STATIC AND DYNAMIC VARIATION IN THE RESPONSE OF AN INSECT TO SUBLETHAL DOSES OF TWO GASES

C. I. Bliss
and
R. L. Beard

**THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN**

Bulletin 577 — November, 1953

STATIC AND DYNAMIC VARIATION IN THE RESPONSE OF AN INSECT TO SUBLETHAL DOSES OF TWO GASES

C. I. Bliss and R. L. Beard[1]

Variation in the response of insects to poisons is unavoidable, as every insect toxicologist is well aware. Even though his insects are from a genetically uniform stock, his environmental control is superb, and the toxicant is administered in exactly equal doses to each individual, at certain dosage levels some will live and others die. At the time of the test, the survivors must have been more resistant to the toxicant than those which died. Assuming the same percentage survival, would the same individuals have survived if the test had been made two days earlier or a week later? If they would have been the same, the variation in resistance would be called "static," but if those surviving both tests were no more numerous proportionately than the square of the proportion surviving one test, the variation in resistance would be called "dynamic." These two types of variation were first recognized in the response of organisms to drugs by Gray in 1931 (Clark 1933), but they have an important bearing upon the development of resistance to insecticides, as has been noted by Beard (1952a). If the variation in susceptibility were entirely static, the rapidity of selection would depend primarily upon the extent to which the resistance of an individual were genetic. Conversely, if the variation were wholly dynamic, individuals surviving any given treatment might not differ inherently from those which failed to survive.

Obviously, the question raised above cannot be answered experimentally with mortality as the response. The relative stability of an individual insect's reaction can be determined only if it can be measured repeatedly. One reaction which meets this requirement is the time required for recovery from a toxicant which stupefies the insect well before a fatal dose is absorbed. Furthermore, in experiments on Drosophila, Broadbent and Bliss (1936) obtained substantially the same toxicological relation with the time of recovery from sublethal doses of hydrocyanic acid gas as from conventional dosage-mortality curves. A marked dynamic variation has been demonstrated by Beard (1952b) in the response of several test species to repeated injections of nicotine and of other toxicants.

The present experiments extend these results by means of two gaseous toxicants, carbon dioxide and nitrous oxide, which can be handled conveniently in repeated applications. In measuring the relative magnitude of static and dynamic variation, several toxicological questions developed. Does the recovery of coordinated movement precede complete physiological recovery from the gas, and if so, how long a period is required for the insect to regain its original state? Does recovery vary with the toxicant? How is recovery time related to length of exposure? These are some of the questions which required answers before static and dynamic variation could be evaluated.

[1] The authors acknowledge with thanks the assistance of Mrs. M. B. Gentry, Jr. in conducting the tests and of Miss T. Santilli in calculating the results.

Methodology

The present study involved a series of experiments and their statistical analysis. It is convenient to list them chronologically, even though the results will not be presented in this order.

Experimental procedure. The adult milkweed bug, *Oncopeltus fasciatus,* served as the test insect throughout. Bugs were reared on milkweed seed and water at a constant temperature of 76° F. and tested five to seven days after the final moult. Each was numbered before testing and its response recorded separately. The test bugs were caged overnight in groups, with water and food.

The toxicants, carbon dioxide and nitrous oxide, were administered from pressure cylinders in a room maintained at a constant temperature of 76°F. The gas, introduced at a constant pressure as measured by a flow-meter, rapidly displaced the air in the container holding the bug. In two tests (Nos. 4 and 7), the bugs were exposed in lots of 15 and 8 respectively in 125 cc. Ehrlenmeyer flasks; in the other five tests, each bug was exposed individually in a 27 cc. vial. The exposure period to carbon dioxide in tests 1 to 3 was 30 seconds (about twice the usual knockdown time) and 45 seconds in test 4, when the corresponding dose of nitrous oxide was 100 seconds. In later experiments, the dosage was varied by gassing individual bugs for two or more different exposure times to study the dosage-response relation within individuals. After exposure to gas, each bug was placed on its back in moving air and the time recorded when it turned over. This righting reflex provided a distinctive end-point that could be recorded to the nearest second. The interval from the end of an exposure until a bug turned over is called here its "recovery time." Bugs were treated repeatedly at varying rest intervals in seven tests, which may be characterized briefly.

(1) On January 7, 1952, each of 20 bugs received eight exposures to carbon dioxide, each exposure starting just one hour following the start of its predecessor. The original recovery times in seconds are given in Appendix Table 1. In comparison with later tests, recovery from the initial exposure was exceptionally slow, so that the calculations have been restricted to the second to the eighth exposure.

(2) On February 26-28, each bug was exposed four times on each of three successive days in a 3 × 3 Latin square design. The rows of the square were represented by three groups of ten bugs, columns by successive test days and letters by rest intervals of 5, 20 and 80 minutes between the righting reaction of each bug and its next exposure. Thus, each rest interval was tested equally on every bug and on each day. In one of the three groups, only eight bugs completed the experiment.

(3) On July 1-3, the second test was repeated with 11 bugs in each group and five exposures on each day instead of four. The original recovery times of these bugs are given in seconds in Appendix Table 2.

(4) Carbon dioxide and nitrous oxide were compared on January 22 and 23, 1953 at exposure periods equal approximately to three times the average knockdown time. On each day, four lots of 15 bugs were exposed four times, twice to CO_2 and twice to N_2O. Two lots on each day were exposed twice to one gas before exposure to the other gas. In the other two lots the gases were alternated. Seventy-five minutes elapsed between

the recovery of the last bug in the first lot and its next treatment, the other lots following in sequence without relation to their recovery times. The original data for this test may be found in Appendix Table 3.

(5) The dosage-response curve was determined from the successive responses of individual bugs to seven different exposure periods on March 2 to 6. Each bug was exposed three or four times on each test day, with a constant rest interval of 80 (March 2) or 90 (March 3-6) minutes between recovery and the next treatment of each of 14 bugs. The individuals tested on March 2 were replaced by a new set of 14 on March 3, and these, in turn, were retested on March 3 to 6, except for the replacement of two individuals which died. On any one day, the exposure periods or doses represented two Youden squares (1940); on March 3-4, they constituted together two 7×7 Latin squares and in computing dosage-response curves were so analyzed. The treatments on the first three days with CO_2 were followed by similar experiments with N_2O on March 5-6. The recovery times of the second set of bugs to both CO_2 and N_2O are shown in Appendix Table 4.

(6) To insure complete recovery between exposures, the dosage-response curves were redetermined with seven doses, gassing each bug once on four successive days (March 17-20). On each day, 28 bugs were treated with CO_2 and 28 with N_2O in eight 4×7 Youden squares. From this series the original recovery times from CO_2 are given in Appendix Table 5.

(7) The variation among individual bugs in mean recovery time was compared with that in the slope of their dosage-response curves by exposing groups of eight bugs to CO_2 for 130 or 520 seconds on four successive days, treating eight groups on April 13-16 and four additional groups on April 21-24. Each pair of groups provided two successive 2×2 Latin squares on the four days of each test.

Statistical analysis. For statistical analysis, each individual recovery time in seconds was converted to its logarithm, and this served as the metameter (y) of response. In these units the response was distributed approximately normally, which made it manageable statistically. The data were examined by the analysis of variance, first to test the significance of potential sources of variation and then to estimate the *components of variance* associated with dynamic and static variation. Because they are essential for an understanding of the experimental results and are relatively new to many biologists, the idea behind components of variance may be reviewed.

Variability in the response is measured conveniently in terms of the variance. Let us assume that N bugs forming a homogeneous group were exposed to CO_2 in a single test and we have the log-recovery time y of each individual. The variance could be computed from the deviations of the individual responses from their mean $(y-\bar{y})$ by squaring each deviation, summing these squares over all N values, and dividing by the degrees of freedom $(N-1)$, to obtain $s^2=S(y-\bar{y})^2/(N-1)$, where $S(\ )$ indicates the sum of the terms in parentheses. Thus, by definition, a variance averages the squares of all deviations from a given mean, regardless of their source.

These sources may represent many types of variation, and of these, we are interested here in estimating the dynamic and static components.

This could not be done with only one observation from each bug. Instead, each of the N bugs in the group might be exposed twice, say on two successive days, to the same dose of CO_2 and a record made of its recovery time on each occasion. After conversion to logarithms, the relation between the two responses of each bug (y_1 and y_2) might be examined by two different techniques.

We could look at the data graphically, by plotting the second response of each bug (y_2) on the ordinate against its first response (y_1) on the abscissa. If the resulting diagram seemed to be a circular scatter of points with no trend, corresponding to a correlation of zero between y_1 and y_2, an individual's first response would give no better indication of its second response than the mean ($\bar{y}_1$) of the whole group. In this case, variation would be wholly dynamic. Alternatively, the plotted points might define a marked trend, with the bugs which recovered more quickly from the first exposure tending to recover earlier than the others from the second exposure. In this case, a bug's second response y_2 could be predicted better by taking account of its first response y_1 than by using the group mean $\bar{y}_1$ alone. To the extent that this occurred, there would be a static element in the variation.

Alternatively, we could isolate the dynamic and static elements in terms of their respective variance components. Not only is this technique more convenient, but it can be extended readily to more complex cases (Crump 1951). Two mean squares would be required. One would be determined from the differences between the two responses of each individual bug (y_1-y_2). When squared, summed, and corrected for the average difference between days, the resulting mean square

$$\{S(y_1-y_2)^2-S^2(y_1-y_2)/N\}/2(N-1)=\hat{\sigma}_1^2$$

would measure the dynamic variance component, or the extent to which the second response of each bug failed to duplicate its first response. The other mean square would be computed from the variation among the sums (or averages) of the two responses for each bug, leading to a second, independent estimate A. In symbols it would be equal to

$$\{S(y_1+y_2)^2-S^2(y_1+y_2)/N\}/2(N-1)=A.$$

If the variation among bugs were no greater than would be predicted from that within bugs, this second mean square, A, would be another estimate of the dynamic variation and the ratio $F=A/\hat{\sigma}_1^2$ would not differ significantly from 1.

More often, however, the second mean square (A) will be significantly larger than the first ($\hat{\sigma}_1^2$). In this case the mean square among bugs would contain demonstrably an additional "static" variance component, that between bugs $\hat{\sigma}_2^2$, which is comparable to the dynamic component $\hat{\sigma}_1^2$ within bugs. Since variances are additive, this second component could be isolated as $\hat{\sigma}_2^2=(A-\hat{\sigma}_1^2)/2$, where the divisor 2 is equal to the number of measurements on each bug. The relative size of these two components under varying experimental conditions is our clue to the potential importance of the static factor. In the analyses which follow, this will be expressed as the ratio $\hat{\sigma}_2^2/\hat{\sigma}_1^2$. We would expect the static component in fact to be less important here than this ratio would indicate, since the responses upon which it is based are separated at most by three days.

Other relations have been studied by the analysis of variance and of covariance, such as the effect of the interval between treatments, the relation between dosage and response within individual bugs, and the comparative response to carbon dioxide and to nitrous oxide. In computing the data from Youden squares, the sum of squares between bugs was determined as described by Yates (1940).

Some bugs were lost before a test could be completed, and were replaced provisionally from the original stock. Losses were few in number, but to preserve the identity of individual bugs, new values were substituted later for either the original bug or its replacement by the missing plot technique or its equivalent (Cochran and Cox 1950). To avoid distorting mean squares and variance components, two aberrant recovery times, which contributed disproportionately to the sums of squares in tests 5 and 7, have been replaced similarly.

Experimental Factors Governing Recovery Time

Recovery time depended upon three factors under experimental control: the length of the rest interval between the righting reflex and the next exposure to gas, the dose of toxicant as determined by the length of exposure, and the gas used, whether CO_2 or N_2O. The effect of the rest interval was studied with CO_2 only, but presumably the response to N_2O would be modified similarly. The differential response to the two gases may be characterized initially by a comparison of their dosage-response curves, although it will be shown later that the variance was larger for N_2O.

Effect of length of the rest interval. In test 1, individual insects were exposed repeatedly to CO_2, starting a new exposure every 60 minutes. The average recovery time (Appendix Table 1) decreased initially and then increased more or less consistently after repeated exposures. Evidently, the effects of the gas had not disappeared completely when the bugs had recovered sufficiently to turn over. Despite the consistency of the average trend, the curves for the individual bugs in the test varied significantly. Some recovered fully in the hour between exposures and showed no trend; others "accumulated" the toxicant progressively.

The effect of rest intervals of 5, 20 and 80 minutes between the righting reaction, which marked the end of the recovery time, and the start of the next exposure was examined in two experiments. Because of their design, differences between successive days and their interaction with the length of the rest interval could be removed statistically, either partially (test 2) or completely (test 3). Each group was tested with a single rest interval on each day. If recovery from carbon dioxide were complete overnight, the curves for the three intervals would be expected to converge to the same initial response. In this respect, the two experiments resembled slope ratio assays (Bliss 1952). Their means have been plotted in Figure 1 and fitted in each case by three converging straight lines.

It is evident from Figure 1 that straight lines described effectively the relation between order of treatment and response; the longer initial recovery time in the first test had substantially vanished. The responses of 11 bugs in each lot to each of the five exposures to CO_2 in test 3 have been summarized as totals in Table 1; their averages are plotted in

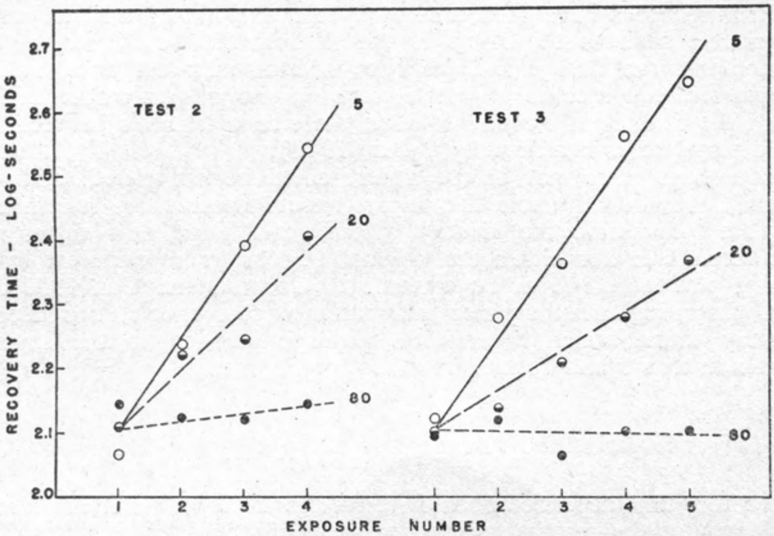

Figure 1. Increase in the recovery time from successive 30-second treatments with CO_2 for rest intervals of 5, 20 and 80 minutes between each righting reflex and the next exposure.

Figure 1. These data and the corresponding values for test 2 have been examined by the analyses of variance in Table 2. The sources of variation that could be segregated by virtue of the experimental design are listed in rows 1 to 3 of the table, the other terms have been computed as described elsewhere (Bliss 1952) for slope-ratio assays.

TABLE 1. ORIGINAL DATA FOR TEST 3 IN TERMS OF THE TOTAL RESPONSE $S(y)$ OF 11 BUGS IN EACH LOT ON EACH DAY AFTER 30-SECOND EXPOSURES TO CO_2. THESE FOLLOWED ONE ANOTHER AT REST INTERVALS OF 5, 20 AND 80 MINUTES AS INDICATED. THE MEANS FOR EACH TREATMENT HAVE BEEN PLOTTED IN FIGURE 1.

Interval in min.	Date 1952	Lot of bugs	$S(y)$ for response number 1	2	3	4	5
5	7/1	C	13.370	15.182	16.046	17.293	18.213
	7/2	B	12.195	14.406	15.369	18.294	18.992
	7/3	A	11.512	12.603	13.578	15.894	17.016
20	7/1	B	12.193	12.502	13.697	14.354	15.547
	7/2	A	12.805	13.022	13.535	14.325	15.220
	7/3	C	11.402	12.065	12.658	13.533	14.360
80	7/1	A	12.579	12.412	10.816	12.361	12.060
	7/2	C	11.929	13.262	12.370	11.838	11.888
	7/3	B	11.621	11.275	11.967	12.188	12.434*

* Missing results for five bugs replaced in the same proportion as their first four responses to those of the remaining six bugs.

TABLE 2. ANALYSIS OF VARIANCE FOR TESTING AGREEMENT WITH THE COMPUTED CURVES IN FIGURE 1, AS DETERMINED FROM THE TOTALS FOR TEST 3 IN TABLE 1 AND FROM THE CORRESPONDING VALUES FOR TEST 2.

Row No.	Term	Test 2 DF	Test 2 Mean Square	Test 2 F	Test 3 DF	Test 3 Mean Square	Test 3 F
1	Among lots of bugs	2	.02305	.61	2	.08892	3.61
2	Among test days	..	......	....	2	.45144	18.31
3	Day × lot × interval ..	..	......	....	2	.10613	4.30
4	Effect of slope	3	2.13727	56.26	3	4.83751	196.2
5	Discrepancy of mean initial response	1	.00154	.04	1	.00255	.10
6	Variation in initial response	2	.04214	1.11	2	.00722	.29
7	Non-convergence	2	.00192	.05	2	.03432	1.39
8	Scatter about computed lines	3	.03067	.81	6	.02317	.94
9	Remainder or error	22	.03799	1.00	24	.02466	1.00

The critical terms in Table 2 tested the agreement of the plotted points with the lines in Figure 1. The observed mean initial response agreed excellently with the point of convergence when the three lines were computed only from the reactions at later exposures (row 5), and the three estimates of the initial response agreed satisfactorily (row 6). Curves computed without forcing convergence agreed at the initial level (row 7). The deviations about the three lines in each experiment (row 8) were somewhat less than the error (row 9) based upon the variation among lots. The smaller error in test 3 than in test 2 was due in part to the segregation of additional sources of potential variation. Both analyses, in short, justified the fitting of three straight lines with a common origin.

The lines plotted in Figure 1 converged at Y=2.105 and 2.104 in tests 2 and 3, with respective slopes for each rest interval of b_5=.1446 and .1405, b_{20}= .0932 and .0607, b_{80}=.0122 and −.0025. The slopes for an interval of 20 minutes in tests 2 and 3 differed just significantly (t=.0325 ± .0140, $P < .05$). The corresponding values of the other three constants agreed very well. As will be shown later, bugs varied more after a rest interval of 20 minutes than after either 5 or 80 minutes of rest.

The slopes of these lines were themselves indices of relative recovery and were related linearly to the logarithm of the rest interval (Figure 2). Although of unequal reliability, both tests could be plotted as parallel straight lines, indicating the same toxicological relations. Given an exposure to CO_2 of 30 seconds, the bugs apparently returned to their original physiological state within a rest period averaging about 80 minutes. As interpolated from Figure 2, the average rest intervals for complete recovery in tests 2 and 3 were 104 and 73 minutes respectively, the latter having about one-fourth as large a variance as the former.

Dosage-response curves for CO_2 and N_2O. In tests 5 to 7, dosage-response curves were determined by measuring the recovery time from varying lengths of exposure to CO_2 and N_2O. Since the same individuals received several different doses, the curves were based upon comparisons within bugs. In test 5, each bug was treated three or four times each day at rest intervals of 80 or 90 minutes, long enough to permit complete

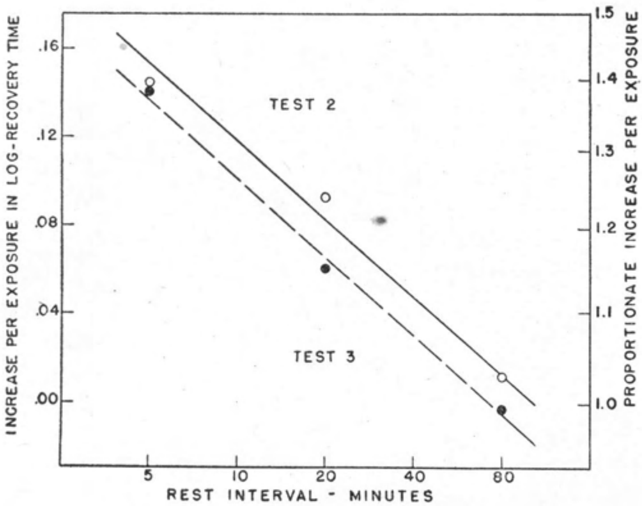

Figure 2. Relation of the increase in log-recovery time, as measured by the slope of the lines in Figure 1, to the length of the rest interval.

recovery from the shorter exposures at least. To insure against incomplete recovery, dosage-response curves were redetermined in test 6 with only one exposure on each of four successive days. Because of their design in Youden squares, neither test 5 nor 6 showed how well individual bugs agreed in the slope of their dosage-response curves. This was the purpose of test 7, in which each bug was exposed twice to each of two dosage levels, with one exposure on each of four successive days.

The log-recovery time in the three experiments with CO_2 has been plotted against the log-exposure time in Figure 3, the curves with a rest interval of 80 or 90 minutes at the left and of one day at the right. In both series, the relation could be fitted by a straight line at exposures longer than about 80 seconds. At shorter exposures, recovery was slower than would be expected from a downward projection of the line, as if recovery were controlled by two reactions, one limiting its rate at the shorter exposures and the other taking over above a critical level. In the upper range the dosage-response curves were the same both in position and slope for rest intervals of either 80 or 90 minutes. Moreover, increasing the rest interval to one day did not change the slope, so that both lines in Figure 3 have been drawn with a combined slope of $b_c = 0.779 \pm .014$. The curves in tests 5 and 6 (both in March) agreed substantially in position as well as in slope, so that any displacement in the curves for test 7 (in April) has been attributed to changes in bug sensitivity. To allow for such a difference, an adjustment has been subtracted from each log-dose in the two-dose tests to bring their means on the line fitted to test 6.

Similar dosage-response curves were determined for N_2O either with the same bugs (test 5) or at the same time with other bugs (test 6). The results have been plotted in Figure 4 in log units. As with CO_2, the plotted points for all curves could be fitted over most of the dosage range by parallel straight lines, in this case with one omission from the

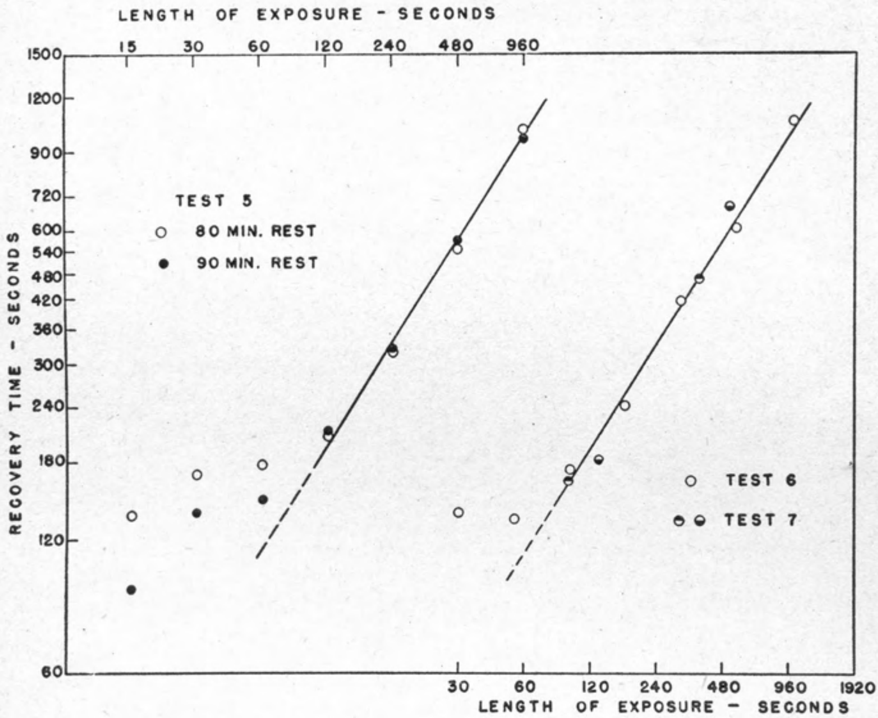

Figure 3. Dosage-response curves for the recovery time from different lengths of exposure to CO_2; the computed curves were determined from the range of doses covered by solid lines.

upper or lower end of each curve. The combined slope of $b_c = 1.060 \pm .038$ was 36 percent steeper than that for CO_2, a very significant difference. Variation of the means about the lines exceeded the error within bugs but not significantly. The curves also differed from those for CO_2 in suggesting an upper limit to the recovery time from a sublethal dose and giving little evidence of a secondary reaction below a lower limit which would change the slope. In test 6, the pressure control of N_2O during gassing behaved badly and this may have caused the aberrant low value in its curve.

Static Components of Variance

Many factors contributed potentially to the variation in recovery time. Those which could be identified have been segregated in separate rows of one or more analyses of variance for each test. In some rows the mean square represented an average difference in response to a selected treatment or environmental effect with a significance which could be tested by the statistic *F*, such as in rows four to eight of Table 2. Other mean squares estimated the variability associated with different kinds of experi-

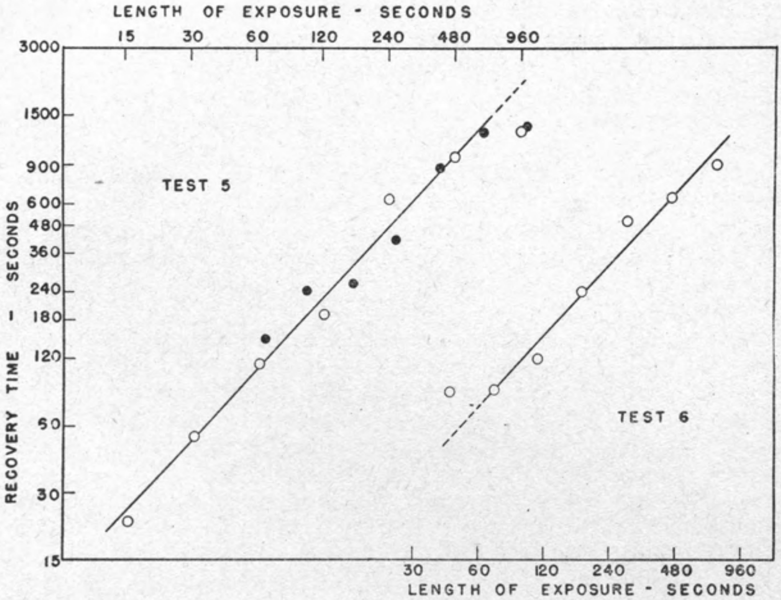

Figure 4. Dosage-response curves for the recovery time from different lengths of exposure to N_2O.

mental units, which, in turn, were assumed to be random samples from populations of similar units. These included the variation in recovery time within individual bugs, among different bugs, and from one day to another. Each response depended upon some factors which were restricted by the design of the experiment to specific rows in the analysis of variance. Other factors, including the dynamic component or random error, occurred in all mean squares. Variance components have been isolated from the relevant mean squares for the assignable sources of variation.

The variance components between and within bugs were directly comparable, the former measuring a static and the latter a dynamic element. Their meaning was limited in each case, however, by the conditions of the experiment. Thus, if a "between bug" component were determined from several exposures on the same day, it would probably include dynamic as well as static variation. If the same insects had been exposed over a longer period of several days, some of the variation that in a single day's test was apparently static might well have shifted to the dynamic component, increasing its relative magnitude.

Other persistent similarities in the response of individual bugs were expressed more readily in other terms, such as correlation coefficients. They were also evidence of static variation that would be essential for the development of resistance. Quantitatively, they were subject to the same reservations in timing that qualified the interpretation of static components of variance.

Variance components for different test periods. The variability between and within bugs gassed repeatedly on the same day was compared in tests 1 to 4. Each response was potentially the sum of the recovery time under equivalent physiological conditions plus a component added by incomplete recovery from the preceding treatments. Except for the initial recovery time in the first test, which here has been omitted, the response increased more or less linearly in successive exposures when they followed one another at too close intervals. Any pattern related to the order of testing was first segregated from the variance within bugs by removing the sum of squares for the means of successive treatments, and also, in the first three experiments, the variability from bug to bug in the linear trend of response upon the order of treatment. The mean square between bugs was computed from the total response of each bug on a single day.

The resulting mean squares and variance components within days are summarized in Table 3. All F values between bugs were highly significant ($P \leqslant .01$), testifying to the presence of both static and dynamic variation. The ratio of the static to the dynamic variance component is given for each test in the last column of the table. In test 1, the variance component between bugs was relatively large, but its size could not be attributed to differences in the trend of recovery time on order of testing when this possibility was tested critically. Tests 2 and 3 have been represented here by data for a rest interval of 80 minutes, after which recovery was presumably complete. In both tests, the static component was relatively small, in contrast to that in test 4 where the rest interval of 75 minutes was nearly as long. Thus when variance components were determined from tests completed on the same day, the apparent static component (between bugs) fluctuated widely, from less than one-fourth to more than twice as large as the dynamic component within bugs.

TABLE 3. VARIANCE COMPONENTS ($\hat{\sigma}_1^2$) FROM THE RESPONSE OF INDIVIDUAL BUGS TO CO_2 IN REPEATED TESTS ON THE SAME DAY.

Test No.	Between bugs				Within bugs		Ratio
	DF	MS	F	$\hat{\sigma}_2^2$	DF	$MS = \hat{\sigma}_1^2$	$\hat{\sigma}_2^2/\hat{\sigma}_1^2$
1	19	.14390	20.50	.01955	94	.00702	2.78
*2	25	.02527	2.96	.00418	50	.00854	.49
*3	30	.02276	2.18	.00246	83	.01046	.23
4	112	.01969	4.66	.00773	112	.00422	1.83

* Response at rest intervals of 80 minutes only.

The variability in the average response of individual bugs over three or four days was computed from the initial response on each of three days in tests 2 and 3 and on four days from test 7, in each case after segregating the variability between lots, between days, and lots by days (Table 4). The variances within bugs were estimated from the interactions of bugs by days. These dynamic components were smaller than in replications completed on the same day. However, where the same bugs were involved (tests 3 and 4), the ratio of the "static" component between bugs to the dynamic component within bugs was nearly the same. None of the "static" components in these tests covering several days was as large relatively as in experiments completed on the same day. The importance of the interval covered by an experiment for estimating relative static variability is evident.

TABLE 4. VARIANCE COMPONENTS ($\hat{\sigma}_1^2$) FROM THE RESPONSE OF INDIVIDUAL BUGS EXPOSED TO CO_2 ONCE ON EACH OF THREE OR FOUR SUCCESSIVE DAYS.

Test No.	Test period days	Between bugs DF	Between bugs MS	Between bugs F	Between bugs $\hat{\sigma}_2^2$	Within bugs DF	Within bugs $MS = \hat{\sigma}_1^2$	Ratio $\hat{\sigma}_2^2/\hat{\sigma}_1^2$
2	3	25	.01532	2.68	.00320	50	.00571	.56
3	3	30	.01027	1.57	.00124	60	.00655	.19
7a	4	56	.01145	3.60	.00207	111	.00318	.65
7b	4	28	.00817	3.08	.00138	55	.00265	.52

The static component in rate of recovery. When bugs were exposed to the same treatment at equal intervals that were too short to allow complete recovery, the mean response from successive exposures increased by a constant increment or slope (Figure 1). This slope on order of treatment varied sufficiently from bug to bug that the mean square for the variation in slope between bugs sometimes exceeded the random error significantly. In test 1, for example, the respective mean squares were .0160 and .0070 with 19 and 94 degrees of freedom respectively. Similar mean squares have been computed for each rest interval in tests 2 and 3 (Table 5, row 2). In both tests (Table 5), the slopes for individual bugs varied no more from one another, with rest intervals of 5 and of 80 minutes, than the responses about the fitted straight lines, whether the mean trend was highly significant (5 minutes) or indistinguishable from zero (80 minutes). With a 20 minute interval, however, the bugs differed markedly from one another in slope. Twenty minutes, therefore, marked a critical point in the recovery from a 30 second exposure to carbon dioxide, some bugs nearly recovering and others accumulating progressively more gas in successive tests.

TABLE 5. MEAN SQUARES AND VARIANCE COMPONENTS WITHIN LOTS FOR EACH REST INTERVAL IN TESTS 2 AND 3.

Term for	DF	Mean squares for interval of 5 min.	20 min.	80 min.
Test 2				
Between bugs, total response	25	.03729	.09387	.02527
Between bugs, slope on order	25	.01634	.04261	.00798
Within bugs about regressions	50	.01353	.01829	.00854
Component between bug totals	25	.00594	.01889	.00418
Component for bug totals after removing effect of slope	24	.00452	.00487	.00444
Test 3				
Between bugs, total response	30	.04484	.06028	.02276
Between bugs, slope on order	30	.00975	.02562	.01211
Within bugs about regressions	90	.00974	.00839	.01046*
Component between bug totals	30	.00702	.01038	.00246
Component for bug totals after removing effect of slope	29	.00711	.00766	.00255

* Based on 83 degrees of freedom.

The mean squares and variance components for bug totals within lots were largest for the 20 minute rest interval in both tests 2 and 3, followed in order of decreasing size by those for 5 and for 80 minutes.

The variation in slope between bugs contributed materially to the larger variance component between bug totals at the 20 minute interval. When its effect was removed by covariance, the between bug component was reduced at 20 minutes by 74 percent in test 2 and by 26 percent in test 3, without comparable reductions at intervals of 5 and 80 minutes.

By timing successive treatments so as to maximize the variability among bugs, here at 20 minutes, individuals with a greater ability to detoxify the absorbed CO_2, and hence a smaller slope on order, might be discriminated more readily from those in which the toxicant tended to accumulate. If this had selective value, the slope observed in individual bugs for a 20 minute rest interval should be correlated with that for 5 minutes in the same bugs, as in fact proved the case. The correlation coefficients for test 2 (r=.373) and for test 3 (r=.429) gave a statistically significant weighted average of r=.404 ($P < .003$). Hence the rate at which a bug could detoxify the residual toxicant, after it had recovered sufficiently to turn over, provided another static element of potential selective value.

Comparative response to CO_2 and N_2O. Test 4 compared the recovery time from 45 seconds of carbon dioxide with that from 100 seconds of nitrous oxide. Although these exposure periods were approximately three times their respective knockdown times, recovery from CO_2 took 20 percent longer on the average than that from N_2O. This difference and one of similar magnitude between the two test days have been excluded in comparing the response to the two gases. A preliminary comparison showed consistently more variability with N_2O than CO_2, so that separate analyses of variance have been computed for each gas and also the covariance between them. The variances and covariances within exposures for four comparable lots, each of 15 bugs, have been summarized in Table 6.

TABLE 6. MEAN SQUARES AND PRODUCTS AND THE CORRESPONDING VARIANCE COMPONENTS FOR THE RESPONSE WITHIN LOTS OF BUGS FOLLOWING EXPOSURES OF 45 SECONDS TO CO_2 AND OF 100 SECONDS TO N_2O IN TEST 4.

Row	Term for	*DF*	Mean squares and products $V(CO_2)$	Covariance	$V(N_2O)$	*F* ratio CO_2	N_2O
1	Alternate exposures . . .	56	.00560	−.00130	.01201	1.96	2.66
2	Successive exposures . .	56	.00285	.00119	.00451	1.00	1.00
3	Between bugs	56	.03013	.02010	.07075	7.13	8.57
4	Bugs × days	56	.00924	.00406	.01506	2.19	1.82
5	Within days and bugs .	112	.00422	−.00006	.00826	1.00	1.00
			Variance components			r^2	N_2O/CO_2
6	Between bugs, static . . .	56	.00522	.00401	.01392	.221	2.67
7	Bugs × days, dynamic .	56	.00251	.00206	.00340	.497	1.35
8	Within days, dynamic .	112	.00422	−.00006	.00826	.000	1.96

In the two tests on the same day with each gas, one-half of the differences in response were between successive exposures and one-half between alternate exposures separated by treatment with the other gas. The variability with these two patterns has been separated in the first two rows of Table 6. With both gases, the mean square for alternate responses was twice or more that for successive responses, a difference which was statistically significant. Whether this was due to timing or to a change in fumigant cannot be determined from the present data.

With both gases, the response of individual bugs varied more from one day to the next than within the same day, and their totals for two days gave a still larger mean square for the variability between bugs (Table 6, rows 3-5). So far as these can be identified with either static or dynamic variation, over-all differences between bugs, in excess of the interaction for bugs by dates, represented the static element. With each gas it was the largest single variance component (row 6). Overnight the bugs changed enough to add another dynamic variance component (row 7) to that for repeated tests on the same day (row 8), averaging in the latter component the results of both alternate and successive treatments. For CO_2, the static component between bugs in row 6 was smaller than the sum of the two dynamic components in rows 7 and 8, giving a ratio of $\hat{\sigma}_2^2/\hat{\sigma}_1^2=.78$. For N_2O the static component was larger than the two dynamic components, with $\hat{\sigma}_2^2/\hat{\sigma}_1^2=1.17$. With both gases, some of the "static" variation would no doubt prove to be dynamic if the test period had been longer than two days. The statistical significance of this difference in the response to the two gases has not been determined. Both the mean squares and variance components in Table 6 showed consistently a greater variability for N_2O than for CO_2. Since the same individuals were used with both gases, the differences in variance were apparently characteristic of the two toxicants.

The square of the coefficient (r^2) for the correlation in the response to the two gases measured what proportion of the variation observed with one gas could be accounted for by the variation observed with the other. As computed from the components of variance and covariance, 22 percent of the over-all variability among bugs was shared by both toxicants, representing a correlation coefficient of $r=.48$. There was no correlation in the responses to the two gases from replications on the same day, but one-half of the additional dynamic variation in tests on successive days was common to both toxicants, representing a correlation of $r=.70$. It is evident that individuals which were more susceptible to one gas tended to be more susceptible to the other.

Static elements in tests with a variable dose. Variance components have been computed from the dosage-response data for both CO_2 and N_2O (Table 7). The component within bugs was consistently larger than in the earlier one-dose experiments; presumably it contained an additional variance component for slope that could not be isolated effectively in tests 5 and 6. As in test 4, the variance within bugs for N_2O exceeded that for CO_2. Here the components between bugs had substantially the same relation to those within bugs for both gases.

The extent to which individual bugs differed in the slope of their dosage-response curves for CO_2 was determined in test 7. With exposures of 130 and 520 seconds, both in the linear zone of the complete curve, the average slope agreed well with that of previous tests. The simultaneous exposure of groups of eight bugs reduced the variance within bugs

TABLE 7. VARIANCE COMPONENTS ($\hat{\sigma}_1^2$) FROM THE RESPONSE OF INDIVIDUAL BUGS TO DIFFERENT LENGTHS OF EXPOSURE TO CO_2 AND N_2O IN TESTS 5 AND 6; FROM YOUDEN OR LATIN SQUARES.

Gas	Test No.	Between bugs *DF*	*MS*	*F*	$\hat{\sigma}_2^2$	Within bugs *DF*	$MS = \hat{\sigma}_1^2$	Ratio $\hat{\sigma}_2^2/\hat{\sigma}_1^2$
CO_2	5	13	.02869	2.57	.00438	33	.01115	.39
		13	.04406	3.27	.00437	70	.01347	.32
	6	27	.02936	1.83	.00332	75	.01607	.21
N_2O	5	13	.04110	1.24	.00199	32	.03316	.06
		13	.03910	2.27	.00729	20	.01723	.42
	6	27	.09279	1.83	.01050	75	.05077	.21

and groups (Table 8) to a fraction of that observed (Table 7) when bugs were treated differently. From the significant *F*-ratios for bug slopes in Table 8, it is evident that bugs varied in their relation to the length of exposure as well as to the length of the rest interval between exposures. In this case, however, the covariance of means by slope was negligible (r=.07 and .27). In the absence of such a correlation, the small differences between the mean squares at the high and low doses in the lower part of Table 8 was not surprising. This also confirmed the assumption made in computing the dosage-response curves that the variability in the response did not depend upon the dose.

TABLE 8. COMPARISONS IN TEST 7 OF VARIANCES AND COVARIANCES FOR LOG-RECOVERY TIME WITHIN GROUPS OF BUGS EXPOSED FOR 130 AND 520 SECONDS TO CO_2.

Term	April 13-16 *DF*	Mean square	Ratio	April 21-24 *DF*	Mean square	Ratio
Variance: bug means	56	.01145	3.60	28	.00817	3.08
Covariance: means × slope	56	.00052*	...	28	.00154*	...
Variance: bug slopes	56	.00508	1.60	28	.00393	1.48
Variance within bugs, $\hat{\sigma}_1^2$	111	.00318	1.00	55	.00265	1.00
Between bugs: high dose	56	.00878	1.13	28	.00759	1.68
Between bugs: low dose	56	.00775	1.00	28	.00450	1.00
Within bugs: high dose	56	.00329	1.07	28	.00276	1.09
Within bugs: low dose	55	.00306	1.00	27	.00254	1.00

* Mean product, not significant.

Summary

The rate at which an insecticide-resistant race can be developed by selection may be governed by the relative magnitude of static and dynamic variation in the response of individual insects to the toxicant. In order to estimate the relative magnitude of these two types of variation, bugs were exposed repeatedly to sublethal doses of CO_2 and N_2O. On removal from the gas, anaesthetized bugs were placed on their backs and the time noted when they turned over; the logarithm of this recovery time served as the response.

The response was conditioned by several toxicological characteristics. With repeated exposures of 30 seconds to CO_2 and rest intervals of less than 80 minutes between the righting response and the next exposure to gas, the time for the righting response increased progressively. This increase was more rapid and greater in extent with shorter rest intervals, indicating that the righting response preceded complete physiological recovery. For exposures to CO_2 of more than 80 seconds, the response increased linearly with the logarithm of the exposure period. At shorter exposures, the dosage-response curve tended to flatten out. A similar relation was observed with N_2O, except that its slope was 36 percent steeper than that for CO_2 and showed little evidence of a reduction in slope at shorter exposures.

Variance components between and within bugs were computed by standard methods. When restricted to the response of individual bugs on the same day, the average variation between bugs exceeded that within bugs by 50 percent, with a tenfold range in the ratio of the two components. When tests were continued over three or four days, the static component averaged only one-half as large as the dynamic component, their ratio ranging from 0.19 to 0.65. Both static and dynamic variance components for N_2O averaged 2.2 times as large as the corresponding values for CO_2. About 22 percent of the variation between bugs was common to both gases, as measured by the square of the correlation coefficient.

Bugs exposed for 30 seconds to CO_2 differed not only in their mean response time but also in the rate of their recovery after the righting reflex, in one test when exposed at intervals of 75 minutes and in two tests when rested for 20 minutes. This characteristic accentuated differences in the mean response of individual bugs and persisted from one day to another, as shown by the significant correlation in the rate of increase per exposure in individual bugs between 5 minute and 20 minute rest intervals. Bugs also differed significantly in the slope of their dosage-response curves, although this factor did not seem to accentuate the variation between bug means.

Although different ratios of static and dynamic components can be expected in responses to different chemicals, the fact that significant dynamic components exist gives validity to earlier speculations that some individual resistance to applied toxicants can be phenotypic rather than genotypic. The identification of these components with physiologic events associated with toxic action might go a long way in effecting an understanding of the development of resistance in insect populations.

APPENDIX TABLE 1. TEST 1. RECOVERY TIME IN SECONDS FOLLOWING 8 SUCCESSIVE 30-SECOND EXPOSURES TO CO_2 AT 60 MINUTE INTERVALS ON JANUARY 7, 1952.

Bug No.	Seconds recovery time from exposure							
	1	2	3	4	5	6	7	8
1	155	141	95	99	259	258	338	170
2	164	120	149	115	165	158	348	271
3	168	119	154	180	211	264	218	393
4	137	239	350	253	228	323	220	391
5	225	132	159	172	255	204	179	318
6	220	171	199	205	230	199	328	415
7	238	163	201	204	220	203	295	283
8	239	333	383	398	442	360	589	890
9	271	258	197	223	261	355	345	326
10	333	215	203	217	390	362	335	175
11	726	353	495	458	481	585	690	818
12	465	300	210	210	348	205	343	468
13	141	173	156	113	149	193	225	225
14	172	121	192	123	215	346	517	581
15	264	115	161	102	216	153	215	251
16	192	118	197	305	282	214	361	167
17	216*	144	205	185	218	206	332	321
18	135	117	111	118	146	136	110	163
19	412	165	324	213	418	487	483	418
20	236	168	259	117	352	189	342	325*

* Calculated (in logarithms) by the missing value technique.

APPENDIX TABLE 2. TEST 3. RECOVERY TIME IN SECONDS FOLLOWING FIVE SUCCESSIVE 30-MINUTE EXPOSURES TO CO_2. THE REST INTERVAL IN MINUTES FOR THE BUGS IN EACH LOT (A, B OR C) IS SHOWN AT THE TOP OF THE COLUMN FOR JULY 1, 2 AND 3, 1952, RESPECTIVELY. THE LOG-RECOVERY TIMES ARE TOTALLED IN TABLE 1.

Bug No.	Rest Interval			Bug No.	Rest Interval			Bug No.	Rest Interval		
	80	20	5		20	5	80		5	80	20
A1	158	94	99	B1	165	136	112	C1	167	129	117
	182	92	114		195	248	119		241	149	120
	92	95	175		140	247	170		284	114	118
	117	188	219		247	496	242		304	118	124
	129	219	316		298	589			569	110	151
A2	100	140	106	B2	208	112	123	C2	219	126	125
	102	206	154		152	135	131		360	165	215
	68	206	209		247	238	138		458	132	148
	171	246	324		380	507	135		450	120	124
	138	252	332		446	488			418	115	205
A3	185	156	88	B3	91	129	119	C3	106	111	86
	98	133	106		107	173	108		174	181	78
	74	100	202		175	346	98		240	93	153
	160	151	191		238	543	107		280	86	197
	122	158	324		380	601			439	91	319
A4	206	153	125	B4	159	138	132	C4	159	133	130
	139	134	121		168	150	108		260	164	160
	99	204	181		173	197	80		329	165	233
	131	182	343		181	364	98		497	195	219
	117	530	559		226	540			663	130	207

APPENDIX TABLE 2 (Continued)

Bug No.	Rest Interval			Bug No.	Rest Interval			Bug No.	Rest Interval		
	80	20	5		20	5	80		5	80	20
A5	161	176	116	B5	154	167	109	C5	207	130	87
	193	269	211		141	319	99		381	126	126
	211	378	102		275	439	85		524	110	131
	170	500	431		162	480	133		417	107	346
	185	747	667		360	803			501	102	270
A6	106	146	96	B6	104	143	98	C6	202	169	101
	224	119	114		133	171	110		253	210	122
	86	108	101		176	192	90		248	181	192
	127	146	176		156	260	147		323	171	285
	101	113	219		233	410	186		388	116	294
A7	115	137	110	B7	146	105	124	C7	188	126	129
	101	133	145		193	275	46		164	154	133
	75	133	177		326	210	248		273	153	130
	107	91	233		209	370	189		334	137	144
	125	144	259		233	525	223		419	137	229
A8	159	162	145	B8	92	140	110	C8	147	139	109
	115	172	212		118	180	207		278	197	134
	121	251	257		137	205	119		270	132	114
	161	333	362		118	869	112		580	93	164
	134	290	449		176	550	107		555	166	149
A9	104	178	111	B9	99	118	110	C9	121	58	103
	99	152	117		97	168	90		229	150	105
	172	130	151		72	160	96		262	107	133
	96	149	249		228	417	94		310	86	107
	101	147	232		175	281	79		345	100	207
A10	135	137	126	B10	97	121	110	C10	205	125	94
	152	143	176		100	436	105		175	140	100
	63	208	127		208	475	159		191	154	119
	148	135	451		225	858	90		327	119	155
	129	269	449		279	1027	132		517	151	144
A11	144	146	114	B11	152	115	110	C11	133	127	129
	135	195	117		144	149	106		220	149	126
	80	233	315		147	212	147		227	126	124
	104	375	235		174	277	129		391	122	134
	111	259	310		184	384	122		293	127	139

APPENDIX TABLE 3. RECOVERY TIMES IN SUCCESSIVE COLUMNS OF INDIVIDUAL BUGS IN EACH LOT, FROM EXPOSURES OF 45 SECONDS TO CO_2 AND OF 100 SECONDS TO N_2O ON JANUARY 22 AND 23, 1953. EACH LOT OF BUGS WAS RESTED 75 MINUTES AFTER THE LAST BUG RECOVERED BEFORE THE NEXT GROUP EXPOSURE OF THE LOT.

Lot	Bug	Treatments on January 22				Treatments on January 23			
		CO_2	CO_2	N_2O	N_2O	N_2O	CO_2	N_2O	CO_2
A	1	195	170	114	139	98	145	99	99
	2	125	116	95	90	89	121	97	105
	3	165	123	116	116	121	134	98	132
	4	206	129	73	86	89	137	76	111
	5	136	108	249	321	115	105	95	84
	6	170	119	86	99	115	141	125	141
	7	129	115	93	147	112	128	108	110

APPENDIX TABLE 3 (Continued)

Lot	Bug	Treatments on January 22				Treatments on January 23			
		CO_2	CO_2	N_2O	N_2O	N_2O	CO_2	N_2O	CO_2
	8	243	151	117	126	108	137	141	121
	9	191	180	65	129	102	359	179	315
	10	133	113	77	101	98	116	79	112
	11	241	129	101	104	111	181	102	121
	12	168	148	207	306	229	136	289	131
	13	166	174	94	134	115	126	115	127
	14	155	120	84	127	89	97	134	79
	15	204	168	95	124	90	168	126	176
		CO_2	N_2O	CO_2	N_2O	N_2O	N_2O	CO_2	CO_2
B	16	126	89	210	86	95	112	162	147
	17	119	79	84	76	88	75	113	100
	18	157	99	109	100	100	108	123	94
	19	168	108	159	78	102	72	182	139
	20	193	133	203	173	125	132	185	163
	21	118	84	122	68	103	90	132	113
	22	173	93	228	117	105	111	154	144
	23	117	81	132	81	84	77	122	106
	24	181	134	187	172	120	162	156	132
	25	167	109	102	297	122	176	146	161
	26	160	101	133	206	91	135	132	109
	27	161	212	169	100	138	136	190	166
	28	166	87	136	258	86	93	152	102
	29	164	206	249	277	119	130	114	105
	30	160	91	90	99	90	89	145	97
		N_2O	CO_2	N_2O	CO_2	CO_2	N_2O	CO_2	N_2O
C	31	289	152	221	228	142	110	127	149
	32	119	137	85	109	97	93	102	68
	33	97	87	78	99	99	91	88	89
	34	121	174	93	134	115	76	199	92
	35	108	169	242	103	117	148	134	91
	36	114	150	106	110	108	98	115	93
	37	341	133	121	131	139	74	134	78
	38	106	182	99	139	141	70	129	69
	39	286	150	244	188	133	106	117	101
	40	124	132	87	122	107	88	107	74
	41	116	139	192	148	132	101	96	111
	42	294	124	162	183	109	68	78	70
	43	156	140	121	138	121	112	141	83
	44	110	121	81	95	121	83	100	67
	45	84	96	71	87	88	66	91	55
		N_2O	N_2O	CO_2	CO_2	CO_2	CO_2	N_2O	N_2O
D	46	290	208	140	122	142	121	146	105
	47	86	88	116	133	99	86	65	110
	48	124	118	194	249	123	118	91	103
	49	120	79	129	106	114	114	67	85
	50	94	83	119	110	116	107	99	90
	51	111	94	230	168	117	112	87	123
	52	137	105	228	150	119	99	132	145
	53	155	158	255	187	151	148	123	117
	54	150	77	162	116	121	113	70	90
	55	118	110	210	160	137	125	114	122
	56	345	340	218	188	105	86	186	299
	57	183	144	220	212	189	335	118	162
	58	123	78	150	113	96	109	73	112
	59	109	116	132	95	114	107	102	84
	60	383	408	244	240	194	160	183	258

APPENDIX TABLE 4. RECOVERY TIME (*R*) FROM SUCCESSIVE EXPOSURES (*E*) OF VARYING LENGTH ON THE SAME DAY, BOTH IN SECONDS. BUGS WERE EXPOSED TO CO_2 ON MARCH 3-4, 1953 AND TO N_2O ON MARCH 5-6, IN ALL CASES WITH A REST INTERVAL OF 90 MINUTES BETWEEN RECOVERY AND THE NEXT EXPOSURE.

Date March	Exposure (*E*) and recovery (*R*) in seconds for bug No. 1		2		3		4		5		6*		7	
	E	*R*	*E*	*R*	*E*	*R*	*E*	*R*	*E*	*R*	*E*	*R*	*E*	*R*
3	240	470	60	468	960	920	15	110	30	165	60	205	120	231
CO_2	60	292	120	207	240	479	60	657	960	1002	15	95	30	126
	480	538	960	745	15	97	30	98	60	176	120	845	240	264
	960	1098	15	107	30	144	60	184	120	210	240	449	480	668
4	30	138	60	125	120	204	240	242	480	558	960	702	15	105
CO_2	15	131	30	83	60	109	120	139	240	340	480	460	960	828
	120	162	240	298	480	593	960	1093	15	93	30	103	60	99
5	60	128	120	184	240	315	480	689	960	1215	15	20	30	66
N_2O	960	924	15	20	30	57	60	150	120	244	240	599	480	936
	240	677	480	816	960	1066	15	20	30	31	60	2779	120	310
	480	1080	960	2137	15	49	30	88	60	132	120	355	240	520
6	71	243	114	330	180	182	287	427	455	1210	723	1029	45	84
N_2O	180	466	287	816	455	883	723	1100	45	99	71	342	114	176
	45	334	71	291	114	213	180	420	287	1144	455	967	723	1570

Date March	8		9		10		11*		12		13		14	
	E	*R*	*E*	*R*	*E*	*R*	*E*	*R*	*E*	*R*	*E*	*R*	*E*	*R*
3	480	691	240	281	120	286	60	128	30	136	15	85	960	868
CO_2	120	210	60	120	30	171	15	47	960	990	480	545	240	230
	30	155	15	85	960	1462	480	558	240	328	120	156	60	125
	240	436	120	204	60	288	30	332	15	93	960	1088	480	400
4	15	92	960	1134	480	650	240	474	120	149	60	101	30	89
CO_2	60	117	30	93	15	105	960	548	480	395	240	210	120	179
	960	1051	480	633	240	271	120	241	60	151	30	141	15	84
5	30	38	15	19	960	1567	480	1244	240	581	120	153	60	113
N_2O	240	1231	120	113	60	94	30	53	15	14	960	2338	480	666
	120	110	60	78	30	58	15	9	960	295	480	1645	240	742
	480	1252	240	701	120	214	60	84	30	53	15	61	960	2506
6	723	1055	455	794	287	727	180	242	114	308	71	121	45	142
N_2O	45	176	723	1274	455	2854	287	1134	180	675	114	244	71	333
	114	259	71	314	45	255	723	1356	455	1727	287	1261	180	1021

* New bug substituted on March 4; original bug dead.

Appendix Table 5. Recovery Time in Seconds of Bugs Exposed Once Each Day to CO_2 for Different Periods (E) in Seconds on March 17-20, 1953. The Subscripts 1 to 4 Designate the Youden Square to Which Each Bug Was Assigned.

Date March	1			2			3			4			5			6			7		
	E	R_1	R_2	E	R_1	R_2	E	R_1	R_2	E	R_1	R_2	E	R_1	R_2	E	R_1	R_2	E	R_1	R_2
17	315	456	548	567	386	477	1020	922	891	30	145	167	54	149	119	97	156	144	175	228	378
18	97	190	232	175	215	298	315	566	415	567	619	685	1020	2104	889	30	142	164	54	123	152
19	567	657	958	1020	969	875	30	152	94	54	134	125	97	143	113	175	219	226	315	343	483
20	1020	1195	975	30	141	217	54	176	127	97	169	162	175	304	176	315	356	450	567	661	641

Date March	8			9			10			11			12			13			14		
	E	R_3	R_4	E	R_3	R_4	E	R_3	R_4	E	R_3	R_4	E	R_3	R_4	E	R_3	R_4	E	R_3	R_4
17	567	572	621	315	435	394	175	494	207	97	148	146	54	129	112	30	99	108	1020	1642	988
18	175	211	215	97	136	1041	54	233	131	30	108	109	1020	314	1679	567	574	495	315	505	394
19	54	103	125	30	102	100	1020	2004	890	567	542	621	315	322	369	175	193	203	97	171	137
20	315	374	432	175	244	217	97	180	157	54	125	120	30	123	334	1020	1025	1135	567	732	660

References

1. BEARD, R. L. 1952a. Insect resistance to insecticides. Science **115** (2996): 608-609.
2. ——————. 1952b. Effect of sub-lethal doses of toxicants on susceptibility of insects to insecticides. Jour. Econ. Ent. **45**(4):561-7.
3. BLISS, C. I. 1952. The Statistics of Bioassay with Special Reference to the Vitamins. Academic Press, Inc., N. Y.
4. BROADBENT, B. M., AND BLISS, C. I. 1936. Comparison of criteria of susceptibility in the response of Drosophila to hydrocyanic acid gas. II. Recovery time. Jour. Econ. Ent. **29**(1):143-155.
5. CLARK, A. J. 1933. The Mode of Action of Drugs on Cells. Edw. Arnold and Co., London.
6. COCHRAN, W. G., AND COX, G. M. 1950. Experimental Designs. John Wiley and Sons, Inc., N. Y.
7. CRUMP, S. L. 1951. The present status of variance component analysis. Biometrics **7**(1):1-16.
8. YATES, F. 1940. The recovery of inter-block information in balanced incomplete block designs. Ann. Eugenics **10**(4):317-325.
9. YOUDEN, W. J. 1940. Experimental designs to increase accuracy of greenhouse studies. Contrib. Boyce Thompson Inst. **11**(3):219-228.