pyrethrum, rotenone, and mannitan monolaurate.

5. Decyl thiocyanate and beta-butoxy-beta-thioctianodiethyl ether were considerably more toxic to the eggs of the red
spider than hexachlorocyclohexane, 2'-hydroxy-2,4,4,7,4'-pentamethylflavan, and 1-trichloro-2,2-bis (p-chlorophenyl) ethane.

6. Eggs laid by red spiders inhabiting rose plants were more resistant to decyl thiocyanate than eggs oviposited by mites feeding on bean plants.

7. When used alone "Duponol O. S." (the emulsifier used with the thiocyanates) was considerably more toxic to the eggs which were sprayed after the eye-spots were visible than to the younger eggs. Decyl thiocyanate plus "Duponol" and beta-butoxy-beta-thiocyanodiethyl ether ("Lethane 440") were also more toxic to the older eggs, but the differences were not as great as those obtained with "Duponol O. S." alone.
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