ELIMINATION OF NATURAL MORTALITY AS A FACTOR IN DETERMINING THE EFFECTIVENESS OF HYDROCYANIC ACID GAS ON THE CALIFORNIA RED SCALE [1]

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INTRODUCTION

In tests on the fumigation of the California red scale (*Chrysomphalus aurantii* Mask.) with hydrocyanic acid gas, natural mortality as ordinarily considered has ranged from 1 to 71 per cent. This has introduced so large an error in experimental tests as to render precise results almost impossible. Therefore, an attempt has been made to establish qualitative tests, based on such phenomena as changes in color and conformation, by which the scales killed by treatment could be distinguished from those that died naturally. Though a complete separation has not been possible, the number of dead scales occurring on untreated, or check, lemons which are indistinguishable from those killed on recently fumigated fruit have proved to be few. However, it has been found that the scales dying from natural causes bear a nearly constant ratio to the number of living scales plus the number of dying scales. This paper is concerned with the amount of fluctuation that one may expect in natural mortality, as defined by these new criteria.

When the California red scale is killed by treatment with hydrocyanic acid gas, it becomes darker in color and then gradually dries out. Scales dying naturally go through a similar change. An attempt was made to determine what factors control the numbers of these dying scales. To simplify the problem, it was decided to use only lemon fruits, and to make counts exclusively of the female in the later periods of her development. At this time the female is relatively large and bodily changes can be noted with less chance of error than is possible during the earlier periods of her development. She is also more resistant to hydrocyanic acid gas. The stages used were the fully developed second stage, the gray adult, and the mature adult.[2] On dying, the scales in these three stages first grow darker. Color changes are practically completed before the drying-out process begins. The color of the insect during the fully developed second stage becomes a dull brown to a dark reddish brown, that of the gray adult a dull gray to almost black, that of the mature adult a dirty reddish to blackish red brown. In drying out, the individuals become increasingly viscous, as can be recognized by probing but by no other means, so far as the writers know, up to the time they are nearly dry. Shriveling may or may not accompany the drying-out process.

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[2] The term "fully developed second stage" is applied to that part of the second stage from the time the individual has reached approximately its largest diameter and is becoming shiny, but before the derm has thickened, up to ecdysis; "gray adult" is applied to that part of the third or adult stage from the time of ecdysis until the individual has reached approximately its largest diameter and is becoming shiny and beginning to darken; "mature adult" is applied to the adult stage beyond the gray adult one.

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The experimental data show that the ratio of dying to living plus dying scales [3] of the total number in the three periods from untreated fruits in fair condition varies but little, even though the factors involved may differ. This is not true for the ratio of living or of dead scales to the total scale count, where those already dead and dried out are also included. The ratio of dying scales to living plus dying scales on untreated fruits shows so little variation that by taking the weighted mean of a moderate number of samples a satisfactory value for expressing mortality can be obtained. This value may then be used in eliminating the natural mortality from counts on treated samples. The terms "mortality" and "natural mortality" occurring hereafter, when used without explanation, refer to the ratio of dying scales to living plus dying scales present, expressed as a percentage.

STABILITY OF MORTALITY VALUES ON UNTREATED CHECKS

To be of broad applicability for experimental tests, the value for expressing natural mortality should be satisfactorily stable under a variety of conditions encountered in practice. The writers have found that the following factors do not definitely influence this value as obtained by their method: Different locations on the same tree; corresponding locations on different trees; different locations on different trees; size of fruit (samples [4] ranged from 1⅛ to more than 2 inches long); severity of infestation (single samples ranged in total counts from 16 to 600 scales); protection by overhanging foliage (records were from a grove rather sparsely foliated); time of year (December 3, 1930, to January 12, 1931, for one grove, and January 22 to May 21, 1931, for another); and time and conditions of storage after collection (from none to 50 days under temperature conditions, all approximately uniform, ranging from 13° to 26° C.). As for fruits of varying conditions, counts from those scales that are beginning to dry out noticeably still show the same general mortality at first, but eventually the mortality rises rapidly. In comparing mature or yellow fruits of good condition with immature or green fruits of like condition, the writers have observed a slightly higher mortality for the yellow fruits in the average of a considerable number of counts. The above-mentioned conclusions have been drawn from a total count of almost 25,000 scales, where all factors except the one under investigation were made as nearly alike as possible.

To give an idea of the stability of the mortality values obtained by the writer's method as compared with the usual practice of obtaining the net mortality of treated material from the ratio of live scales to the total number of scales on checks, two frequency distributions have been plotted. (Fig. 1.) The data were obtained from counts on 63 lemons, all green or immature, in good condition and collected from a single grove during two periods in 1931, that is, from January 22 to February 20 in the winter and from May 8 to 21 in the spring. The collections in the winter period were stored from 3 to 21 days at 13° C., those in the spring period were not stored. Counts of the latter were made on the day the samples were collected or on the following day. The abscissa of the chart gives, in terms of percentage, the ratio of living to living plus dying

[3] All scales discolored or in process of drying out are referred to as dying. Living scales denote live scales exclusive of those dying.
[4] In this paper a sample refers to an individual lemon fruit.

scales obtained from the formula $\frac{A}{A+Dy}$, and the ratio of living to total number of scales (excluding empty shells) obtained from the formula $\frac{A}{A+Dy+Dr}$, in which A refers to living scales, Dy to dying scales, and Dr to dead, dried scales. The percentage of mortality, expressed by the formula $\frac{Dy}{A+Dy}$, is, of course, obtained by subtracting the value for $\frac{A}{A+Dy}$ from 100 per cent. The abscissa is

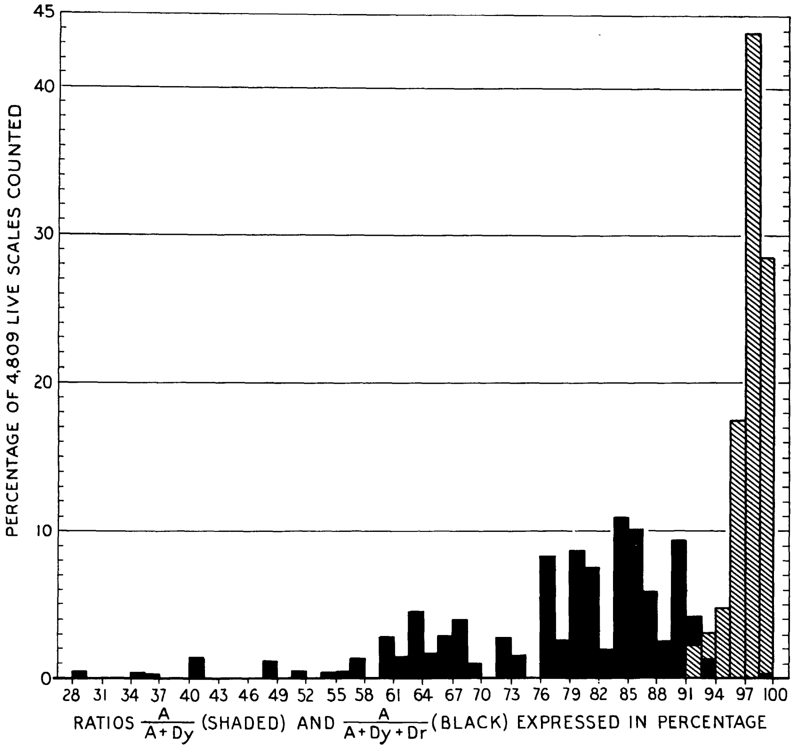

FIGURE 1.—Comparison of variability of the ratio of living scales to living plus dying scales $\left(\frac{A}{A+Dy}\right)$ and the ratio of living scales to the total number of scales $\left(\frac{A}{A+Dy+Dr}\right)$ from the same counts

divided into 1.5 per cent intervals. The numbers of live scales on each sample which fell in each 1.5 per cent interval on the abscissa were totaled and the ratio of this total to the total count of 4,809 live individuals, expressed as a percentage, was placed in the appropriate interval on the abscissa and read from the scale on the ordinate. This was done both for the ratio of living to living plus dying scales (shaded columns) and for the ratio of living to living plus dying plus dead and dried scales (black columns). It is seen that the range of mortality obtained by the writers' method is narrow (0 to 9 per cent), whereas that obtained by the usual method is so wide as to introduce a real error in its use.

TABLE 1.—*Comparison of variability of the ratio of living scales to living plus dying scales* $\left(\frac{A}{A+Dy}\right)$ *and the ratio of living scales to the total number of scales* $\left(\frac{A}{A+Dy+Dr}\right)$ *from counts on fruits with the following factors involved: Grove from which collected, period of collection, storage temperature, and condition of fruit*

Grove	Period of collection	Group No.	Fruits used	Storage temperature	Condition of fruit	Scales: A	Scales: Dy	Scales: Dr	Scales: A+Dy+Dr	Ratios: $\frac{A}{A+Dy}$	Ratios: $\frac{Dy}{A+Dy}$	Ratios: $\frac{A}{A+Dy+Dr}$
			Number	° *C.*		*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
A	Jan. 22 to Feb. 20, 1931	1	20	13	Good (green)	2,793	69	576	3,438	97.6	2.4	81.2
	May 8-21, 1931	2	43	([a])	do	2,016	51	882	2,949	97.5	2.5	68.4
	Jan. 22 to Feb. 20, 1931	3	5	13	Good (yellow)	1,284	64	360	1,708	95.3	4.7	75.2
	Jan. 22, 1931	4	4	13	Drying	931	28	224	1,183	97.1	2.9	78.7
	May 21, 1931	5	6	([a])	do	541	17	591	1,149	97.0	3.0	47.1
	Jan. 22 to Feb. 20, 1931	6	3	26	Good (green)	680	14	117	811	98.0	2.0	83.8
	Mar. 30, 1931	7	33	13, 17, 20, 23, 26.	Good (green or yellow or partly both)	2,092	95	1,776	3,963	95.7	4.3	52.8
		8	47	10	Good (green)	3,898	217	1,807	5,922	94.7	5.3	65.8
B	Dec. 3, 1930, to Jan. 12, 1931	9	13	10	Good (yellow)	864	49	912	1,825	94.6	5.4	47.3
		10	10	26	Good (green)	1,098	50	488	1,636	95.6	4.4	67.1
C	Jan. 26, 1931	11	3	13	Good (yellow)	184	8	21	213	95.8	4.2	86.4

[a] Counted promptly after collection.

In Table 1 a series of total counts is presented to show the relation of the mortality ratios to date of collection, temperature at which the fruit was stored (if the counts were not made promptly after collection), and condition of fruit, in three widely separated groves in the Whittier (Calif.) district. Considering the data from grove A, it is seen that counts of fruits collected between January 22 and February 20 and stored from 2 to 10 days in a relatively cold medium (Group 1) show practically the same mortality as counts from fruits collected 3 months later and not stored (Group 2); that counts on fruits stored for about the same length of time in a distinctly warmer environment (Group 6) show approximately the same mortality as those stored at the lower temperature (Group 1). This is true also for fruits distinctly drying (Group 5) when compared with fruits in good condition (Group 2). The mature fruits (Group 3) are seen to have a slightly higher scale mortality than comparable green fruits in good condition (Group 1). Group 7 represents the data for those fruits collected at the time indicated which did not dry out. The mortality on these fruits, which were stored from 8 to 50 days, was but little higher than that on the fruits in any other group and was slightly less than that on the mature or yellow fruits. The range in mortality for the seven groups in grove A is only 2.7 per cent, whereas the ratios of live scales to total scale count show a range more than twelve times as great, or 36.7 per cent.

The mortality data for groves B and C are in harmony with the corresponding data for grove A. Grove B had been much neglected. The fruits were collected earlier than those from grove A and the storage conditions were not under such satisfactory control as for the fruits from groves A and C; also the humidity was lower in both storage media. Any or all of these factors may have exerted an influence in producing a slightly higher mortality on fruits from grove B. When the counts on fruits composed of groups most nearly equivalent from groves A and B were compared, however, the difference in mortality was found to be only 2.3 per cent (Table 2), but the percentage of live scales in the total scale count differed by 13.5.

TABLE 2.—*Comparison of the ratio of living scales to living plus dying scales* $\left(\frac{A}{A+Dy}\right)$ *and the ratio of living scales to the total number of scales* $\left(\frac{A}{A+Dy+Dr}\right)$ *from combined counts on fruits consisting of groups most nearly equivalent from two different groves*

Grove	Period of collection	Group No.	Fruits used	Scales				Ratios		
				A	Dy	Dr	$A+Dy+Dr$	$\frac{A}{A+Dy}$	$\frac{Dy}{A+Dy}$	$\frac{A}{A+Dy+Dr}$
			Number	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
A	Jan. 22 to May 21, 1931.	1, 2, 3, 6	71	6,773	198	1,935	8,906	97.2	2.8	76.0
B	Dec. 3, 1930, to Jan. 12, 1931.	8, 9, 10	70	5,860	316	3,207	9,383	94.9	5.1	62.5
	Total or weighted mean			12,633	514	5,142	18,289	96.1	3.9	69.1

The mortality in the three periods, when considered separately, was found to check well with the combined mortality. The data in

Table 3 were obtained on fruits in like condition collected from one grove on two different occasions. In both cases the mortality was slightly lower for the fully developed second stage than for the two adult stages. The results for both gray adult and mature adult were somewhat different for the two sets of observations, but the average mortalities derived from the sum of the two sets of figures were practically the same for both stages.

TABLE 3.—*Comparison of variability of the ratio of living scales to living plus dying scales* $\left(\frac{A}{A+Dy}\right)$ *and the ratio of living scales to the total number of scales* $\left(\frac{A}{A+Dy+Dr}\right)$ *from counts of female scales in the three later periods on fruits comparable in condition but collected at two different occasions from the same grove*

Period of collection	Scales							
	Fully developed second stage				Gray adult			
	A	*Dy*	*Dr*	*A+Dy+Dr*	*A*	*Dy*	*Dr*	*A+Dy+Dr*
1931	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Jan. 22	711	11	64	786	958	36	310	1, 304
May 19 to 21	844	16	290	1, 150	1, 079	25	718	1, 822
Total or weighted mean	1, 555	27	354	1, 936	2, 037	61	1, 028	3, 126

Period of collection	Scales—Continued				Ratios					
	Mature adult				$\frac{A}{A+Dy}$			$\frac{A}{A+Dy+Dr}$		
	A	*Dy*	*Dr*	*A+Dy+Dr*	Fully developed second stage	Gray adult	Mature adult	Fully developed second stage	Gray adult	Mature adult
1931	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Jan. 22	964	23	183	1, 170	98. 5	96. 4	97. 7	90. 5	73. 5	82. 4
May 19 to 21	401	19	303	723	98. 1	97. 7	95. 5	73. 4	59. 2	55. 5
Total or weighted mean	1, 365	42	486	1, 893	98. 3	97. 1	97. 0	80. 3	65. 2	72. 1

In Figure 2 six frequency distributions have been plotted, in the same manner as in Figure 1, to illustrate variability of $\frac{A}{A+Dy}$ as related to the grove from which the fruit was collected, period of collection, whether or not the fruit was stored, and, if so, at what temperature. The first four distributions (A to D, inclusive) are represented by Groups 1, 2, 7, and 8, respectively, in Table 1, and the fifth and sixth distributions (E and F) by the data for groves A and B, respectively, in Table 2. The percentages for the other groups in Table 1 are not plotted, since the number of samples was too small to bring out any significant results. However, Groups 3 and 6, and 9 and 10, are included in frequency distributions E and F, respectively.

The general trends indicated in the six frequency distributions may be stated briefly as follows: In the aggregate practically the same percentage of live scales occurs in A and B, but in A the percentage falling in the interval 97 to 98.5 per cent is more than three times that in any other interval, those in the intervals on either side of this one are nearly equal, and there is a sharply reduced percentage in the next lower interval; whereas in B the largest percentage, which is not twice that of any other interval, falls in the interval 98.5 to 100 per cent, and there is a decreasing percentage in the lower intervals. In C no interval shows a decided concentration of scales, the percentages being fairly evenly distributed in the six intervals extending from 91

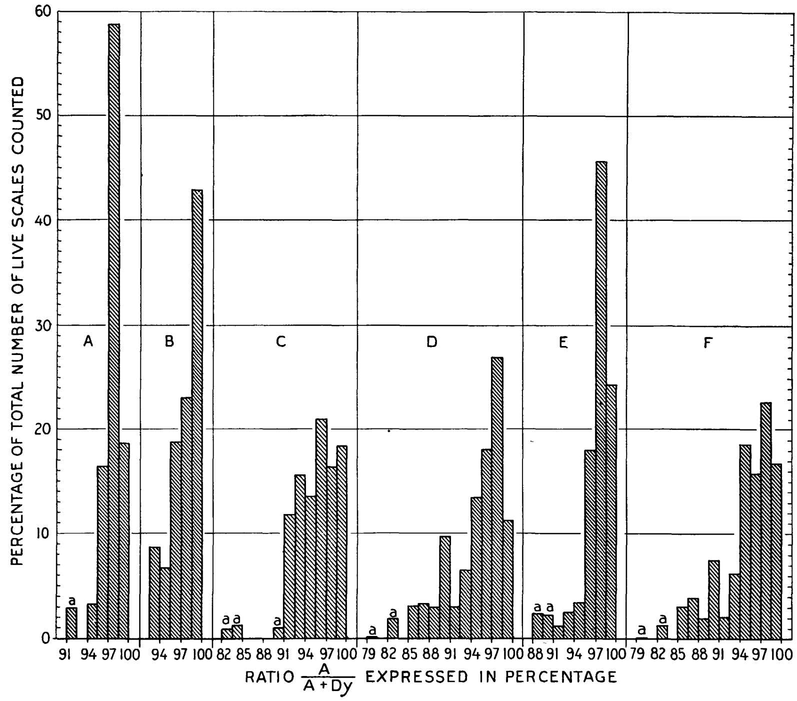

FIGURE 2.—Comparison of variability of the ratio of living scales to living plus dying scales $\left(\frac{A}{A+Dy}\right)$ from counts on fruits with the following factors involved: Grove from which collected, period of collection, whether stored or not, and, if so, the storage temperature. The frequency distributions are represented by the following groups in Table 1: A, Group 1; B, Group 2; C, Group 7; D, Group 8; E, Groups 1 2, 3, and 6; F, Groups 8, 9, and 10. *a* represents the count from a single sample

to 100 per cent. In D there is some concentration of scales in the interval 97 to 98.5 per cent, but the remainder are well distributed in the next higher and in the next eight lower intervals. In E the distribution resembles that of A, but with a less marked concentration in the interval 97 to 98.5 per cent, and a correspondingly larger percentage in each of the other intervals on either side of it. In F the general range of distribution is like that of D, but more evenly distributed in the four intervals between 94 and 100 per cent. The percentage values of one to three intervals in each of the frequency distributions except one appear to be more or less aberrant; that is, these values are larger than would be expected from the positions of their intervals, which also may be separated from the others by blank intervals. This state of affairs may be due, at least in part, to the fact that these apparent aberrant values are derived from counts on single samples.

METHOD OF ELIMINATING NATURAL MORTALITY IN TESTS ON FUMIGATED SPECIMENS

Thus far only the natural mortality on untreated checks has been considered. In order to use these results in experimental tests, the fumigated scales must be counted after all those killed by treatment are distinguishable from living survivors by the criteria already presented, but before they have dried out enough to be confused with those that died before treatment. When fruits were stored promptly after treatment with hydrocyanic acid gas in the controlled rooms of the lowest (13° C. or 55.4° F.) and highest temperatures (26° C. or 78.8° F.) in which approximately 78 and 72 per cent relative humidities were maintained, respectively, all dying scales in the three periods could be distinguished four days afterwards. Counts could be continued with certainty for a week in the 26° C. environment and for two weeks in the 13° C. environment.

To eliminate the natural mortality in tests on fumigated samples counted in this way, the procedure is simply to multiply the number of living plus dying scales on the treated fruits by the percentage mortality on the corresponding checks as determined by the methods given here. This gives the number of individuals that are considered to be naturally dying. On subtracting this number from the number counted as dying, the number of scales actually killed by treatment is obtained, which is then compared with the number of survivors to secure the correct percentage of mortality due to the treatment. Two examples are given in Table 4 to illustrate the elimination of natural mortality from treated samples by means of the ratios $\frac{A}{A+Dy}$ and $\frac{A}{A+Dy+Dr}$ of the checks. The data for the checks, besides being presented collectively (records 7 and 13) for each treatment concerned, are given individually to bring out the variability in the numbers of scales involved and in the values for the ratios. The net mortality for each set of treated samples is calculated from the collective ratio values (records 8 and 14) and also from the extreme ratio values (records 9 and 10, 15 and 16). It is observed that in both sets the collective net mortality values obtained from the ratio $\frac{A}{A+Dy}$ are lower, and the extremes show distinctly less variability, than those obtained from the ratio $\frac{A}{A+Dy+Dr}$.

TABLE 4.—*Estimation of percentage of scales killed by hydrocyanic acid gas treatments using the ratio of dying scales to living plus dying scales* $\left(\frac{Dy}{A+Dy}\right)$ *and the ratio of living scales to the total number of scales* $\left(\frac{A}{A+Dy+Dr}\right)$ *for the elimination of natural mortality*

EXAMPLE 1

Record No.	Fruits used	Hydrocyanic acid schedule	Date treated	Date collected	Storage temperature	Counted after collection	Scales				Ratios		
							A	*Dy*	*Dr*	*A+Dy+Dr*	$\frac{A}{A+Dy}$	$\frac{Dy}{A+Dy}$	$\frac{A}{A+Dy+Dr}$
	Number				° *C.*	*Days*	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
1						3	80	2	67	149	97.6	2.4	53.7
2						9	23	0	3	26	100.0	0	88.5
3						9	73	3	19	95	96.1	3.9	76.8
4	1	Check		Jan. 13, 1931	10	9	150	3	156	309	98.0	2.0	48.5
5						9	94	0	36	130	100.0	0	72.3
6						9	96	5	53	154	95.0	5.0	62.3
7	Total or weighted mean						516	13	334	863	97.5	2.5	59.8
8, 9, 10	7	110 per cent	Jan. 12, 1931	Jan. 13, 1931	10	11–14	43	239	390	672	15.2	84.8	6.4

Record No.	Fruits used	Hydrocyanic acid schedule	Date treated	Date collected	Mortality by writers' method					Mortality by usual method				
					A+Dy	$\frac{Dy}{A+Dy}$ of check	Dying naturally	Killed by treatment		$\frac{A}{A+Dy+Dr}$ of check	Survivors		Killed by treatment	
											Natural	Observed		
	Number				*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Per cent*	*Number*	*Number*	*Number*	*Per cent*
8					282	2.5	7.1	231.9	84.36	59.8	401.9	43	358.9	89.30
9	7	110 per cent	Jan. 12, 1931	Jan. 13, 1931	282	0	0	239.0	84.75	88.5	594.7	43	551.7	92.77
10					282	5.0	14.1	224.9	83.95	48.5	325.9	43	282.9	86.81

EXAMPLE 2

Record No.	Fruits used	Hydrocyanic acid schedule	Date treated	Date collected	Storage temperature	Counted after collection	Scales: A	Scales: Dy	Scales: Dr	Scales: A+Dy+Dr	Ratios: $\frac{A}{A+Dy}$	Ratios: $\frac{Dy}{A+Dy}$	Ratios: $\frac{A}{A+Dy+Dr}$
	Number				° *C.*	*Days*	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
11	1	Check		Dec. 6, 1930	10	12	33	1	44	78	97.1	2.9	42.3
12							115	2	28	145	98.3	1.7	79.3
13	Total or weighted mean						148	3	72	223	98.0	2.0	66.4
14, 15, 16	8	133 per cent	Dec. 2–5, 1930	Dec. 3–6, 1930	10	9–12	27	775	442	1,244	3.4	96.6	2.2

Record No.	Fruits used	Hydrocyanic acid schadule	Date treated	Date collected	Mortality by writers' method: A+Dy	Mortality by writers' method: $\frac{Dy}{A+Dy}$ of check	Mortality by writers' method: Dying naturally	Mortality by writers' method: Killed by treatment		Mortality by usual method: $\frac{A}{A+Dy+Dr}$ of check	Mortality by usual method: Survivors: Natural	Mortality by usual method: Survivors: Observed	Mortality by usual method: Killed by treatment	
	Number				*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Per cent*	*Number*	*Number*	*Number*	*Per cent*
14	8	133 per cent	Dec. 2–5, 1930	Dec. 3–6, 1930	802	2.0	16.0	759.0	96.56	66.4	826.0	27	799.0	96.73
15					802	1.7	13.6	761.4	96.57	79.3	986.5	27	959.5	97.26
16					802	2.9	23.3	751.7	96.53	42.3	526.2	27	499.2	94.87

If it proves impossible to make adequate counts on natural mortality for use as given here, there is sufficiently small variation in the results obtained to justify an approximation. For female red scales from the fully developed second stage to the mature adult stage on lemons under field conditions similar to those at Whittier, Calif., a natural mortality of 4 per cent, exclusive of dried individuals, may be assumed. Though this figure is not based upon a full year's record, the work covers six months and includes the winter and spring seasons.

SUMMARY

In tests of hydrocyanic acid gas as a fumigant against the California red scale (*Chrysomphalus aurantii* Mask.), natural mortality as ordinarily considered varies so widely that better means of estimating it have been sought. By using lemon fruits exclusively and the three later stages in the development of the female only—that is, the fully developed second stage, the gray adult, and the mature adult—qualitative tests based on such factors as changes in body color and conformation have not given a satisfactory separation between natural mortality and mortality produced by treatment, which are mixed on treated samples, for any of these three periods. It has been found, however, that in each of the three periods natural mortality bears nearly a constant ratio to the number of living plus dying scales on untreated samples irrespective of a large number of varying factors ordinarily encountered in practice.

When counted at the proper time after treatment, practically all scales killed by treatment and those dying naturally can be distinguished, chiefly by changes in color and in viscosity of body contents. By using the relatively stable values obtained to express natural mortality, this factor can be eliminated from such counts on treated samples and a considerably higher degree of accuracy can be obtained than is possible by the old method of estimating the natural mortality from the ratio of living scales to total scale count.

This study indicates that it may be possible to determine constants for the correction of natural mortality in the California red scale, or in other insects, without separate counts for every experiment.