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THE STUPEFACTION OF RED SCALE,  
*AONIDIELLA AURANTII*, BY  
HYDROCYANIC ACID

D. L. LINDGREN



# THE STUPEFACTION OF RED SCALE, AONIDIELLA AURANTII, BY HYDROCYANIC ACID<sup>1, 2</sup>

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## INTRODUCTION

BOTH THE RED SCALE, *Aonidiella aurantii* (Mask.), and the black scale, *Saissetia oleae* Bern., become more difficult to kill if they have first been exposed for a short time to a sublethal concentration of hydrocyanic acid gas. The term applied to the effect of small charges of HCN is "protective stupefaction." It may be brought about in the field by the leakage of gas through the tents or by poor diffusion of the gas within the tent. Since most workers agree that the red scale becomes stupefied when pre-fumigated with a sublethal concentration of HCN, an investigation was begun to determine the length of time these insects remain stupefied.

## EARLIER INVESTIGATIONS

Gray and Kirkpatrick (1929) concluded that under the laboratory conditions of their experiments:

Both the resistant and nonresistant strains of black and red scales exhibit a characteristic which is termed "protective stupefaction," that is, when a lot of scale is first exposed to a sublethal, but stupefying concentration of hydrocyanic acid in air, followed by a normally lethal concentration, more of them are able to survive than a lot upon which the reverse procedure has been followed.

.....  
Correlated field and laboratory observations and experiments, not fully described in this paper, furnish good circumstantial evidence that protective stupefaction is sometimes a factor adversely affecting the results of scale kill in commercial fumigation.

Pratt, Swain, and Eldred (1931) found that protective stupefaction is a fact in the case of both black and red scales when exposed to lethal concentrations of HCN after 10- or 3-minute exposures to sublethal concentrations, but that protective stupefaction does not follow exposure for only 1 minute to sublethal concentrations.

<sup>1</sup> Received for publication February 14, 1938.

<sup>2</sup> Paper No. 347, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

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Moore (1933) states:

Under conditions giving high kills prefumigation reduced the kill. Above 98 per cent there were four significant negative differences and two without differences. Below 98 per cent, the differences are all positive but only one is significant. These results indicate that exposure to low concentrations preceding the regular fumigation does not always reduce the kill and field results confirm these conclusions.

## EXPERIMENTAL METHODS

A series of laboratory fumigation experiments was begun in 1935 on lemons infested with resistant red scale obtained from a single grove in the Corona district. In each test 18 to 20 lemons were used, and previous to fumigation they were held at 70° to 74° F for 18 to 60 hours. All experiments except those conducted in 1935 were carried on in pairs, that is, 18 to 20 lemons infested with red scale were subjected to a stupefying charge of 0.5 cc of HCN for 5 minutes in a 100 cu. ft. fumatorium. They were then removed and placed in a second fumatorium of the same size, along with 18 to 20 lemons which had not been exposed to a sublethal charge, and the two groups were subjected to the lethal charge for 40 minutes. The temperatures at which the fumigations were conducted ranged from 73° to 76° F and the relative humidity from 35 to 60 per cent. Within this humidity range, there seemed to be no effect on the fumigation results. Quayle and Rohrbaugh (1934) also found no significant differences in the effect of humidity within a range of from 50 to 80 per cent on fumigation results with red scale.

The lethal charges of HCN were administered in two different ways: one in which a low dosage—usually 3 cc but sometimes 2 or 4 cc—of HCN was added to the fumatorium and allowed to remain 40 minutes; and the other in which 12 cc of HCN was added and the exhaust pump was operated for the 40 minutes. The first produces a low, uniform concentration and the second a high-peak concentration similar to that obtained in commercial fumigation practice.

A sample was kept from each lot of fruit picked to determine the natural mortality of the red scale. About 1,000 mature female scales were examined on the untreated lemons at the same time that counts were made on the fumigated lots. The natural mortality varied from 48 to 57 per cent in the different untreated samples. In the tables the total number of scales indicated is the number of live scales present before treatment. Actually about twice as many red scales were examined in the tests, but since approximately one-half (48 to 57 per cent) of these represent the population which had died from natural causes, they were not included in the tables. In each case the percentage of natural mortality

was assumed to be the same in the treated sample as in the corresponding untreated one.

Only mature females were included in the examinations, which were made from 10 to 14 days after the fumigations. The experiments were numbered and dated only, and thus the several workers examining the scales had no knowledge of the treatment the insects had received.

The significance of the means was determined by the method outlined by Snedecor and Irwin (1933). This method takes into consideration the variance within the samples; and a comparison is made of the differences within groups as well as between groups to determine whether the means of the different methods of treatment are significant. A probability ( $P$ ) of less than 0.05 is regarded as small enough to justify the conclusion that the differences are not due to random sampling.

#### PRELIMINARY TESTS WITHOUT A STUPEFYING CHARGE, COMPARING A LOW, UNIFORM CONCENTRATION WITH A HIGH-PEAK CONCENTRATION

Preliminary tests were made in 1935, to determine the mean concentration with the two methods of administering the dosage. The experiments also afford a comparison of the kill with the two types of concentration when used without a stupefying charge; and when compared with similar fumigations in subsequent experiments, they give an indication of the variation in results from year to year.

In the 1935 tests both the low, uniform concentration (with a 3-cc dosage) and the high-peak concentration (with a 12-cc dosage) were used (fig. 1). The mean average concentrations were calculated from the formula given by Knight (1925),  $\frac{\sum MC \times T}{\sum T}$ , where  $MC$  equals the mean

concentration for each time interval over which  $MC$  is computed. As evaporation and diffusion were very rapid in the fumatorium (less than  $\frac{1}{2}$  minute), the mean average concentration was calculated from the time the cyanide was added. Samples of gas for titration were taken at 1, 4, 7, 15, 30, and 40 minutes from the time fumigation began. The mean average concentrations obtained from the two types of curves did not vary greatly, as can be seen from figure 1.

The 1935 experiments are summarized in table 1. These data indicate but slight difference in the kill with the two types of concentration.

In order to indicate the variation in results from year to year, those results of later experiments (see p. 217–218) that deal with scales fumigated without a stupefying charge are also summarized in table 1. The

red scales used in 1936 and 1937 were obtained from the same grove and in the same season as those used in the earlier experiments. In 1936 the low, uniform type of concentration was the only one tried. In 1937 this type was used with 9 lots, but most of the lots were fumigated with the high-peak type of concentration.

If results with the same type of concentration in different years are compared, they will be seen to vary rather widely from year to year.

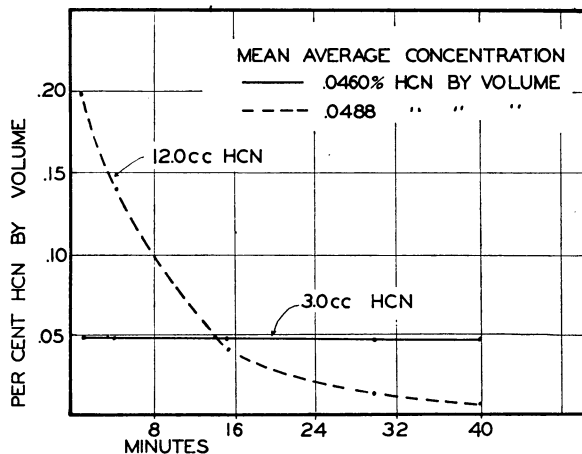


Fig. 1.—Types of concentration to which the red scales were exposed.

TABLE 1

FUMIGATION OF RESISTANT RED SCALE FROM 1935 TO 1937: A COMPARISON OF THE EFFECTS OF HIGH-PEAK (12 OR 14 CC) AND LOW, UNIFORM (3 CC) DOSAGES OF HCN

Dosage, cc	Time of fumigation	Number of experiments	Live scales treated	Number of scales killed	Per cent killed
3	Fall, 1935.....	16	14,699	13,382	91.04
14	Fall, 1935.....	15	12,408	11,463	92.38
3	Fall, 1936.....	37	34,398	28,806	83.74
3	Fall, 1937.....	9	5,280	4,582	86.78
12	Fall, 1937.....	47	41,724	36,972	88.61

Caution must therefore be exercised in comparing the different types of concentration in different years. In comparing the kill of red scale obtained by a low, uniform concentration in 1936 with that obtained by a high-peak concentration in 1937, for example, it must be remembered that, even though the red-scale-infested lemons were picked from the same grove, there was a lapse of one year between the time the two types of dosages were used. During this year, the scale had passed through

some severe and prolonged cold weather and one oil-spray treatment early in the spring of 1937. The difference in favor of the high-peak type of concentration is consequently of little or no significance.

## RESULTS OBTAINED IN 1936 WITH A LOW, UNIFORM TYPE OF CONCENTRATION

The plan at the beginning of this problem was to determine the importance of protective stupefaction and the length of time red scale would remain stupefied after they had been exposed to a sublethal charge of HCN. Experiments were carried out in four groups: with no time inter-

TABLE 2

FUMIGATION OF LEMONS INFESTED WITH RESISTANT RED SCALE WITH VARYING INTERVAL BETWEEN STUPEFYING AND FUMIGATING CHARGES IN 1936

(Stupefying charge, 0.5 cc of HCN; fumigating charge, 3 cc of HCN per 100 cu. ft.)

Interval	Treatment	Number of experiments	Live scales treated	Number of scales killed	Mean per cent kill	Difference of means,* per cent	Probability, <i>P</i>
None	Fumigated .....	11	10,442	8,806	84.33	} -10.36	<0.01
	Stupefied and fumigated...	11	9,940	7,353	73.97		
1 hour	Fumigated .....	8	7,544	6,295	83.44	} -7.12	0.03
	Stupefied and fumigated...	8	8,077	6,165	76.32		
2 hours	Fumigated .....	9	8,458	7,093	83.86	} +2.33	0.11
	Stupefied and fumigated...	9	7,910	6,818	86.19		
3 hours	Fumigated .....	9	7,954	6,612	83.13	} +4.81	0.04
	Stupefied and fumigated...	9	7,926	6,970	87.94		

\* Mean per cent kill for the stupefied and fumigated tests minus the mean per cent kill for the fumigated.

val and with 1 hour, 2 hours, and 3 hours between the stupefying charge and the regular fumigation. Work on all four groups of experiments was simultaneous, no one group being completed before another was started.

In the no-time-interval experiments, fumigation with 3 cc HCN followed immediately after the stupefying charge. (See "Experimental Methods," p. 214, for description of procedure.) In the other groups of experiments, the procedure was the same except that the lemons were removed from the fumatorium and remained under atmospheric conditions for the interval designated. In each case a check lot of lemons infested with red scale that had not been exposed to the stupefying charge was fumigated at the same time. Each time interval was tested from 8 to 11 times.

Table 2 summarizes the results of these experiments. The red scale



exposed to a small dose of HCN 1 hour or less before fumigation survived the regular fumigation better than those not so exposed; but when exposed to the stupefying charge 2 or 3 hours before fumigation, they did not survive so well as those not exposed. The difference between mean percentage killed when a stupefying charge was given and that when none was given was — 10 per cent with no time interval, — 7 per cent with a 1-hour interval, + 2 per cent with a 2-hour interval, and + 5 per cent with a 3-hour interval. The difference is significant in each case except in that with the 2-hour interval, which is low enough to be due to chance variation. Apparently the resistant red scale succumbs to the stupefying charge immediately and remains stupefied for at least 1 hour.

The reversal of the difference in the 2-hour and 3-hour intervals may be the result of a higher rate of respiration of the insects after having been in a stupor for some time. Up to the present time, no other explanation can be offered. The close association of the red scale with its host makes the rate of respiration difficult to measure, and its waxy covering prevents any observation on its activity.

A few tests were conducted in which there was an interval of 4 hours between the sublethal and the lethal charges of HCN. In this series the difference in kill resulting from the two types of treatment was not significant, which indicates that at the end of 4 hours after receiving a stupefying charge, the resistant red scales react normally to the regular fumigation procedure.

#### RESULTS OBTAINED IN 1937 WITH THE HIGH-PEAK TYPE OF CONCENTRATION

On checking over the results of the experiments carried on in 1936, the question arose as to whether red scale after having been stupefied would react similarly to a high-peak type of concentration. It was thought that a high-peak concentration might overcome the effects of a stupefying charge to some extent. Therefore a series of experiments was conducted in 1937 along lines similar to those of 1936, with the exception that 12 cc of HCN were used in the fumatorium as the lethal charge and the gas was gradually withdrawn from the chamber to give the type of concentration curve shown in figure 1.

Table 3 summarizes the results obtained in 1937 with the high-peak type of concentration. The results are very similar to those obtained in 1936 with a low, uniform concentration. The sudden high charge of HCN does not overcome the effects of the stupefying dose of HCN. The red scale remain stupefied for at least 1 hour, as shown by the fact that

fewer scales are killed by the regular fumigation after this interval. Two hours after the scales have been stupefied, they have come out of their stupor and are actually easier to kill; and this is also the case after the 3-hour interval.

TABLE 3

FUMIGATION OF LEMONS INFESTED WITH RESISTANT RED SCALE WITH VARYING INTERVAL BETWEEN SUBLETHAL AND LETHAL CHARGES IN 1937

(Sublethal charge, 0.3 cc of HCN; lethal charge, 12 cc of HCN per 100 cu. ft.)

Interval	Treatment	Number of ex- periments	Live scales treated	Number of scales killed	Mean per cent kill	Difference of means,* per cent	Prob- ability, P
None	{ Fumigated.....	12	9,371	8,050	85.90	} - 7.30	0.01
	{ Stupefied and fumigated...	12	9,267	7,284	78.60		
1 hour	{ Fumigated.....	12	11,318	10,119	89.41	} -11.44	<0.01
	{ Stupefied and fumigated...	12	11,462	8,936	77.96		
2 hours	{ Fumigated.....	12	11,419	10,303	90.23	} + 5.85	<0.01
	{ Stupefied and fumigated...	12	12,585	12,091	96.07		
3 hours	{ Fumigated.....	11	9,616	8,500	88.39	} + 9.56	<0.01
	{ Stupefied and fumigated...	11	10,387	10,174	97.95		

\* Mean per cent kill for the stupefied and fumigated tests minus the mean per cent kill for the fumigated.

### RESULTS OBTAINED WITH LABORATORY-REARED RESISTANT AND NONRESISTANT STRAINS OF RED SCALE

In August, 1936, the rearing of resistant and nonresistant strains of red scale was begun in insect-proof rooms of the insectary of the Citrus Experiment Station. The original stocks of red scale were collected on August 24 and 25, 1936, from widely separated lemon groves: the resistant strain from Corona, and the nonresistant strain from an isolated grove in the foothills east of Glendora. A series of tests (Quayle, 1938) had definitely shown a wide difference in the susceptibility to HCN of red scale collected from these two groves. In the insectary the red scales were transferred to squash, and the two strains reared in separate rooms under identical conditions.

During the summer of 1937, a method was devised by which crawlers in large numbers were transferred from the stock culture on squash to freshly picked grapefruits. As many as 16 grapefruits were infested daily, 8 with crawlers from the nonresistant stock, and 8 with crawlers from the resistant stock. The grapefruits were held in the rooms in which the stock cultures were kept for several days or until all the crawlers had

settled, after which they were transferred to separate cages in a room in which the temperature was held at 80° F and the relative humidity above 85 per cent. At such high humidity, the grapefruits remained in excellent condition. Approximately 40 days elapsed from the time the grapefruits were infested until the females were mature and started to produce young. In this way a daily supply of red scales of definite age

TABLE 4

FUMIGATION OF LABORATORY-REARED RESISTANT RED SCALE WITH VARYING  
INTERVAL BETWEEN STUPEFYING AND FUMIGATING CHARGES

(Stupefying charge 0.5 cc of HCN; fumigating charge  
4.0 cc of HCN per 100 cu. ft.)

Interval	Treatment	Number of ex- periments	Live scales treated	Number of scales killed	Mean per cent kill	Difference of means,* per cent	Prob- ability, P
None	Fumigated . . . . .	7	12,683	10,604	83.61	} -13.47	<0.01
	Stupefied and fumigated . . .	7	12,673	8,889	70.14		
1 hour	Fumigated . . . . .	6	9,413	7,849	83.38	} - 8.59	0.01
	Stupefied and fumigated . . .	6	9,894	7,400	74.79		
2 hours	Fumigated . . . . .	8	13,489	11,122	82.45	} + 4.93	<0.01
	Stupefied and fumigated . . .	8	14,350	12,539	87.38		
3 hours	Fumigated . . . . .	5	7,631	6,157	80.68	} + 8.37	0.03
	Stupefied and fumigated . . .	5	7,818	6,962	89.05		

\* Mean per cent kill for the stupefied and fumigated tests minus the mean per cent kill for the fumigated.

could be had for experimental purposes. The date of infesting the grapefruits was marked on each with India ink.

The red scales reared in the laboratory are more homogeneous than those picked at random in the field. Factors which may cause variation such as natural mortality, climatic conditions previous to picking of infested fruit, variation in condition of host plant, age of the scales, and a heterogeneous population of the scales to begin with, are all eliminated when they are reared in the laboratory. Counting of the scales is simplified because all of them on the grapefruit are within a few hours of being the same age; therefore, all the scales on the grapefruit can be counted, whereas fruit picked from the field have scales of all ages. (Scales that are in certain stages only are considered in evaluating fumigation results.)

The experimental procedure with the laboratory-reared insects was the same as that conducted on the red scales obtained from the field. Adult females 40 to 42 days old and just starting to produce young crawlers were used in all the tests. Only the low, uniform type of con-

centration was used, a dosage of 2.0 cc of HCN being used on the non-resistant scales and 4.0 cc on the resistant strain. Since as many as 300 to 450 adults could be reared on a single large grapefruit, only 4 or 5 infested grapefruits were used in each experiment.

The results obtained by the stupefaction and fumigation of the laboratory-reared strain of resistant red scales were similar to those obtained with the same strain from the field. If the resistant red scales reared in

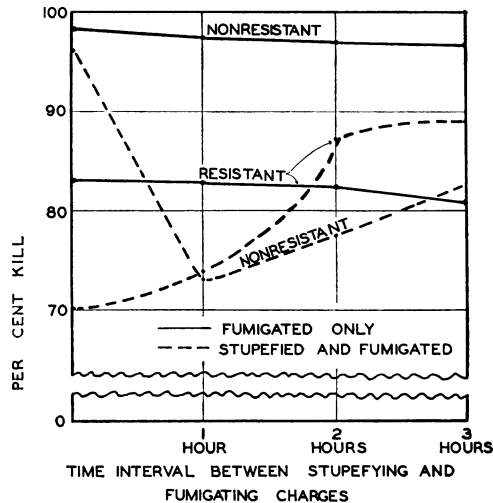


Fig. 2.—Fumigation of laboratory-reared resistant and non-resistant red scale: effects of a stupefying charge. The stupefying charge was 0.5 cc of HCN; the fumigating charge, 2 cc of HCN with the non-resistant strain and 4 cc of HCN with the resistant strain. Data taken from tables 4 and 5.

the laboratory are exposed to a stupefying charge of HCN immediately before, or 1 hour before the regular fumigation, more of them are able to survive the fumigation than those not exposed to the stupefying charge; but if they are exposed to a stupefying charge 2 hours or 3 hours prior to the regular fumigation, fewer are able to survive the fumigation than those not so exposed (table 4 and fig. 2).

It will be noted that 4.0 cc of HCN gave approximately the same kill of the resistant strain of red scale reared in the laboratory as 3.0 cc of HCN gave with the same strain obtained in the field. This may be due to the very favorable conditions under which scales are reared in the laboratory; or continual inbreeding in the laboratory may eliminate non-resistant individuals which are perhaps present in a resistant population in the field.

The nonresistant strain of red scale reacts in an altogether different manner to a stupefying charge of HCN, which seems additional proof of the existence of two strains of the red scale in southern California (table 5 and fig. 2). More of the nonresistant strain are able to survive a normally lethal dose of HCN if they are exposed to a stupefying charge immediately before the regular fumigation; but the difference of the

TABLE 5

FUMIGATION OF LABORATORY-REARED NONRESISTANT RED SCALE WITH VARYING INTERVAL BETWEEN STUPEFYING AND FUMIGATING CHARGES

(Stupefying charge 0.5 cc of HCN; fumigating charge  
2.0 cc of HCN per 100 cu. ft.)

Interval	Treatment	Number of ex- periments	Live scales treated	Number of scales killed	Mean per cent kill	Difference of means,* per cent	Prob- ability, P
None	{ Fumigated . . . . .	7	10,478	10,295	98.25	} - 2.28	0.01
	{ Stupefied and fumigated . . . . .	7	10,498	10,075	95.97		
1 hour	{ Fumigated . . . . .	9	17,487	17,083	97.69	} -24.08	<0.01
	{ Stupefied and fumigated . . . . .	9	17,239	12,690	73.61		
2 hours	{ Fumigated . . . . .	9	14,092	13,707	97.27	} -19.71	<0.01
	{ Stupefied and fumigated . . . . .	9	14,890	11,548	77.56		
3 hours	{ Fumigated . . . . .	9	13,475	13,014	96.58	} -13.75	<0.01
	{ Stupefied and fumigated . . . . .	9	13,362	11,067	82.83		

\* Mean per cent kill for the stupefied and fumigated tests minus the mean per cent kill for the fumigated.

means is only 2.28 per cent, which indicates that only a few of the non-resistant strain were affected by the stupefying dose. However, if the nonresistant strain of red scale is exposed to a stupefying charge 1, 2, or 3 hours before the regular fumigation, a greater number (24.08 per cent; 19.71 per cent; and 13.75 per cent, respectively), are able to survive the regular fumigation. It appears that the nonresistant strain reacts slower to a stupefying dose of HCN than does the resistant strain. It requires 1 hour before the nonresistant strain becomes fully stupefied, whereas the resistant strain reacts immediately. At the end of 2 hours the effects of stupefaction on the resistant strain are present but operating in another direction, while the nonresistant strain may show the effects of stupefaction in the same direction even at the end of 3 hours.

## STUPEFACTION OF OTHER INSECTS

Peters (1936, p. 72) states:

Small traces of hydrocyanic acid cause, in the case of the granary weevil, a shock or stupefying effect that results in cessation of respiration. The hydrocyanic acid can then only enter the body by diffusion, for which high concentrations or long exposures are necessary (therefore the relatively great resistance of granary weevils for hydrocyanic acid) . . . . . At lower temperatures, however, the granary weevil, because of a cold phlegma occurring at about 5° C, generally loses the ability to effect this defense reaction, whereby its resistance is lowered. . . .

TABLE 6

COMPARATIVE RESULTS OF FUMIGATION OF GRANARY WEEVIL, CONFUSED FLOUR BEETLE, AND CONVERGENT LADY BEETLE WITH AND WITHOUT STUPEFYING CHARGE  
(Stupefying charge, 5 min.; lethal charge, 45 min.)

Insect	Treatment	Stupefying charge, cc	Lethal charge, cc	Number of experiments	Total insects treated	Mean per cent kill	Difference of means,* per cent	Probability, P
Confused flour beetle.....	Fumigated.....	....	5.0	6	1,182	84.77	-0.51	0.90
	Stupefied and fumigated.....	1.0	5.0	6	1,067	84.26		
Lady beetle....	Fumigated.....	....	3.0	7	2,278	82.95	-1.11	0.80
	Stupefied and fumigated.....	0.5	3.0	7	2,489	81.84		
Granary weevil	Fumigated.....	....	120.0	6	957	36.47	-19.51	<0.01
	Stupefied and fumigated.....	10.0	120.0	6	843	16.96		

\* Mean per cent kill for the stupefied and fumigated tests minus the mean per cent kill for the fumigated.

As a result of their recent experiments, Mackie and Carter (1937) concluded:

One factor not generally considered among those engaged in fumigation of grain is what may be called protective stupefaction, which occurs when a concentration of a gaseous insecticide is not sufficiently strong to kill an insect immediately but knocks it out and causes a suspension of its normal breathing function, thus protecting it against the action of a fumigant.

At the time the experiments were being conducted on red scale, three other insect species, confused flour beetle, *Tribolium confusum* Duval, granary weevil, *Sitophilus granarius* (Linn.), and lady beetle, *Hippodamia convergens* Guérin, were available in numbers large enough for experimental purposes. In all of the experiments with these three species the exposure to the lethal charge of HCN immediately followed the exposure to the stupefying charge.

The data in table 6 indicate that of the three species treated the granary weevil was the only one which definitely showed signs of stupefaction. A reduction in kill of 19.5 per cent was obtained when these insects were first exposed to a sublethal charge of HCN. In the fumigation of stored products "protective stupefaction" may be an important factor, for insects in grain are usually exposed to a low concentration of the gas as it penetrates into the mass of grain.

### SUMMARY

Under laboratory conditions with rapid and complete diffusion of hydrocyanic acid gas, a high-peak concentration offers only a slight advantage, if any, over a low, uniform type of concentration on the resistant strain of red scale, *Aonidiella aurantii* (Mask.).

A greater percentage of resistant red scale survive a normally lethal charge of HCN if they have first been exposed to a sublethal dosage of the gas immediately before the regular fumigation.

The effects of the sublethal charge are about the same on the resistant red scale after a 1-hour interval, but after a 2-hour interval the insects have come out of their stupor and are actually easier to kill. They remain easier to kill for 3 hours after they have been subjected to a sublethal charge, but after 4 hours the insects appear to react normally again to a uniform concentration of HCN, that is, as though no stupefaction had occurred.

A sudden high-peak concentration does not overcome the effects of a stupefying charge on the resistant red scale.

Results obtained by the stupefaction and fumigation of laboratory-reared resistant red scale are similar to those obtained with resistant red scale from the field.

Nonresistant red scale require 1 hour before the stupefying charge is effective, as indicated by a large reduction in kill. Nonresistant red scale remain stupefied even after an interval of 3 hours between the sublethal charge and the normally lethal charge of HCN.

The results of this work indicate that there are two definite strains of red scale in southern California.

The granary weevil, *Sitophilus granarius* (Linn.), is readily stupefied by a low concentration of hydrocyanic acid gas, whereas the confused flour beetle, *Tribolium confusum* Duval, and the convergent lady beetle, *Hippodamia convergens* Guérin, are not thus affected.

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