AN APPARATUS FOR TESTING THE TOXIC VALUES OF CONTACT INSECTICIDES UNDER CONTROLLED CONDITIONS.

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(Plates XXI–XXII.)

Introduction.

The apparatus described in this paper was constructed for the purpose of comparing quantitatively the lethal properties of contact insecticides. It was designed on the assumption that if successive numbers of similar insects are placed upon a plane surface of constant area and sprayed with a spray evenly distributed over that area under constant conditions as regards the amount of fluid, pressure of spraying, spreading, wetting and adhering properties of the fluid, for the same concentration of toxic substance, the amounts of poison received by each insect will always be equal. On varying any one of these factors any difference in the effect observed on the insects will be directly due to this variation.

For the immediate purposes of our investigation the factors varied were the poison and its concentration, so that provided that the foregoing assumption is correct, the proportion of dead to numbers sprayed will at each concentration give a measure of the toxicity at that concentration. If a number of chemical substances are tested at different strengths, curves can be plotted indicating how toxicity varies with the concentration and chemical constitution.

From some suitable point upon each of these curves a direct comparison of toxicity between the chemical compounds can be made. Statistically the 50 per cent. death-point, that is the concentration of poison which kills 50 per cent. of the insects sprayed, is the best. If, for example, the concentration of a standard poison such as nicotine giving a 50 per cent. mortality is known, the ratio of this amount to the amount of another substance giving the same mortality may be regarded as an insecticidal index for that substance, while if the curves are continued from their lower to their upper limits, indications will be obtained of the strengths that the insects can sustain without injury and of those required to kill 100 per cent.

Description of Apparatus.

The parts of the apparatus can be grouped as follows :—

(1). The arrangements for supplying air at a constant known pressure.

(2). A glass jar and means of levelling both its wooden cover and a small glass dish inside the jar.

(3). An arrangement for holding and adjusting the spray nozzle on the wooden cover of the jar.

The general lay-out of the apparatus is shown in Plate xxi, fig. A. Air is supplied from a cylinder of compressed air (1). When the cylinder is full this is at a pressure of about 120 atmospheres, but by means of a valve (2) it is released at any desired pressure, the pressure being regulated by a thumb-screw (3). The pressures within the cylinder and of the issuing air are indicated by dial gauges (4).

To obtain a more accurate indication of the pressure of the air, a Y-piece is inserted in the tube between the cylinder and the nozzle, one arm of which is connected to a mercury manometer (5) graduated to indicate pounds per square inch, and the other arm is connected to the nozzle. Between the cylinder and the Y-piece a filter (37) is

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inserted in the air tube, composed of a glass tube containing copper gauze and cotton-wool. This was found to be necessary owing to the amount of dust in the air, which gradually choked up the small outlet holes of the nozzle.

The air supply to the nozzle is turned on and off by means of a tap above the valve (6). The usual size of compressed air cylinder used holds 100 cubic feet of air.

The glass jar (Plate xxi, figs. A, B–7) in which the spraying is carried out has an internal depth of 44 cm. and an internal diameter of 19·5 cm.

This jar stands on a levelling platform (Plate xxi, figs. B, C–10), resting on three ivory points (8), which are fixed on small plates (9) arranged to slide on the arms of the levelling platform, to which they can be locked in any position. The platform is levelled by means of three screws (11), the points of the screws resting on small blocks on the table.

Within the jar is a smaller levelling platform (Plates xxi, xxii, figs. B, D–12), which supports in a similar manner a small glass dish (13) in which the insects to be sprayed are placed. In addition, this platform bears screws with ivory tips (14), at the outer ends of the arms, by means of which the platform is held in a central position in the jar. Two of these screws can be locked so that after being removed from the jar, the platform can be returned to the same position.

By means of marks on the jar and on the two levelling platforms, they can always be returned to the same position after removal.

The cover of the glass jar (Plates xxi, xxii, figs. B, E–15) is of mahogany, and rests on the top of the jar. It is held in position by three blocks resting against the inside of the jar (16). One of these blocks is fixed, while the other two are movable, and can be locked in any position by screws (17). By means of marks on the cover and on the jar, the cover can always be replaced in the same position. A large hole is cut in the cover, within which is fastened the arrangement for holding and adjusting the nozzle.

The nozzle (Plate xxii, figs. E, F–18)* is composed of two tubes; one, the air tube (text-fig. 1, B), has one end enlarged for connecting with the air supply, and the other end is closed by a screw (A), which can be removed to clean the tube. The outlet for the air is through two small holes (C) close together on the lower side of the tube near its closed end. The other, the liquid tube (E), is bent at right angles, and one arm is attached along the lower side of the air tube, so that the end comes opposite the two outlet holes in that tube. This end of the liquid tube is of smaller internal diameter than the remainder. The other arm of the liquid tube bends down at right angles from the air tube.

Part of the length for which the air and liquid tubes are joined is covered by a short length of wider tube (Plate xxii, fig. F–21), so that the nozzle can be held and turned in a clamp. The clamp (22) is attached by a screw to a small block (23), which is again attached by a screw to a small plate (Plate xxii, figs. E, F–24). By adjusting the block and clamp, the nozzle can be moved forward and back, turned over either way, tilted up or down, and turned to either side.

To the upper side of the air tube is attached a gallows (Plate xxii, figs. E, F–25), from which a plummet (26) is suspended over a scale of squared paper on the cover, by means of which it is possible to tell whether the nozzle is correctly adjusted, after the correct position has once been found. The thread holding the weight passes through a hole at the end of the gallows and is attached to a slide on the arm, so that the height of the weight can be regulated.

The small plate to which the clamp is attached bears two small holes (27) which connect with two points (28) on a larger intermediate plate (29), and the small plate

* This nozzle was designed for us by Mr. Leopold Ward, 2–3, Duke Street, S.W. 1.
The apparatus was constructed to our design by Messrs. Pellant, Harpenden.

is then held in position by two clamps, each tightened by a screw (30). The object of having this small removable plate is in order that the nozzle may be conveniently removed for cleaning and then replaced without losing its adjustment. This is only necessary very occasionally, as the liquid tube can be cleaned by spraying water through from the reservoir.

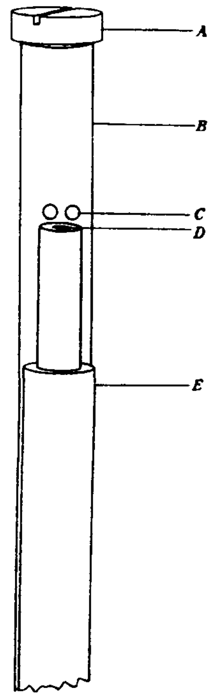


Fig. 1. Detail of nozzle: A, screw to allow of cleaning air tube; B, air tube; C, air outlet holes; D, liquid outlet; E, liquid tube.

The intermediate plate bears at one side a ball (Plate xxii, fig. F–31) which fits into a socket (32) on the basal plate (36) and can be locked by two screws. These two plates are further joined by two screws (33) at the opposite side to the ball joint. These screws are slightly curved and are attached to the basal plate, passing through slots in the intermediate plate, the position of the intermediate plate being altered by two milled nuts on each screw (38*a*, *b*). This arrangement of screws and a ball and socket joint is perhaps an unnecessary refinement, but gives a means of making small adjustments with greater accuracy than by the other methods described.

The free arm of the liquid tube of the nozzle passes through holes in the intermediate and basal plates. The liquid to be sprayed is contained in a small glass tube (Plate xxii, fig. F–34) which is held in a clip (35) so that the liquid tube of the nozzle reaches just to the bottom, the clip being fastened over the air tube.

The current of air blowing out through the two small outlet holes in the air tube across the open end of the liquid tube causes a partial vacuum, which draws the spray fluid from the reservoir and throws it out in the form of a very fine spray.

In Plate xxi, figs. A and B, the internal platform is shown resting on a glass plate † which is raised above the bottom of the jar on a glass tripod. The reasons for this are set out later, but, in brief, it renders spraying more accurate and easy. With this modification the distance from the nozzle to the dish is about 36 cm.

† This plate has a central perforation 2 cm. in diameter by means of which it can be readily placed in position.

A considerable experience with this apparatus has shown that it might be simplified in certain details for less critical work without any material loss of accuracy. The intermediate plate with its ball and socket joint and the two screws for fine adjustments might be omitted, and the small upper plate clamped directly on the basal plate. As there is a dial gauge attached to the cylinder, which can be read with moderate accuracy down to 10 lb., the mercury manometer is unnecessary for any but the most careful work.

The apparatus is set up in the following way :—The spray nozzle is connected to the air cylinder as shown in Plate xxi, fig. A, and by a preliminary trial the valve is adjusted to give the desired pressure in the gauge and manometer (in our case 15 lb. per square inch). The glass jar is placed upon the ivory points of the large external platform and adjusted to a central position ; the top is then put on with the arrow marked on the mahogany cover just over the arrow marked on the lip of the jar. It is then fixed in position by the sliding blocks on the lid, until a minimum of lateral movement is possible, and levelled by means of the milled-head screws on the platform.

The cover is then removed and the internal platform is placed in the jar, centred by means of the ivory-tipped screws, and levelled. A glass disc 5 cm. in diameter is then placed centrally on this platform, in the position which will be occupied by the dish containing the insects to be tested (Plates xxi, xxii, figs. B, D–13).

The mahogany lid is replaced, the spray nozzle placed in position and the orifice brought perpendicularly over the centre of the disc, as indicated by a plummet let down into the jar.

The lid is removed and a circular glass plate of diameter 18·5 cm. (preferably with a central perforation to allow of easy manipulation) is lowered into the jar and placed with its centre over the centre of the disc. Small quantities of water or dilute saponin solution are sprayed over the glass plate and the position of the nozzle adjusted until the spray has an even distribution about the smaller disc underneath. For the final adjustments we placed a second small disc of diameter 5 cm. on the glass plate so as just to cover the one on the platform. The disposition of the spray on the small disc could then be examined, as well as the distribution round the area marked out by it.

The position of the indicating plummet attached to the nozzle is noted on the slip of squared paper fixed on the mahogany top, and the nozzle screwed up tight in its position. The apparatus is now ready for calibration and for use.

Calibration of the Apparatus.

The calibration can be carried out as follows :—After ascertaining that the spray is evenly distributed, a previously weighed disc or dish of 5 cm. diameter is placed in position on the central platform ; 1 c.c. of water or saponin solution is pipetted into the reservoir and sprayed under known pressure. A disc is only suitable for amounts of spray of about 0·1 gm. After spraying, the disc is placed in a covered weighed dish and re-weighed. For quantities larger than 0·1 gm. a small dish with upright sides, such as is actually employed in spraying insects, is used. After spraying it is carefully wiped on the outside with filter paper, covered with a watchglass and re-weighed.

The data of a number of sprayings obtained in this way are set out in Table I.

All spraying was done at a pressure of 15 lb. per square inch.

Series A sprayed on to a disc 5 cm. in diameter, no air-filter used, and spraying timed with a stop watch. Air-tube of nozzle found to be slightly choked with dust after spraying.

Series B, C, D, E, G, H and I sprayed on to a dish 5 cm. in diameter with perpendicular sides 1 cm. deep, air-filter used, and sprayed until reservoir empty.

In series A and B distance from nozzle to dish or disc was 43·5 cm. ; in series C, D, E, G, H and I distance was 36 cm.

Series D sprayed after dismantling and re-erecting apparatus ; series H after detaching nozzle, washing and replacing.

Series A, B, C, D and E sprayed with nozzle No. 1, G, H and I with nozzle No 2.

Mean of D is slightly but significantly different from mean of C.

Mean of E is slightly but significantly different from mean of D.

There is no significant difference in variability in series E.

There is no significant difference in the means of series G, H and I.

In series A and B spraying was carried out with the platform at the bottom of the jar, 43·25 cm. from the nozzle, and in the former the operation was carefully timed on and off. Under these tedious conditions it was found possible to spray with a high degree of accuracy at this depth, but if a change is made to the method actually used when insects are being sprayed, the Probable Error of any single spraying rises from 0·84 per cent. (in A) to 3·7 per cent. (in B). This is due to the fact that near the bottom of the jar eddy-currents are formed, and the spray can be observed drifting across the surface of the disc, any slight change in working conditions apparently profoundly altering the degree of drift.

TABLE I.

Data relating to Weights of Spray falling on an Area 5 cm. in Diameter.

Series.	A	B	C	D	E	G	H	I
Pressure in cylinder.						20 atmos.	20–5 atmos.	110 atmos.
	Weight of spray falling on disc or dish (gms.)							
	(1) 0·1016 ·1003 ·1022 ·1014 ·1038 (2) ·1010 ·1044 ·1020 ·1036 ·1033	(1) 0·1331 ·1200 ·1192 ·1344 ·1330 (2) ·1195 ·1290 ·1291 ·1355 ·1405	0·2629 ·2773 ·2723 ·2709 ·2735 ·2649 ·2759 ·2725 ·2670 ·2744 ·2684 ·2687	0·2604 ·2680 ·2650 ·2633 ·2596	Dish No. 1. Jan. 6 0·2715 ·2820 Mar. 2 ·2763 ·2771 Dish No. 2 Mar. 2 ·2772 ·2826	0·2515 ·2470 ·2523 ·2485 ·2540	0·2515 ·2430 ·2463 ·2473 ·2522 ·2480	0·2425 ·2472 ·2500 ·2522 .2509
Mean ...	(1) 0·10186 (2) 0·10286	(1) 0·1279 (2) 0·1307	0·27072	0·26326	0·2777	0·25066	0·24805	0·24856
Probable error of single spraying %	±0·84	±3·7	±1·06	±0·79	±0·96	±0·89		

In view of this the internal platform was raised, the depth from nozzle to dish being reduced to 36 cm. The remaining data apply to this condition. It was found that it was much easier to spray at this depth, and that without taking any tedious or undue precautions the accuracy was considerable, the P.E. of series C being about 1 per cent.

Series D was carried out to test the effect of dismantling. The apparatus was taken down and then rebuilt. The results obtained in this series agree very closely amongst themselves, but are significantly different from those in series C. This difference is, however, only slight, and only becomes appreciable owing to the close agreement of the individual sprayings in the same series. It would hardly have any considerable bearing in actual practice, because the variation in the individual resistance amongst the insects sprayed is far greater than this, and because it is unnecessary, once the instrument is assembled, to dismantle it entirely for considerable periods of time. These series indicate, however, that some care is necessary in centring the spray before use.

The sprayings in series E were carried out to test the stability of the instrument and to find out whether a change of dish had any effect on the accuracy of the results. Two sprayings were carried out on 6th January and four on 2nd March. On the latter date different dishes were used. Between these two periods a considerable number of practical spraying tests were carried out. An analysis of the results showed that there was no significant difference in the variability of the results.

The data in series G, H and I refer to a second spraying nozzle made of wider tubing. It was constructed to avoid clogging during practical tests. The distribution of the spray is hardly so even as with No. 1, and it appears to be somewhat less in amount over the same area.

An opportunity was taken during these tests to ascertain the effect of detaching the nozzle and the plate to which it is clamped from the intermediate plate. This was carried out, the nozzle washed and put back, and a further series of sprayings, set out in column H, was done. There is no significant difference in the results arising from this operation.

Series G, H and I indicate that the amount of air in the cylinder, provided that it is above the minimum pressure required to give 15 lb. per square inch, has no influence on the amount of spray delivered.

The following analysis of the data in series A, B, C, D, E of Table No. I was carried out by "Mathetes," a voluntary worker in the Statistical Department at Rothamsted, to whom we desire to express out best thanks.

"The data were tested by the use of "Student's" Tables* (1) as recommended by Fisher.† Applying the formula $Z = \frac{\bar{x} - \bar{x}^1}{\sqrt{S(x-\bar{x})^2 + S^1(x^1-\bar{x}^1)^2}} \sqrt{\frac{n\,n^1}{n+n^1}}$, where n, n^1 are the numbers of observations on the two occasions, $\bar{x}$, $\bar{x}^1$ the corresponding means, "Student's" Tables give the probability of obtaining by chance an algebraically less value of Z, whence can be deduced the probability of obtaining by chance a value numerically greater than that observed, as shown in the following table :—

TABLE II.

	Z	n $(=n+n^1-1)$	P	Probability of obtaining a larger value of Z by chance.
A_1, A	·424	9	·8667	·2666
B^1, B_2... ...	·200	9	·7066	·5868
C, D	·866	16	·9977	·0046

There is thus no evidence of a significant difference between means of determinations done on different days under the same experimental conditions, so that A_1 may be pooled with A_2 and B_1 with B_2, for comparison with C as to the relative accuracy of the various methods of spraying, and the results of such a comparison are set out below. It should be noted, however, that there is a significant difference between

* "Student" (1917) Tables for estimating the Probability that the means of a unique sample of observations lies between $-\infty$ and any given distance of the Mean of the Population from which the Sample is drawn (Biom. xi, pp. 414–17).

† R. A. Fisher (1922). The goodness of fit of regression formulae and the distribution of regression coefficients. J. R. Stat. Soc. lxxxv, pp. 597–612.

C and D, so that experiments carried out after dismantling and resetting up the apparatus are not directly comparable, so far as the mean weight of spray is concerned, with those carried out before, although there is no significant difference in variability.

TABLE III.

	Mean gms.	S.D.	Coefficient of variation V.	P.E. of single observation (actual) milligrammes.	Percentage P.E.
A	·10236	·0012808	1·2513	±0·86	±0·84
B	·12933	·0070900	5·4821	±4·78	±3·70
C	·270725	·0042370	1·5651	±2·86	±1·06
D	·26326	·0030684	1·1655	±2·07	±0·79
E	·27778	·0039699	1·4292	±2·68	±0·96

It will be seen that B is much more variable than A or C, while C is very little worse than A, and having regard to the ease and speed with which determinations are carried out under the conditions obtaining in method C as compared with A, it is much to be preferred.

Series E closely resembles C, except that the mean weight of spray is significantly higher. There is no significant difference in variability, but the difference in mean weight points to the advisability of making control weighings after re-centring the apparatus."

The evenness of distribution of the spray was examined by spraying small quantities of Indian ink diluted with saponin solution on clean glass plates and on blotting paper.

These tests indicated that while the spray was by no means evenly distributed over the whole area covered by it, the spray thinning off rapidly towards the edges as might be expected, it was nevertheless fairly even over the small area in the centre which was actually used in the experiments, about 25 per cent. of the liquid sprayed actually falling on this area, as indicated in Table I.

Spraying Practice.

As the apparatus is intended to test contact insecticides, the most suitable test subjects are sucking insects. *Aphis rumicis* (apterous agamic females) in the adult stage have been chiefly used by us. The mode of operation is as follows :—

A number of these insects are placed upon a disc of flannel, held in position in the glass dish by three clips (Plate xxii, fig. D). The slightly frayed surface of the flannel prevents the insects from moving too freely and so allows time for them to be sprayed before they can escape from the dish.

The dish is then placed in position in the jar, the required amount of the substance to be tested is placed in the small glass tube (Plate xxii, fig. F–34), and the air turned on from the cylinder.

After spraying, the disc of flannel is removed with the aphides upon it and placed with a supply of the food-plant in a large petri dish, which is then covered with fine cotton gauze stretched on an iron ring, the diameter of which is greater than that of the dish. The weight of the iron ring keeps the gauze in position and prevents it from being accidentally knocked off.

The dishes of sprayed insects are carried on trays to a shady, well-ventilated greenhouse, where they are kept for examination. A moderately warm, but damp, shady place is found most suitable for this purpose.

The aphides are examined and given fresh food after periods of one and two days. By prolonging the period of inspection the subsequent grouping of results is somewhat simplified, but as the effectiveness of an insecticide can generally be judged after two days, the result hardly compensates for the extra labour involved.

Those aphides which are unaffected by the spraying are usually found feeding on the leaves or walking on the dish or cover. Others are found lying on the flannel or on the bottom of the dish. They are all examined and their condition recorded as being in one or other of four categories :—

Alive.—Aphides apparently unaffected by the spray.

Slightly affected.—Aphides affected to a certain extent, but still able to move, and likely to recover.

Moribund.—Aphides considerably affected and only able to make slight aimless movements of the appendages.

Dead.—Aphides apparently dead.

By prolonging the inspection period these categories tend to simplify out into dead and alive ; this, however, is attended by a certain amount of risk, as some of the insects may die from causes other than that due to the experiment. When further data are available it may be possible to give values to these groups based upon statistical analysis. The numbers of dead or alive can then be expressed in percentages of the numbers sprayed, or better, of the number of survivors in the controls.

The number of aphides sprayed at a time has been usually ten, a larger number increasing the difficulties of handling before, and of examination after, spraying. Spraying so small a number as this at each concentration enables one to separate out useless compounds from those that are worth further investigation.

For the final evaluation of the latter it is imperative to use larger numbers of insects for each concentration. This arises from the variations—which may be wide—in the individual resistance of the insects. As it is extremely difficult during examination to count, say, a hundred aphides moving about in an enclosed and comparatively small space, and to record with accuracy the results, and as when a large number are sprayed together some insects are likely to crawl over others and so prevent the even distribution of spray over the whole number, it has been our practice to make several repetitions of sprays at each concentration, using ten at a time.

The aphides used in these experiments are specially reared for the purpose and are all descended from a single fundatrix. The successive generations are raised on broad-bean plants in pots under muslin covers in a greenhouse. Each set of plants is infected from one plant of the previous set, a single apterous agamic female being placed on each plant. As all the aphides for a series of tests are taken from the same set of plants, they all belong to approximately the same generation. The aphides used for spraying were always adult apterous agamic females. By taking these precautions it was hoped to reduce the variation in resistance of the aphides to a minimum.

The removal of the aphides from the plants for spraying requires care to avoid injuring them in any way, as they only remove their stylets slowly from the tissues upon which they are feeding. It can, however, be safely accomplished by cutting off the parts of the plants infested, when it is found that after a short time the insects withdraw their stylets spontaneously and begin to wander. They can then be easily and safely handled with a camel's-hair brush.

Our experience has shown us that for very critical test spraying it is advisable to spray a control series with a standard insecticide on the same day. This is due to the extraordinary seasonal variations that may occur in the general resistance of insects (aphides in particular). So far we have not been able to ascertain precisely the causes of these variations, but meteorological conditions would seem to play some part. It seems probable that insects are somewhat less resistant on cool and overcast days, than on days that are bright and warm.

On one or two occasions tests have been carried out on Lepidopterous larvae, using this apparatus, various methods for preventing their escape being tried. This can be successfully accomplished by placing the larvae in the glass dish and covering it with a piece of washed tulle stretched in a small wooden embroidery frame. The mesh of the tulle used was sufficiently small to prevent the larvae from escaping quickly, and the threads were so fine that the amount of the spray fluid held up was very small.

Test Sprays with Nicotine.

These were carried out at a number of concentrations ranging upwards from 0·0025 per cent. Owing to temporary difficulties in rearing large numbers of suitable aphides only 50 insects were sprayed for each concentration. As the accuracy of results obtained in experiments of this type is proportional to the square root of number of test subjects, it is probable that 50 per test is not a sufficient number for great statistical exactitude.

The results are, however, set out in Table IV and text-fig. 2 to show the type of curve that one is likely to obtain from a series of spraying experiments.

TABLE IV.

Showing Toxicity of various Concentrations of pure Nicotine to A. rumicis (*adult apterous agamic females*).

1st Series. 50 sprayed, 10 at a time.

Concentration %	Number of Insects used (actual).	Number unaffected.	Number affected.*	% Affected of total number treated.	% Affected in test calculated to control.
0·0025	48	47	1	2	2
·005	49	46	3	6·5	6·5
·01	50	47	3	6·0	6·0
·02	47	35	12	25·5	25·5
·04	50	20	30	60	60
·06	50	4	46	92	92
·08	49	1	48	98	98
Control	47	47	0	0	—
2nd Series.					
0·0025	50	45	5	10	3·7
·005	46	42	4	8·7	2·3
·01	50	47	3	6·0	0
·02	46	35	11	24	18·7
·03	46	26	20	43·5	39·6
·04	49	18	31	63·3	60·8
·06	50	10	40	80	78·6
·08	50	7	43	86	85·0
·1	50	2	48	96	95·7
·15	50	2	48	96	95·7
·2	50	0	50	100	100
Control	45	42	3	6·5	—

Where the number tested differs from 50, one or two insects have escaped after spraying.

In all the above tests, including controls, 1 per cent. of saponin was used as a wetting reagent.

* The term "affected" indicates resultant death.

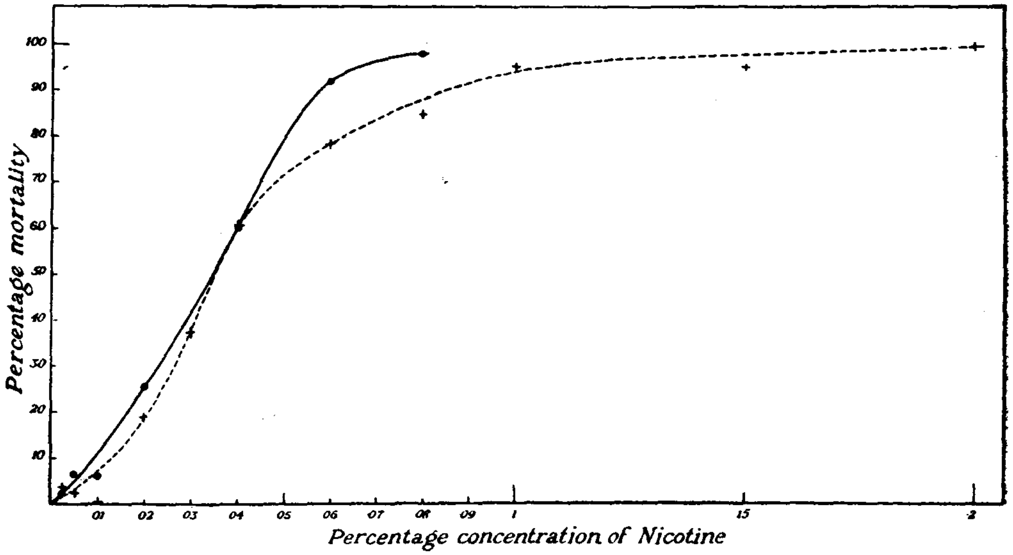


Fig. 2. Typical curves showing relation between concentration and toxicity for nicotine.

The figures in the last column are obtained by deducting the percentage affected in control (a) from the percentage affected in the test (b) and multiplying by $\frac{100}{100\text{-a}}$, the percentage affected calculated to control is then (b-a) . $\frac{100}{100\text{-a}}$.

The curves are drawn freehand to cut as many points as possible, and we have expressed both of them in the sigmoid form, but the lower points vary too much to make this type, although very probable, an absolute certainty.

An inspection of Table IV and fig. 2. shows that the results obtained agree most closely in the neighbourhood of the 50 per cent death-point, substantiating the conclusion arrived at on statistical grounds that this is the best point for direct comparison of results.

One very important feature of these curves is the way in which they tail off gradually as the 100 per cent. death-point is approached, rendering any comparison of toxicities near this point, a matter of considerable uncertainty.

We are greatly indebted to Mr. R. A. Fisher, Chief Statistician to the Rothamsted Experimental Station, for the following note setting out the best means of comparing the toxic values of insecticides.

" In any given experimental conditions the probability of any particular insect dying must be regarded as a continuous function of the concentration of the insecticide used. The control gives any experimental value of the probability of death corresponding to zero concentration, and with any effective insecticide we must imagine that as the concentration is increased, the probability of death increases from this minimum value, until possibly a final concentration is reached at which death is certain.

" The relation between concentration and probability of death could theoretically be determined by experiment by exposing a large number of insects to the action of the insecticide at each concentration. The number of insects required, however, increases enormously if we wish to explore in this manner the region in which the probability of death is high. If as many as 99 per cent. of the insects were killed, the accuracy of the comparison between any two insecticides would depend upon the comparatively few insects which survived, and to compare them with any accuracy many thousands of insects would have to be used. The same difficulty arises in the comparatively unimportant case when the deaths are few. For a given number of insects the most accurate comparison can be made when the concentrations are such that about 50 per cent. perish. The region between 25 per cent. and 75 per cent. can be fairly easily explored. It is for this reason that the preliminary examination of chemical substances should be made by a comparison of the concentrations required to give a mortality of 50 per cent.; when the equivalence at this point is established, it would further be most valuable to ascertain if the same relative concentrations are equivalent over the range 25–75 per cent. Only in this way does it seem possible to infer a general equivalence of insecticidal properties. The direct comparison of mortality when the probability of survival is very small would seem to be beyond the scope of accurate laboratory investigation."

Summary.

An apparatus for determining the relative toxicities of contact insecticides is described in detail. It is so arranged that successive batches of insects are sprayed under conditions as similar as possible, so that on using various substances at different concentrations, the results are directly comparable.

The apparatus consists of a glass jar in the lid of which an atomiser is fixed. By means of compressed air at known pressure the atomiser throws a constant quantity of fine spray upon insects placed in a dish inside the jar. Photographs illustrating the details of the apparatus are given.

The method used in practice for the spraying of aphides in this apparatus is described, and examples are given in the form of a table and a graph of the type of results obtained when different concentrations of nicotine are sprayed upon apterous agamic females of *A. rumicis*.

Two notes from the Statistical Department at Rothamsted are included, one analysing the accuracy with which the instrument sprays and the other giving reasons for regarding the concentrations which kill 50 per cent. of the insects sprayed as the most suitable for the direct comparison of the toxicity of insecticides.

EXPLANATION OF PLATES XXI. AND XXII.

A. Complete apparatus. × about $\frac{1}{25}$

B. Glass jar with top and levelling platforms in position. × about $\frac{1}{9}$.

C. Large external levelling platform. × about $\frac{1}{5}$.

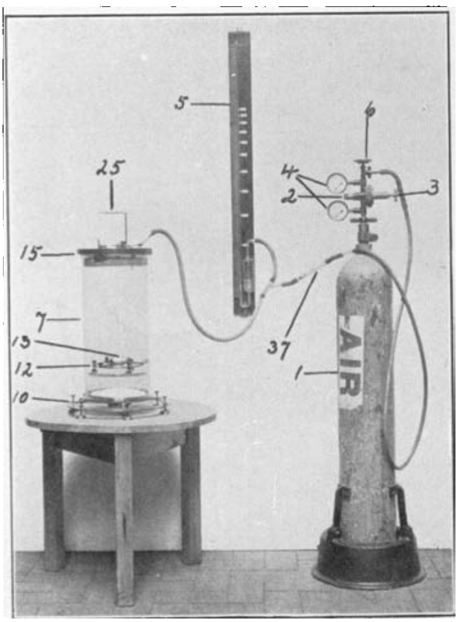
D. Small internal levelling platform. × about $\frac{1}{3}$.

E. Top of jar. × about $\frac{3}{10}$.

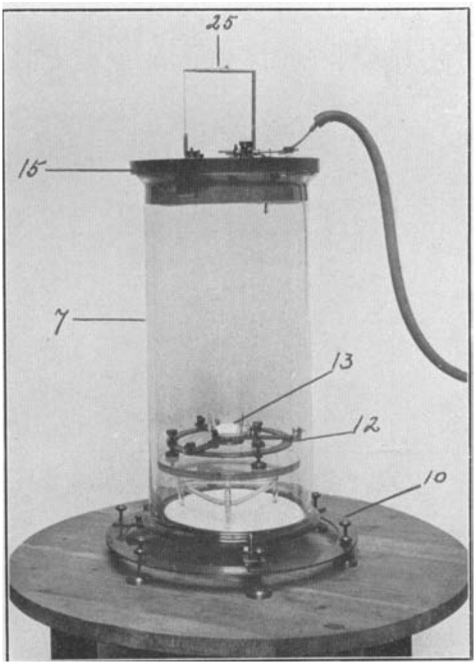
F. Details of top of jar. × about $\frac{1}{5}$.

1. Cylinder of compressed air.
2. Pressure-regulating valve.
3. Thumb-screw for adjusting same.
4. Dial gauges.
5. Manometer.
6. Air tap.
7. Glass jar.
8. Ivory points for levelling platform.
9. Sliding plate carrying same.
10. Large levelling platform.
11. Levelling screws.
12. Small levelling platform.
13. Glass dish.
14. Ivory-pointed centring screws.
15. Mahogany top.
16. Centring blocks of top.
17. Screws locking blocks.
18. Nozzle.
19. Air tube of nozzle.
20. Liquid tube of nozzle.
21. Wider tube to fit in clamp.
22. Clamp.
23. Block holding clamp.
24. Small plate.
25. Gallows and plummet.
26. Plummet.
27. Holes in plate fitting over points.
28. Points.
29. Intermediate plate.
30. Clamp for small plate.
31. Ball.
32. Socket.
33. Adjusting screws between intermediate and basal plates.
34. Glass tube.
35. Clip for glass tube.
36. Basal plate.
37. Air filter.

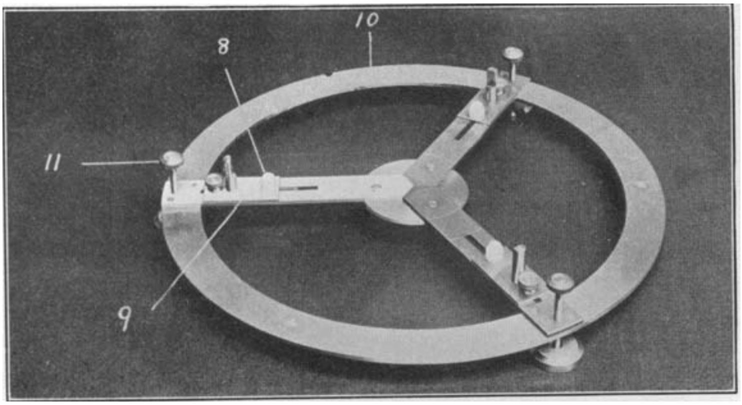
38*a*, *b*. Upper and lower milled nuts, giving means of fine adjustment between intermediate and basal plates.



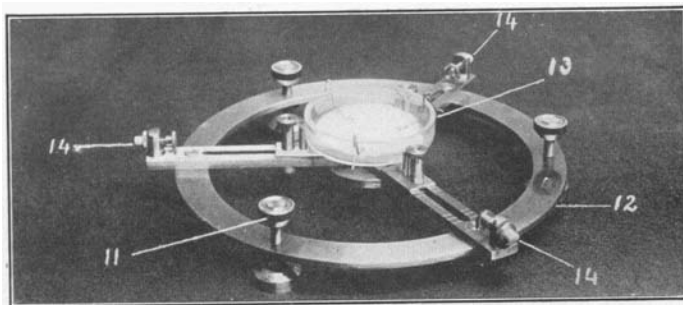
A. General view of apparatus as arranged for use.



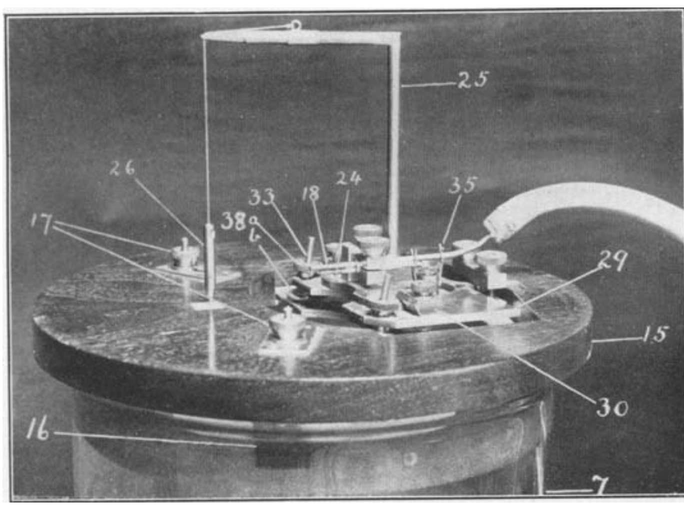
B. Glass jar with levelling platforms and lid.



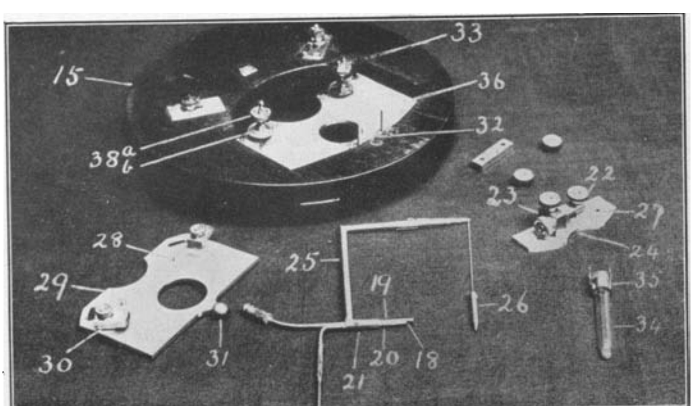
C. External levelling platform.



D. Internal levelling platform.



E. Lid of jar with nozzle.



F. Details of lid of jar and arrangements for adjusting nozzle.