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INVESTIGATIONS ON THE PHYTOTOXICITY OF BORDEAUX MIXTURE TO TOMATOES.

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SUMMARY.

Bordeaux mixture (4-4-40) *was shown in a series of experiments to have a higher toxicity for the tomato plant than Bordeaux* 4-2-40 *and various "inert" copper compounds.*

Experimental results indicated that the lime content of Bordeaux mixture is an important factor in phytotoxicity to tomatoes.

Season, cultural conditions and the vigour of the plants at the time of spraying were shown to influence the phytotoxicity.

There were indications that Bordeaux mixture stimulated tomatoes in some circumstances, and this is attributed to a response to copper of plants growing on copper-deficient soils.

The evidence obtained suggests that Bordeaux mixture adversely affects transpiration, photosynthesis and translocation, and through the general physiological upset impairs flower setting.

A new method of estimating phytotoxicity was devised and is described in detail.

I. INTRODUCTION.

It has been accepted for a considerable time that Bordeaux mixture has a phytotoxic action on various deciduous fruit trees, the responsible constituent being copper. As a result of this injury by copper salts, the tendency for a number of years was to increase almost without limit the ratio of lime to copper sulphate in fungicidal mixtures.

This practice continued until some experimenters noticed that Bordeaux mixture tended to have a detrimental effect on certain vegetables under drought conditions. Attention was gradually focussed on this problem and as a result largely of the work of Wilson and Runnels (1933 *et seq.*) and Horsfall and his collaborators (Horsfall and Harrison, 1939; Horsfall, Hervey and Suit, 1939; Horsfall, Magie and Suit, 1938) it was established that some vegetables, particularly tomatoes and cucumbers, are "lime-sensitive." Following the establishment of lime toxicity, the effect on the plant was investigated, attention being focussed principally on the effect on transpiration, the processes of photosynthesis and translocation being largely neglected.

The early experiments discussed in this paper were laid down primarily to compare the efficiency of various fungicides in the control of the commoner tomato diseases. The occurrence of significant differences in yield, however, without any appreciable incidence of foliage and fruit diseases, led to a more detailed investigation of the reasons for these differences.

II. MATERIALS AND METHODS.

1. Fungicidal Materials Used.

(i.) Home-made Mixtures.

Bordeaux mixtures of formulae 4-4-40, 4-2-40 and 6-2-40 were used. In all cases the second figure refers to the number of pounds of hydrated lime which was used in making up the mixture.

The other home-made mixture used is that known as home-made cuprous oxide (formerly as colloidal copper). This spray was modified in Queensland from the formula originally set out by Raleigh (1933) and was established as an efficient fungicide for tobacco seed-beds and citrus respectively by Mandelson (1933) and Blackford (1941). The method of preparation involves the use of a stock mixture which is prepared from two solutions as described by Blackford. The solutions used and the method of preparation are given below.

Solution A.	*Solution B.*
1 lb. bluestone ($CuSO_4.5H_2O$).	5 oz. caustic soda.
1 pint molasses.	3 pints water.
4 pints water.	

Solution B is poured slowly into Solution A, while mixing thoroughly. A heavy dirty-green precipitate is formed immediately. This is allowed to stand until the mixture has changed to a brownish-yellow colour, usually a fortnight. The stock is then diluted, using one gallon to make 10 gallons of spray, which gives a copper strength equivalent to that of Bordeaux 4-4-40.

(ii.) Commercial Copper Spray Mixtures.

Two types of commercial copper spray mixtures were used. The first comprises those containing insoluble copper compounds—cuprous oxide, copper oxychloride, basic copper sulphate, and basic copper carbonate. All were stated by the manufacturers to contain 50 per cent. metallic copper. Except for copper carbonate, which was subsequently discarded, all produced excellent suspensions in water.

The second group somewhat resembled a Burgundy mixture and had a chemical composition similar to the following:—$CuSO_4.5H_2O$ (50%), Na_2CO_3 (40%), and $Ca(OH)_2$ (10%). Materials of this type were not used after the first experiment because of obvious burn when used alone and the possibility of severe damage when used in combination with lead arsenate and other insecticides.

A commercial ammoniacal copper preparation was not used because experience had indicated the inferiority of this type of copper spray for field control of tomato diseases.

(*iii.*) *Commercial Copper Dust Mixtures.*

Three copper compounds—copper sulphate (monohydrate), basic copper carbonate and copper oxychloride—were used in the manufacture of dusts. The first was always mixed with hydrated lime and the others with kaolin or a similar inert material. The standard dust used was one containing 7% metallic copper, and though one with 6·5% was used in several cases, the difference has been neglected in comparing data. The mixtures used were either standard commercial lines as sold to growers or special mixtures which were made up in small quantities using a hand barrel for mixing.

2. Methods and Rates of Application.

(*i.*) *Field Experiments.*

The sprays were standardized to a common copper content (1 lb. copper per 40 gallons) and the rate of application corresponded very closely with the growers' spraying practices. Similarly, the dust applications were readily standardized with the routine growers' practices for dusting, using a 7% copper dust. However, it was quite clear that growers using sprays were applying more copper per acre than those who were dusting. Thus the choice was whether to apply the same amount of copper per acre per application in dusting as in spraying or to follow the usual commercial practice of simply using as much spray or dust as would give a reasonably complete coverage of the foliage and stems, and possibly dusting the plants more often than they were sprayed if weather conditions warranted.

To fulfil the condition of equal quantities of copper per acre, the copper content of the dusts would have had to be increased or higher application rates than growers considered economical would have been necessary. On the other hand, it was considered inadvisable to reduce the copper content of the sprays below the recommended standard of 1 lb. to 40 gallons. Consequently, as the first object of the field work was to compare the materials on the market under practical conditions, the second basis was used.

The sprays were applied at the rate of 240-300 gallons per acre of fully grown plants, so in any one such application 6-7 lb. of copper were used per acre. In dusting plants at the same stage, approximately 50-85 lb.

of dust (i.e., $3\frac{1}{2}$-6 lb. of copper) were applied per acre. There was considerable variation in dust quantities with weather conditions, higher rates being necessary on windy days. In the majority of the experiments the dusts were applied at the same intervals as the sprays. In those cases where the application of dust was more frequent than for sprays, the total amount of copper applied would have approached that used in spraying, but in general it was found impracticable to deposit as much copper on the foliage with a 7% copper dust as with the copper spray containing 1 lb. of copper to 40 gallons. As a result, comparisons between sprays and dusts are limited.

There are over 2,000 plants per acre for crops grown unpruned on the ground, the planting distance being 6 ft. by 3 ft. and plants overlapping across the inter-row spaces. For trellised plants the number is slightly over 7,000 per acre. In this case the planting distance is 4 ft. 6 in. by 1 ft. 4 in. and the stems are approximately 5-7 feet in length.

In grouping the yields from the various experiments into one table, no differentiation between the different frequencies of dusting has been made. The decision to neglect this is based on Experiment 6, in which a copper carbonate dust was used at two different frequencies, one approximately every 10 days (i.e., as often as the sprays) and the other approximately every seven days. There was no significant difference between the yields, the plants treated at 7-day intervals producing the equivalent of 1,260 cases per acre and those treated at 10-day intervals 1,240 cases per acre. The disease incidence in this experiment was nil.

(*ii.*) *Pot Experiments.*

Spray treatments only were used throughout the pot experiments, as they permitted a more uniform application of the materials. It was still difficult, however, to obtain a uniform deposit. The apparatus used in all experiments except one was a form of hand syringe with a very fine aperture (·03 in.), which was found to be the most convenient of the simpler hand types for the materials used. In the one exception to the above method, the plants were dipped in the fungicide. This method may have some advantages over the spray if wetting and spreading properties of the sprays are equivalent, but the question was not investigated further.

A criticism of most experiments on fungicidal phytotoxicity which are conducted with potted plants is that the plants have a thinner epidermis than normal owing to the sheltered greenhouse conditions usually associated with this form of culture. In all of these experiments, however, except in part of Experiment 14, the potted plants were grown and treated in the open air fully exposed to the weather.

The pots used were of the conical porous clay type, 5 in. or 7 in. in diameter, and rendered impervious by treatment with paraffin wax.

Unless otherwise specified, a plot consisted of one plant and only one plant was grown per pot.

III. THE SYMPTOMS INVOLVED.

This paper is not concerned with the more obvious types of tomato plant injury which are manifested as necrosis, wilting and falling of the leaf or fruit russeting, but rather with those cases where there has been a reduction in yield or growth without any obvious symptoms. Phytotoxicity has been widely discussed in recent years, the subject matter ranging from that of weedicides to an elucidation of the essential features of a successful insecticide or fungicide. For the last-mentioned the case must be considered from the points of view of both the host and the parasite. The delicacy of the position is obvious when one realises that the purpose of the fungicide is to exert a differential effect on the two plants concerned. The information on the phytotoxicity of fungicides up to 1945 was summarized by Horsfall (1945).

Throughout the field experiments where sprays were applied repeatedly, necrosis of the leaf was relatively uncommon, the principal symptom being a harshness and stiffness of the sprayed leaves, but even this was not always noticeable. The absence of noticeable symptoms in some of the experiments was probably due to there being no unsprayed plants to compare with those treated with Bordeaux mixture, the comparison being usually between Bordeaux-sprayed plants and those treated with one of the inert copper compounds. Though the latter also tend to give a harsh appearance to the leaf, there was occasionally an appreciable difference in the appearance of the plants as well as a significant difference in yield from those treated with Bordeaux 4-4-40. The other common symptom was a slight dwarfing which was not readily seen under normal conditions.

Necrosis of leaf margins occurred occasionally with Bordeaux 4-4-40 and in Experiment 9 it was apparent at a period in which plants treated with Bordeaux 4-2-40 were showing no necrosis.

Necrosis of the flower parts was not noted, but defloration was observed and is of primary importance.

No obvious abnormalities in fruit development as listed by Horsfall, Magie and Suit (1938) were noticed. An increase in the incidence of blossom-end rot of the fruit was recorded by Wilson and Runnels (1937a) for Bordeaux mixture, but no information on that point was obtained in these experiments. There was no consistent significant reduction in the early yield, or any significant effect on the size of the individual fruits.

IV. METHODS OF ESTIMATING PHYTOTOXICITY.

Three methods were used to estimate phytotoxicity:—(i) by total fruit yield, (ii) by total vegetative growth, and (iii) by vegetative growth rate.

1. Estimation by Total Fruit Yield.

The use of total fruit yield is the obvious practical means of estimating phytotoxic effects for a commercial crop such as tomatoes, where the fruits are the desired product. As these field experiments were all conducted under normal farming conditions and all operations were fitted in with the growers' routine, which was influenced by market prices, it was decided in all cases to take the total harvest rather than attempt a sampling method. Usually the fruit were picked at the mature green stage and never more frequently than once a week.

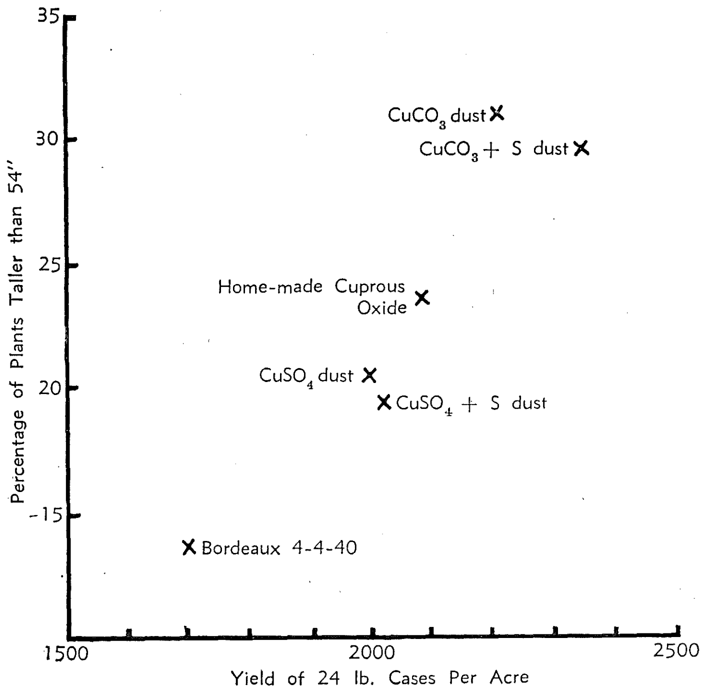

Fig. 1.

Diagram Showing the Relationship Between Height of Plants and Yield of Fruit.

2. Estimation by Total Vegetative Growth.

It cannot always be assumed that the total vegetative growth of the plant is positively correlated with total yield. This has been clearly shown many times when excessive nitrogenous fertilizer has been used. Such a possibility also appears to exist in the case of fungicidal treatment of tomatoes. In an experiment (not reported here) in which a copper fungicide was compared with a mixture whose active constituent was phenyl mercuric triethanol ammonium lactate, it was found that the latter significantly reduced the yield of fruit without any parallel reduction in vegetative growth. Within the group of copper fungicides, however, there appears to be a positive correlation, which is shown clearly in Experiment 5, when differences in height of the plants in different treatments were apparent. This relationship is shown graphically in Fig. 1, where total yields are plotted against the percentage of stems higher than 4 ft. 6 in. The plants in this experiment were pruned to two stems and trellised.

3. Estimation by Relative Growth Rates.

If the total vegetative growth of a plant is reduced by a particular treatment it is reasonable to expect that the treatment might have some immediate effect on the vegetative growth rate. Wilson and Runnels (1937a) recorded that retardation of early vegetative growth is directly related to reduced yields of fruit and that the fruit yield reduction is proportionately greater than the vegetative retardation in early growth. If this could be accepted, young plants could be used to make a rapid test of phytotoxicity. Da Costa (1946), when comparing the growth of the various parts of the tomato plant as indicators of soil moisture shortage, found that leaf area was definitely the most sensitive function to changes in moisture, and that only the eight youngest leaves showed any appreciable growth over any one period; of these, those between 4 in. and 10 in. in length were the most sensitive.

Table 1.

Growth of Leaves of Tomato Plant.

Leaf No.	Original length L_1. cm.	Final length L_2. cm.	$(L_1)^2$.	$(L_2)^2$.	$(L_2)^2 - (L_1)^2$.	Average relative growth rate.	Average length of leaf for period. cm.
Series A—12–9–47 to 15–9–47.							
1	2·25	2·25	5·06	5·06	0·0	0·0	2·3
2	4·25	4·31	18·1	18·6	0·5	1·0	4·3
3	5·69	6·06	32·4	36·7	4·3	4·0	5·9
4	7·31	7·94	53·4	60·3	6·9	5·7	7·6
5	7·88	8·69	62·1	75·6	13·5	6·7	8·2
6	9·16	10·85	83·7	108·9	25·2	11·3	9·9
7	8·28	10·66	68·6	106·7	38·1	17·7	9·4
8	5·97	8·47	35·6	71·8	36·2	23·7	7·1
9	4·35	6·91	18·9	47·7	28·8	30·3	5·5
10	3·66	6·03	13·4	36·4	23·0	33·0	4·7
11	1·72	3·66	3·0	13·4	10·4	51·0	2·5
12	0·85	2·22	0·72	4·93	4·21	64·3	1·4
Series B—19–9–47 to 22–9–47.							
1	2·25	2·25	5·06	5·06	0·0	0·0	2·25
2	4·31	4·31	18·6	18·6	0·0	0·0	4·3
3	6·12	6·12	37·4	37·4	0·0	0·0	6·12
4	8·12	8·19	65·9	67·1	1·2	0·7	8·2
5	8·88	9·06	78·9	82·1	3·2	1·3	9·0
6	11·38	11·50	130·0	132·0	2·0	1·0	11·6
7	11·56	11·81	135·0	139·0	4·0	1·3	11·8
8	10·38	10·75	108·0	115·0	7·0	2·0	10·7
9	10·00	10·38	100·0	108·0	8·0	2·1	10·3
10	9·19	9·75	84·5	95·1	10·6	4·0	9·6
11	6·15	7·19	37·6	51·1	14·5	11·0	6·8
12	4·63	5·81	21·4	33·8	12·4	14·7	5·4
13	3·25	4·38	10·6	19·2	8·6	19·0	4·0
14	1·50	2·31	2·25	5·30	3·05	26·3	2·1
15	0·81	1·31	0·66	1·72	1·06	27·7	1·2

In the case of the experiments discussed in this paper, no moisture shortage was anticipated, and there was no reason to assume that the leaves which showed the greatest sensitivity to water shortage would also show the greatest sensitivity to a check in growth possibly due to reduced efficiency in photosynthesis. The leaves on a number of plants were therefore measured for growth under normal conditions. A typical result is shown in Table 1. The two series are taken from the same plant but represent different intervals of time, as shown by the dates at the head of each series.

In all the growth rate experiments discussed here it has been assumed that the area of a tomato leaf is directly proportional to the square of its length, as demonstrated by Goodall (1945) and Ross (1946). To save further calculations, the values of $(\text{length})^2$ have been used for all calculations involving increases in area for any particular period.

In addition to tabulating the absolute increase in area, a comparison of the average relative growth rate for each leaf was made.

The relative growth rate (R) at any instant is given by the equation

$$R = \frac{1}{A}\frac{dA}{dt}$$

The average value of this quantity over a period $t = t_1$ to $t = t_2$ is given by

$$\bar{R} = \frac{\int_{t_1}^{t_2} R\,dt}{t_2 - t_1} = \frac{\log_e A_2 - \log_e A_1}{t_2 - t_1}$$

where A_2 = the area at time t_2,
A_1 = the area at time t_1,
$\bar{R}$ = average relative growth rate.

This definition of the average relative growth rate over a period is independent of the form of the growth curve (Fisher, 1920).

In calculating the average length of leaf for the period, it was originally assumed that under uniform conditions area of tomato leaf plotted against time gave an exponential curve, at least for the earlier more active period of its growth, with which this investigation is principally concerned. This was found to be unjustified, and as the data were not taken under sufficiently uniform conditions to enable the true form of the curve to be calculated, the length of the leaf in the middle of the experimental interval was taken as the arithmetic mean of the lengths at the beginning and end of the experimental period.

In Fig. 2 the average relative growth rate for each leaf is plotted against the average length of the leaf for the period. In Fig. 3 it is plotted against the leaf number, the oldest leaf being number 1 and allowance being made for the position of the flowers. The general difference between the two curves is due to the lower temperatures prevailing during the period 19-22/9/47.

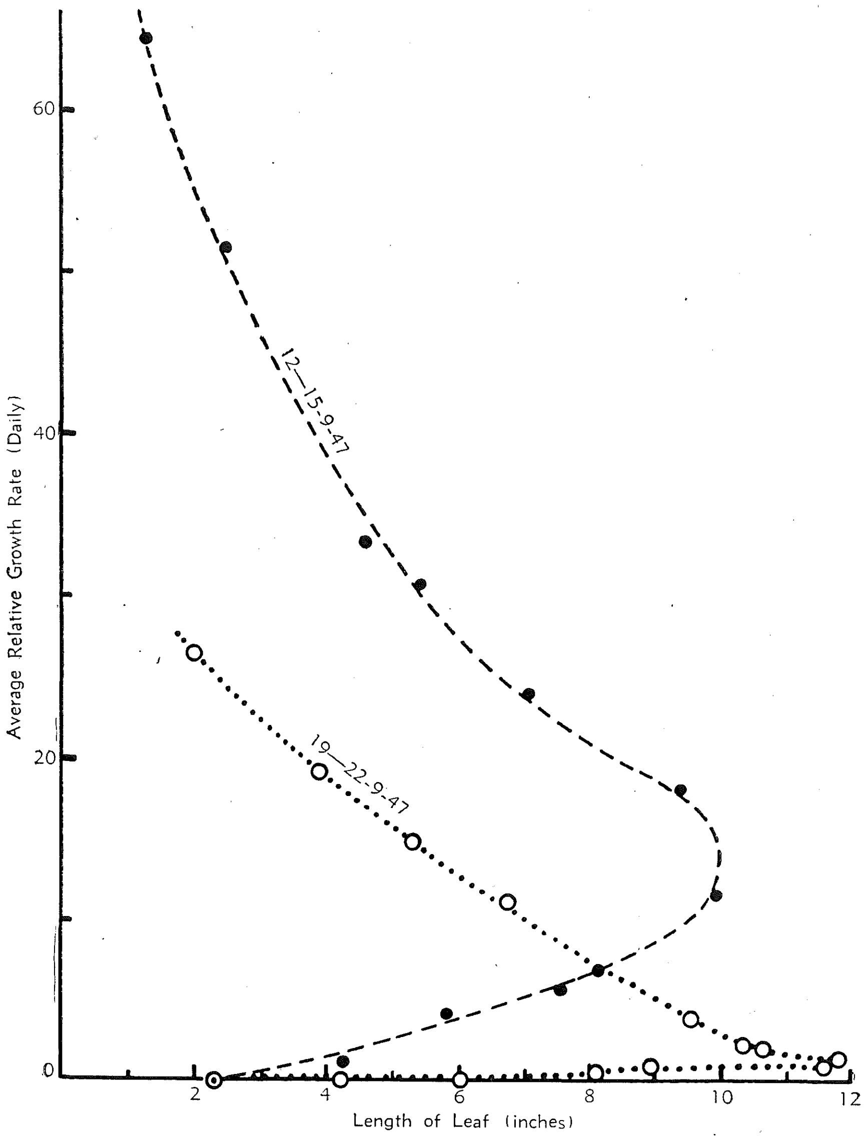

Fig. 2.

Diagram Showing the Relationship Between Average Daily Relative Growth Rate and Length of Leaf.

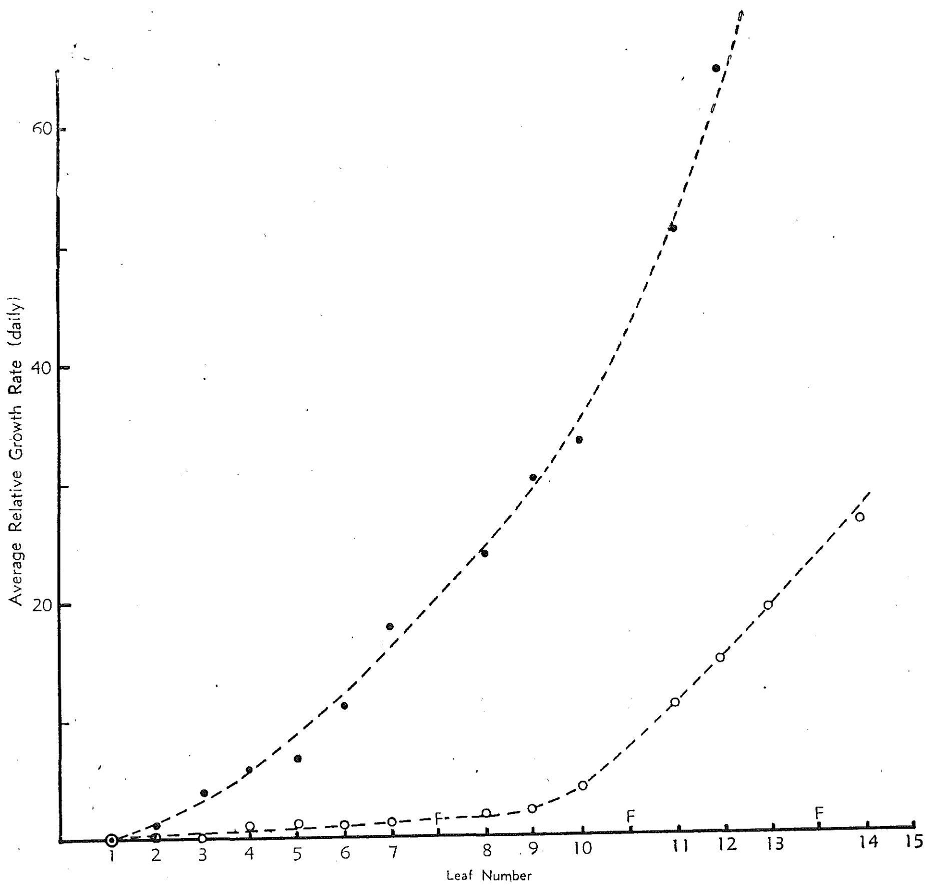

Fig. 3.

Diagram Showing the Relationship Between Average Daily Relative Growth Rate and Leaf Position.

On considering these curves, the following points are revealed:—

(1) The relationship between growth rate and length of leaf reverses about the seventh youngest leaf. These data were obtained from relatively young plants, but with older plants pruned to a single stem this still applies with a larger proportion of leaves of approximately equal length and very low relative growth rates; a number of points on the curve in this region of inflexion result. The reverse section is due to the earliest leaves maturing at a smaller size than the later ones and is characteristic of all tomato seedlings.

(2) For any detailed comparisons, similar sized leaves must be used, as with actively expanding leaves the average relative growth rate varies inversely with the length of the leaf.

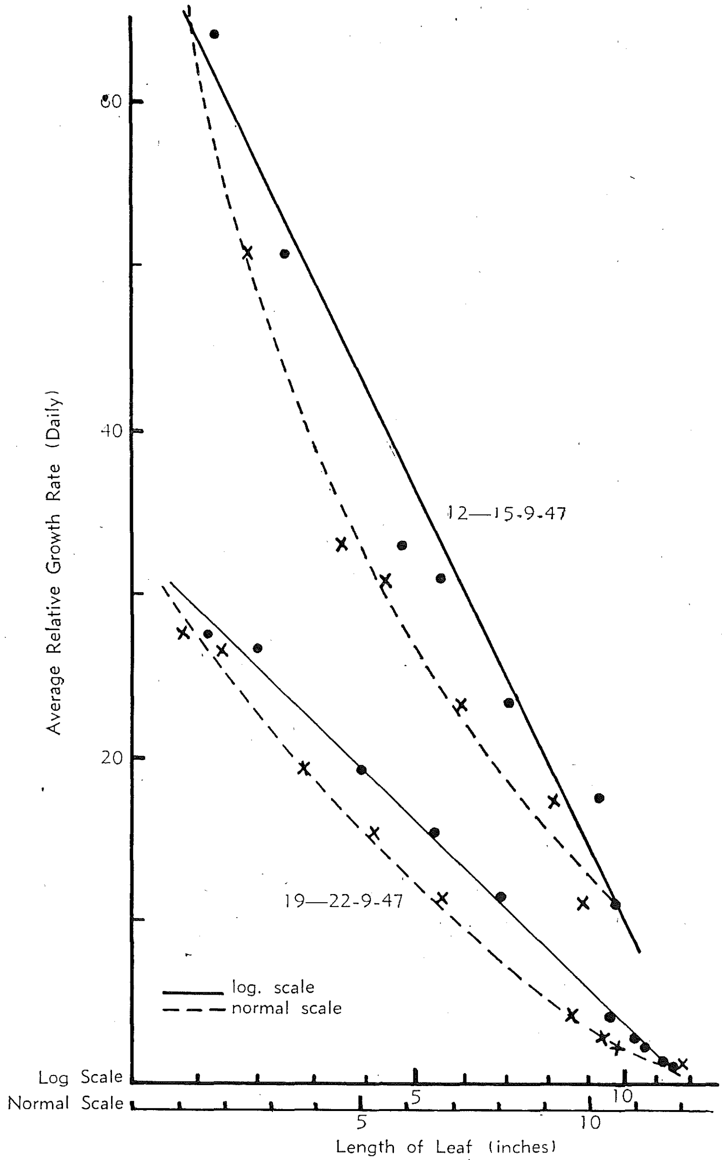

Fig. 4.

Diagram Showing the Relationship Between Average Daily Relative Growth Rate and Length of Leaf for the Younger Leaves Only.

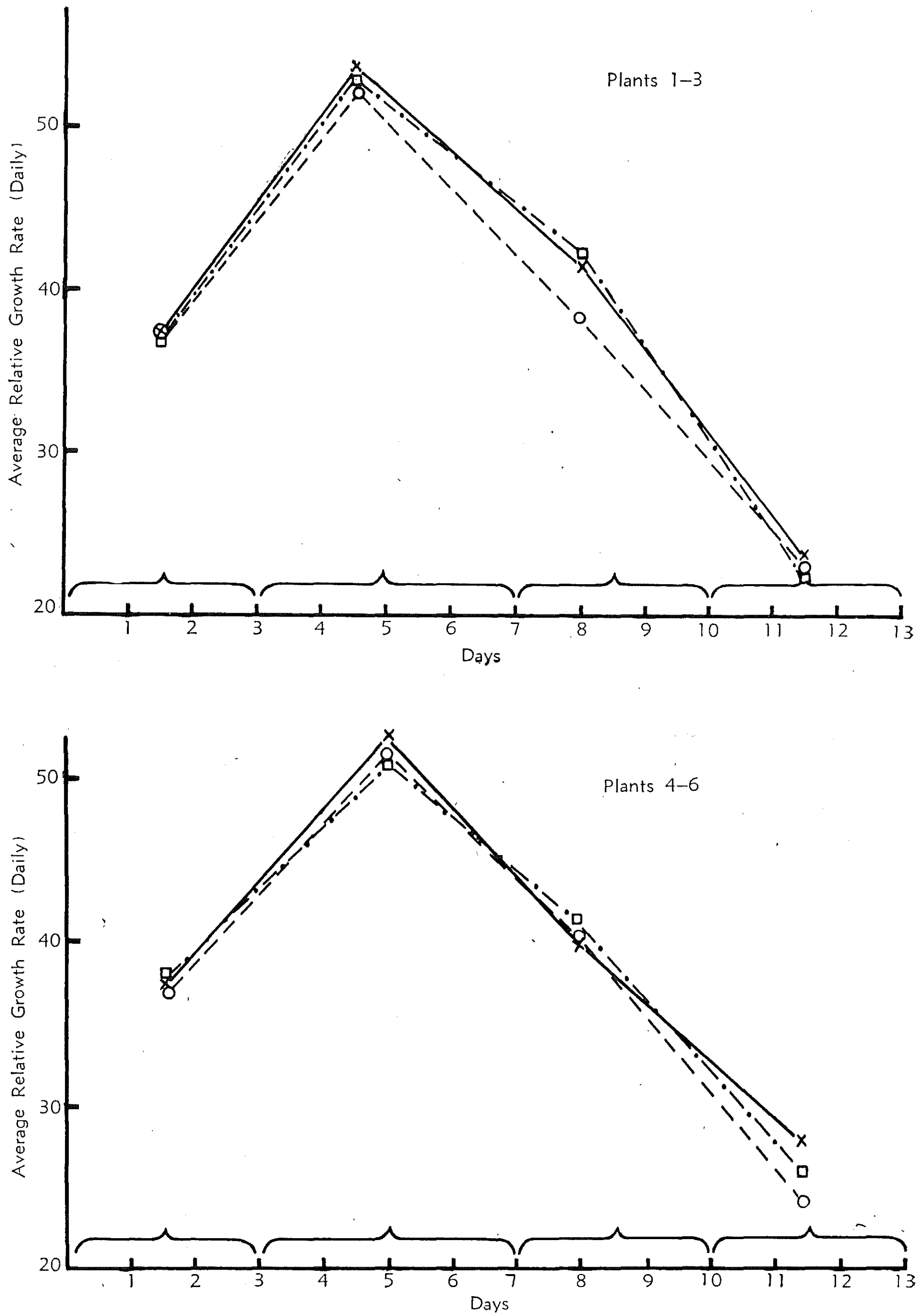

Fig 5.

Diagrams Showing the Average Daily Relative Growth Rate of a $2\frac{1}{2}$-inch Leaf for Six Plants Over a Period of 13 Days.

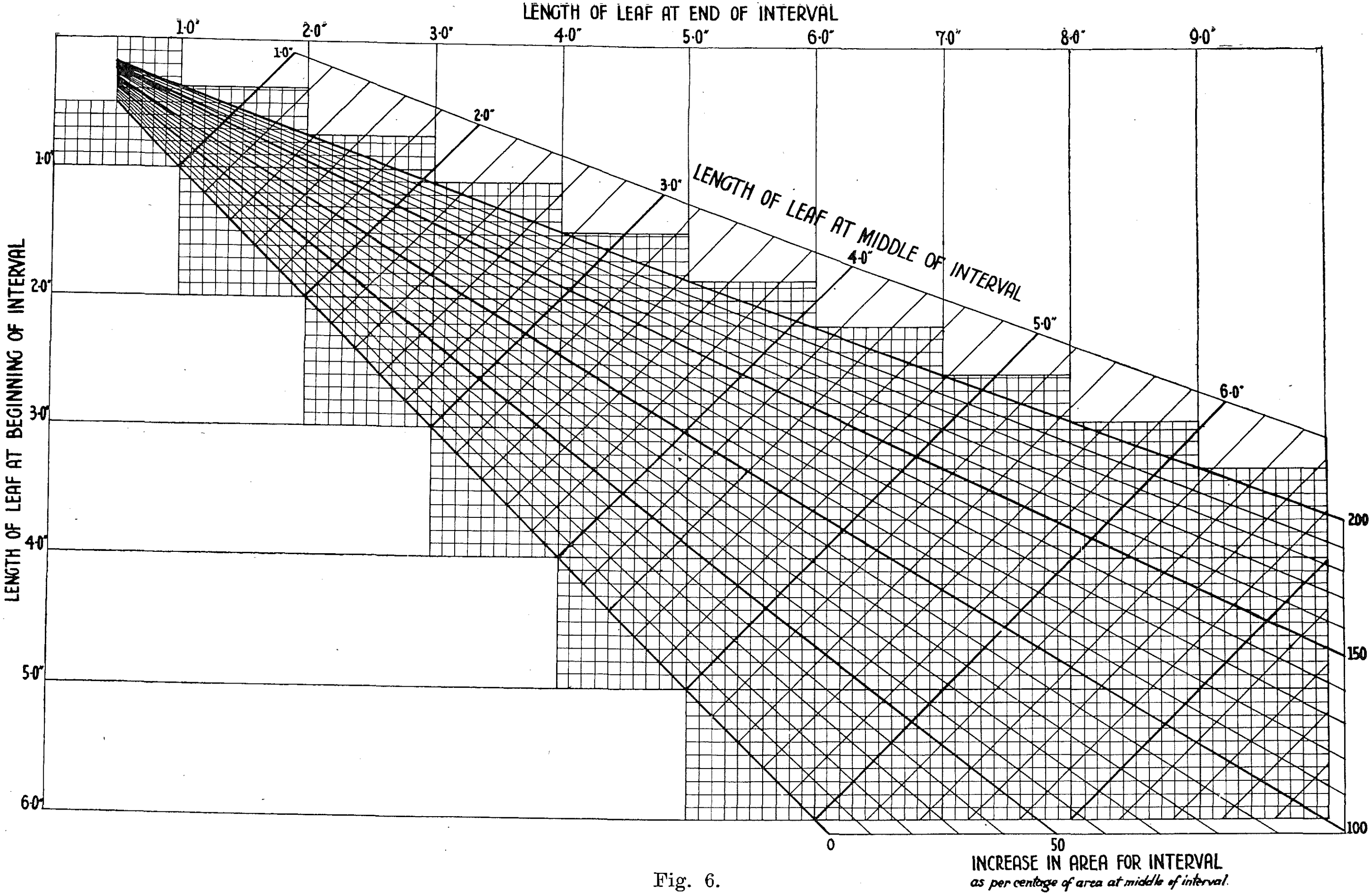

Fig. 6.

Nomogram for the Estimation of Percentage Increase in Area and Average Length of a Tomato Leaf, Given Original Length and Final Length.

To enable comparisons of relative growth rates to be made between plants under different spray treatments or growing conditions, it is desirable that each set of measurements be integrated and expressed as one figure. This appeared to be best done by selecting a standard size of leaf and using the growth rate of that size to express the growth rate of the whole plant for the period under consideration. To enable small plants to be compared with larger plants, the leaf should be as small as practicable. Under the conditions of the experiments it was found that leaves which were less than one inch in length at the commencement of the experimental period could be measured accurately only with considerable difficulty. Therefore, for quick working, leaves smaller than this at the commencement of the experimental period were not measured. To avoid extrapolation beyond the measurements and to suit both large and small plants it was decided to adopt the $2\frac{1}{2}$-inch leaf as a standard.

The fixing of a standard size of leaf introduced the necessity for interpolation, and as the estimations involved numbered hundreds, a straight-line relationship was sought. The best relationship found for all the experiments was obtained by plotting "average relative growth rate" against "log (10 + length of leaf in inches)." Fig. 4 illustrates the two curves for the normal and logarithmic scales on the abscissa. The relationship was checked for a total of 16 plants which were grown in three groups—viz., four, 10 and two plants respectively—and at different times of the year. The two-plant group was pruned to one stem, trellised and grown under field conditions; the others were all in pots and of various sizes, but all were pruned to one stem. The relationship was found to be consistent.

To further check this method of expressing the relative growth rate of the plant in an integrated form, six tomato plants were grown in pots and measurements of the actively growing leaves made periodically. The results are shown graphically in Fig. 5, where the average relative growth rates for a $2\frac{1}{2}$-inch leaf (estimated by the above method) are plotted against time.

The method was then used to check the relative effects of the fungicidal treatments on young plants. As the differences obtained were relatively small (i.e., in comparison with the depressing effect of moisture shortage on the plant), it was found that the sensitivity of the results could be increased by taking into consideration the average relative growth rate for a pre-treatment period. After a number of experiments it was decided that this period should be at least a week. The final figure used for comparison was—

$$\frac{\text{Average relative growth rate after treatment} \times 100}{\text{Average relative growth rate before treatment}}$$

The estimation of average length for each leaf, and the use of a minimum of four leaves per plant, called for the construction of a nomogram. The one shown in Fig. 6 enabled both average relative growth rate and average length of leaf for the period to be read off simultaneously, the only data required being the length of the leaf at the beginning of the period and the length of the leaf at the end of the period.

4. Notes on the Use of Average Relative Growth Rates for Estimation of Phytotoxicity.

A considerable amount of experimental work has been devoted to laboratory and greenhouse experiments for estimating the relative efficiency of various potential fungicides, but little time has been given to the development of a suitable method for small-scale exploratory work on phytotoxic properties. Wellman and McCallan (1943) suggested a system which was applicable only to those cases where direct necrosis is produced, but there is still scope for subjective errors.

The method used in this paper enables an estimate which is capable of statistical analysis to be obtained, and being dependent on certain specific measurements it is not liable to subjective error. The basic relationship depends on the fact that when the average relative growth rate of a leaf for a short period of several days is plotted against the average length of the leaf for that period, there is a straight-line relationship between the average relative growth rate and log (length of the leaf in inches + 10). The fact that the relative growth rate decreases steadily with the size and age of the leaf eliminates the possibility of the growth rate of even the young leaves following an exponential curve. The type of curve suggested as possibly being applicable in such cases of leaf growth, where the growth curve is sigmoid, is that attributed to Robertson (quoted by Miller, 1938, p. 1026) and conveniently expressed by the equation—

$$\log\left(\frac{x}{A - x}\right) = k(t - t_1)$$

where x = dry weight at any time, t_1,
A = maximum dry weight of the leaf,
t = time at which the leaf is one-half of its final dry weight,
k = a constant.

On examining this curve it is found that it is characterized by a steadily decreasing relative growth rate. The growth of a tomato leaf under uniform conditions may satisfy the above equation, but it is not possible to check this with the data on hand. Growing conditions were extremely variable for the experiments in this series, and the value for each point is based on three- and four-day intervals. Further, each of the present curves is derived from plotting average relative growth rates of a number of different sized leaves all taken over the same interval of time.

While the fundamental growth curve would be of interest, it is not essential for the practical use of this relationship. It is considered that the method used here would be very useful for growth experiments generally, but there are certain aspects which, if more completely investigated, would possibly increase its usefulness. One such point is illustrated by Fig. 7. The four lines graphed illustrate the growth rate relationships for one plant for four successive periods which were characterized by differences in temperature.

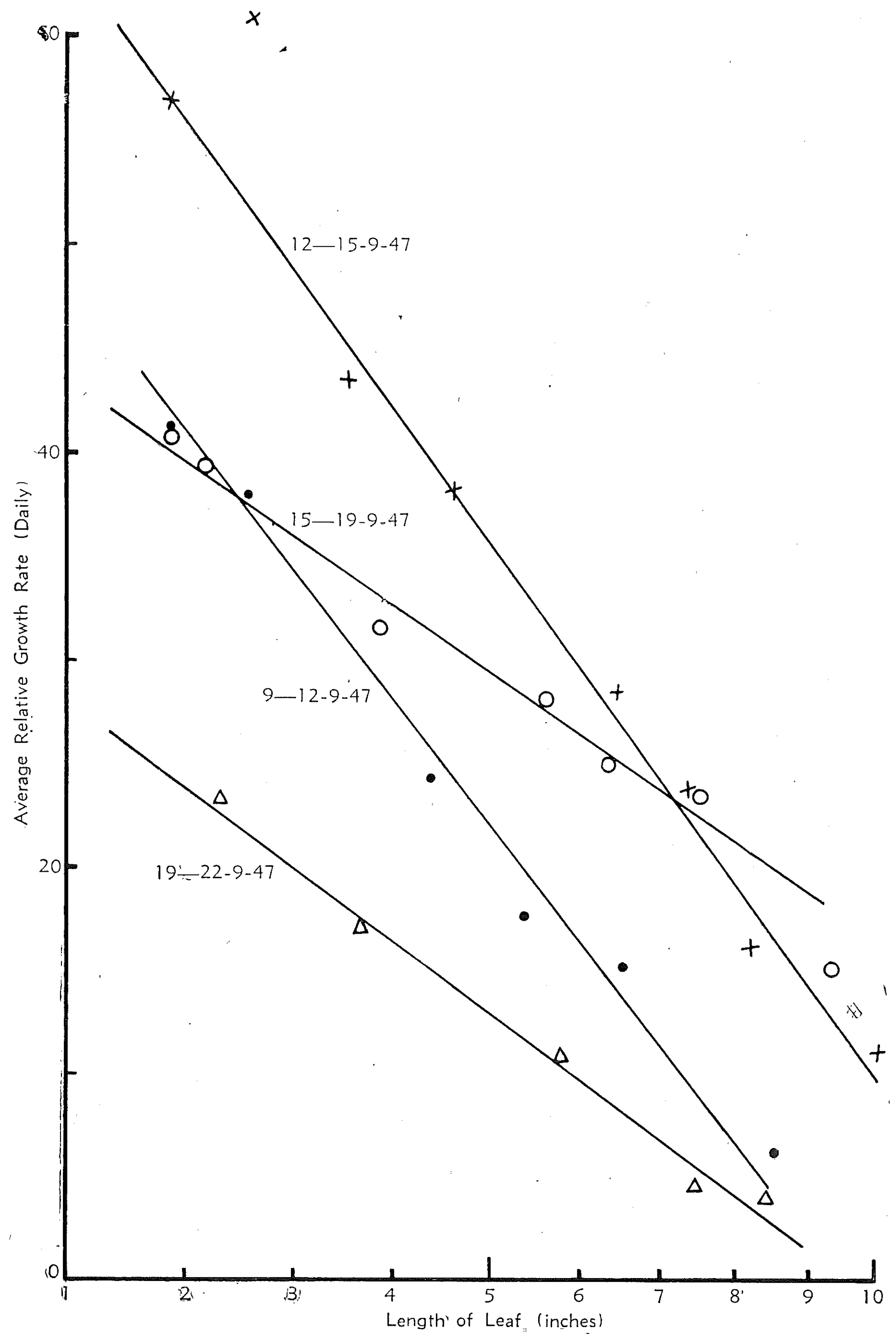

Fig. 7.

Diagram Showing the Average Daily Relative Growth Rate of Leaves of Various Lengths for Four Periods Differing in Temperature.

The relationship in each case is plotted by using only the actively growing leaves. The lines for two of the periods (12-15/9/47 and 15-19/9/47, particularly the latter) indicate that the larger leaves have manifested a proportionately greater increase in relative growth rate in comparison with the period 19-22/9/47 than the smaller leaves. In the experimental results given, no allowance has been made for such occurrences.

There was present throughout the pot experiments a relatively large variability. This was accepted as possibly the normal consequence of using one-plant plots. It may, however, be reduced if allowance is made for those cases as above. In addition, it may be possible to correlate any aberrant behaviour with some morphological character; for example, Goodall (1945) found it necessary to eliminate all seedlings with the first two leaves opposite instead of alternate.

V. THE DEMONSTRATION OF PHYTOTOXICITY.

The experiments discussed in this section were conducted partly in the field and partly in pots, the former during the years 1937 to 1940 and the latter during 1946.

Table 2.

ESSENTIAL DETAILS OF EXPERIMENTS 1–6.

Experiment No.	Year.	Date plants set in field and season.	Variety and growth.	Number of applications. of fungicide.	Incidence of leaf and fruit diseases.	Experimental layout.
1	1937	14–6–37 Spring crop	Break o' Day Unpruned	6	Slight ..	8 treatments in 4 randomized blocks. Single row plots of 25 plants
2	1938	16–6–38 Spring crop	Break o' Day Pruned to two stems	6	Nil ..	4 treatments in 5 randomized blocks. Single row plots of 18 plants
3	1939	30–3–39 Autumn crop	Break o' Day Unpruned	9	Moderate	5 treatments in 4 randomized blocks. Double row plots of 20 plants
4	1939	17–6–39 Spring crop	Marvana Unpruned	9	Slight ..	5 treatments in 4 randomized blocks. Single row plots of 10 plants
5	1939	10–6–39 Spring crop	Break o' Day Pruned to two stems	9	Nil ..	6 treatments in 6 randomized blocks. Single row plots of 27 plants
6	1940	28–3–40 Autumn crop	Break o' Day Unpruned	5	Nil ..	6 treatments in 6 x 6 Latin square. Single row plots of 28 plants.

1. Experiments and Results.

(i.) Experiments 1 to 6.

Objective.—To estimate the relative effect of the standard copper fungicides on tomatoes.

Materials, methods and layout.—The data are drawn from six field experiments carried out in the Brisbane-Redlands area. Cultural conditions varied considerably, plants being trellised or unpruned as described on page 4. The fertilizing, irrigation and general cultivation were in all cases left to the farmer, who treated the experiment plot as part of his own crop. The experiments were distributed over spring and autumn crops.

The sprays and dusts were all applied by the writer, so reducing the variability due to the personal element. In no case was an untreated check plot used, because all experiments were carried out on commercial farms and untreated areas could not be left as possible sources of infection. As home-made Bordeaux 4-4-40 had been the most widely used fungicide up to this time, this spray was adopted as the standard for comparison. Disease incidence (see Table 2) was insignificant in five out of the six experiments. In the other experiment, total yields demonstrated the same order of phytotoxicity and showed no correlation with incidence of disease.

Summary of results.—The objectives of these field experiments were similar, so similar data from the various experiments are grouped for the purpose of discussion. The essential details of each experiment are listed in Table 2.

The figures from these field experiments are listed together under the following headings:—(*a*) total yield of fruit; (*b*) early yield of fruit; (*c*) vegetative growth; (*d*) fruit size and flower set.

Table 3.

RELATIVE EFFECT OF COPPER SPRAYS AND DUSTS ON TOTAL YIELDS OF TOMATOES.
(YIELDS GIVEN IN HALF-BUSHEL CASES PER ACRE.)

Treatment.	Experiment Number.					
	1	2	3	4	5	6
Sprays—						
Bordeaux 4–4–40	989	1,212	389	490	1,700	1,140
Cuprous oxide (home-made) ..	856	1,565	428	487	2,000	1,450
Cuprous oxide (commercial) ..	..	..	432	523	..	..
Copper oxychloride (commercial) ..	..	..	..	..	..	1,420
Copper carbonate (commercial) ..	873	..	..	..	..	..
Burgundy type (commercial) ..	653	..	..	..	..	..
Dusts—						
Copper carbonate and kaolin ..	920	1,550	..	..	2,210	..
Copper carbonate, kaolin and sulphur	1,020	..	448	..	2,340	1,240
Copper sulphate and lime	908	1,340	..	..	2,020	..
Copper sulphate, lime and sulphur ..	..	..	374	569	2,070	1,080
Differences necessary for significance (5% level)	162	325	Not significant	Not significant	215	154

(*a*) *Total yields of fruit.*—These are listed in Table 3 and expressed in terms of half-bushel cases (24 lb.) of fruit per acre. The discussion of these results appears on pages 22-24.

(*b*) *Early yield of fruit.*—The effect of the treatment on the early yield of fruit was investigated by comparing the proportion of the crop which had matured at an early point in the harvesting programme—viz., when approximately one-quarter to one-third of the crop had been harvested. The figure for comparison was estimated from the fraction

$$\frac{\text{Weight of early harvest} \times 100}{\text{Weight of total harvest}}.$$

This eliminates the effect due to an overall reduction in yield. There was an apparent reduction in early yield of Bordeaux 4-4-40 plots as compared with cuprous oxide plots for Experiments 1-4, but the figures were reversed for Experiments 5 and 6. Thus there was no consistent evidence suggesting that Bordeaux 4-4-40 reduces the early harvest.

(*c*) *Vegetative growth.*—In Experiment 5, which was used to show the effect of the treatments on vegetative growth, the plants were pruned to two stems and trellised, each stem being supported in an upright position by twine until it reached a height of 4 ft. 6 in., after which the training ceased. Differences in the growth of the plants in the various treatments were obvious to the eye at the stage when the stems had grown to approximately this height, and as a rapid method of demonstrating this difference, the percentage of stems greater than the particular height (4 ft. 6 in.) was calculated for each treatment, all stems being counted. The results are given in Table 4.

Table 4.

THE EFFECT OF COPPER TREATMENTS ON VEGETATIVE GROWTH (EXPERIMENT 5).

Treatment.	Percentage of stems over 4 ft. 6 in. high.	Significantly exceeds at 5 % level.
Sprays—		
1. Bordeaux 4–4–40	14	
2. Cuprous oxide (home-made) ..	20·3	1
Dusts—		
3. Copper carbonate and kaolin ..	31	1, 2, 5
4. Copper carbonate, kaolin and sulphur	29·5	1, 2, 5
5. Copper sulphate and lime ..	19·7	
6. Copper sulphate, lime and sulphur	23·7	1

(*d*) *Fruit size and flower set.*—In several experiments the fruit was counted as well as weighed. Table 5 lists the average weight of the fruit from the various treatments.

Table 5.

AVERAGE WEIGHT OF AN INDIVIDUAL FRUIT (IN LB.) FROM VARIOUS TREATMENTS.

Treatment.	Experiment 2.	Experiment 5.	Experiment 6.
Sprays—			
Bordeaux 4–4–40	·217	·241	·301
Cuprous oxide (home-made)	·200	·255	·311
Copper oxychloride	..	..	·318
Dusts—			
Copper carbonate and kaolin	·197	·237	..
Copper carbonate, kaolin and sulphur ..	..	·242	·300
Copper sulphate and lime	·199	·232	..
Copper sulphate, lime and sulphur ..	..	·255	·291
	No significant differences		

In Experiment 2, the average number of flowers set per hand for the first five hands and the early fruit yields (weight) were recorded (Table 6).

Table 6.

CORRELATION OF FLOWER SET AND FRUIT YIELD (EXPERIMENT 2).

Treatment.	Flower Set.		Yields.	
	Average number of flowers set per hand.	Percentage of mean.	Cases per acre.	Percentage of mean.
Sprays—				
Bordeaux 4–4–40	1·23	68	262	71
Cuprous oxide (home-made) ..	2·48	138	460	124
Dusts—				
Copper carbonate and kaolin ..	1·88	105	409	110
Copper sulphate and lime	1·62	90	358	96
Mean	1·80	..	372	..

Counts of the number of flowers set on the early hands of the tomato had also been made on plants in two other experiments not included in these trials. By combining these two groups, a further five replications of figures were obtained for the above four treatments. The results are shown in Table 7.

(*ii.*) *Experiment* 7.

Objective.—To investigate whether Bordeaux 4-4 40 has any immediate effect on plant growth.

Materials, methods and layout.—Tomato plants approximately six inches high were used and the general conditions of culture were as outlined previously for pot trials. Two treatments were used and randomized in four replications. The date of commencement was 29/6/47.

Table 7.

EFFECT ON FUNGICIDE TREATMENT ON FLOWER SET.

Treatment.	Flower Set.	
	Percentage per hand of flowers.	Percentage of mean.
Bordeaux 4–4–40	38	74
Cuprous oxide (home-made)	50	97
Copper carbonate dust	74	144
Copper sulphate dust	44	86
Mean	51·5	..

Summary of results.—The growth rates before and after treatment are listed in Table 8.

Table 8.

AVERAGE DAILY RELATIVE GROWTH RATES (EXPERIMENT 7).

Treatment.	Six days pre-treatment.	Days 1–3 post-treatment.	Days 1–7 post-treatment.
Bordeaux 4–4–40 ..	3·42	2·21	3·17
Unsprayed	3·76	4·04	4·67
Difference	·34 ± ·206 Not significant	1·83 ± ·086 Significant at 1% level	1·50 ± ·389 Significant at 5% level

In Table 9 the significance of the reduction in average daily growth rate is shown after expressing the figure for the first three days post-treatment as a percentage of the pre-treatment figure. This factor will be referred to as the growth rate ratio, and equals

$$\frac{\text{Average relative growth rate for post-treatment period} \times 100}{\text{Average relative growth rate for pre-treatment period.}}$$

Table 9.

GROWTH RATE RATIOS (EXPERIMENT 7).

Treatment.	Growth rate ratio.
Bordeaux 4–4–40	66·0
Unsprayed	112·3
Difference	46·3 ± 7·04

The difference is significant at the 1 % level.

(*iii.*) *Experiment* 8.

Objective.—To investigate whether Bordeaux 4-4-40 or copper oxychloride has any immediate effect on plant growth.

Materials, methods and layout.—Tomato plants approximately six inches high were used and the general conditions of culture were as outlined previously for pot trials. Three treatments were used and the layout was in the form of five randomized blocks. The experiment commenced on 6/7/49.

Summary of results.—Table 10 summarizes the results for this trial, which are expressed on the percentage basis as mentioned previously.

Table 10.

GROWTH RATE RATIOS (EXPERIMENT 8).

Treatment.	Growth rate ratio.
Bordeaux 4–4–40	78·1
Copper oxychloride (2 lb. to 40 gall.)	80·7
Control (untreated)	82·2
Difference necessary for significance at 5% level	1·98

The differences here are relatively smaller than in Experiment 7, but this is probably accounted for by the more vigorous growth due to higher temperatures.

2. Discussion of Phytotoxicity.

(i.) *Bordeaux Mixture and Other Copper Sprays.*

In the experiments under consideration, Bordeaux 4-4-40 was compared with cuprous oxide (home-made and commercial products), copper oxychloride and copper carbonate compounds in the form of sprays. The results from the field trials (Experiments 1-6), together with those from Experiment 9, are summarized on a comparative basis in Table 11, the yield of Bordeaux 4-4-40 being taken as 100 in each trial.

Table 11.

YIELDS AFTER TREATMENT WITH INERT COPPER COMPOUNDS.
(BORDEAUX 4–4–40 = 100.)

Experiment No.	Cuprous oxide (home-made).	Cuprous oxide (commercial).	Copper oxychloride (commercial).	Copper carbonate (commercial).
1	95·4	..	..	97·3
2	128·3	..	..	..
3	110·2	111·0	..	..
4	99·4	106·7	..	..
5	117·7	..	..	..
6	127·3	..	124·7	..
9	..	..	94·1	..

In four of the six trials in which Bordeaux 4-4-40 and home-made cuprous oxide were compared, the latter gave yields at least 10 per cent. higher, but in the other two gave slightly lower yields. Plots treated with the other compounds considered as a group exceeded the Bordeaux 4-4-40 treated plots three times and were lower in the other two.

Toxicity due to Bordeaux 4-4-40 also had a dwarfing effect on the vegetative growth (Table 4) and reduced flower set (Tables 6 and 7) and growth rates (Table 10).

Though there were several exceptions (e.g., in Experiments 1 and 4), these figures indicate generally that Bordeaux 4-4-40 has a decidedly greater phytotoxicity than the inert copper compounds, a fact which was demonstrated by Wilson and Runnels (1940) and Horsfall, Magie and Suit (1938). The extra toxicity was attributed by these workers to the excess lime present in the mixture and this matter is discussed in further detail in a later section (pages 31-32).

The exceptional results are interesting cases and are considered to be due to the operation of two other factors—viz., differences in the plant growth rate at the time of spraying, and an additional stimulation by Bordeaux mixture as compared with the other copper sprays on particular soils. These factors are discussed in more detail later (pages 32-34).

(*ii.*) *Comparison of Copper Dusts.*

Copper dusts have become increasingly popular for use on tomatoes in Queensland. In the early stages of these experiments, the copper carbonate and kaolin mixture and the copper sulphate and lime mixtures were the only two types available, but during recent years copper oxychloride has occupied a prominent part.

In these trials the yield from the copper carbonate dust treatment exceeded that from the copper sulphate and lime dust in every case; the ratios of the yields, assuming copper sulphate and lime to be 100, were 101·5, 115·7, 119·7, 109·4, and 114·8.

These results are of interest in view of the fact that as tomato growers in other countries have not made wide use of copper carbonate as a field dust, there does not appear to have been any direct comparison of this copper compound as a dust with copper sulphate and lime mixture. While copper oxychloride was not compared directly with copper sulphate and lime, it is considered that it would have the same relative position as copper carbonate, as they are both relatively insoluble.

As for the sprays, the extra phytotoxicity of copper sulphate dust is attributed to the high lime content, usually from 40 to 80 per cent. of the dust.

(*iii.*) *Comparison of Dusts and Sprays.*

On comparing the yields of similar compounds in the dust and spray form—e.g., copper sulphate and lime dust with Bordeaux 4-4-40 in Experiments 1-6, copper carbonate in Experiment 1 and copper oxychloride spray in Experiment 9—the evidence is slightly in favour of the dust. The comparative yields are shown in Table 12.

Table 12.

COMPARATIVE YIELDS OF DUSTS.

(SPRAY = 100.)

Experiment Number.	Copper sulphate and lime.	Copper carbonate.	Copper oxychloride.
1	101·0	111·0	..
2	110·5	..	..
3	93·8	..	..
4	116·2	..	..
5	120·2	..	..
6	94·8	..	..
9	..	..	100·9

The differences were statistically significant for the individual experiment in Experiment 5 only. As the phytotoxic effects of a dust would possibly vary with the amount of moisture present, part of the observed variation can be attributed to differences in that factor.

VI. ANALYSIS OF THE CAUSES AND EFFECTS OF PHYTOTOXICITY.

1. Experiments and Results.

In this section of the work the objective and experimental conditions varied, so each trial is discussed separately. Unless otherwise stated, the general conditions for application of the fungicides and methods of cultivation were as outlined under "Materials and Methods."

Table 13.

MEAN YIELDS (LB./PLOT) (EXPERIMENT 9).

Fungicide.	Strength of mixture.			
	1 lb. copper to 40 gall. or 5 lb. dust.	½ lb. copper to 40 gall. or 5 lb. dust.	¼ lb. copper to 40 gall. or 5 lb. dust.	Means.
	1	2	3	
Bordeaux mixture .. .. $\left(\frac{CuSO_4.5H_2O}{Ca\,(OH)_2} = \frac{1}{1}\right)$	108·0	98·5	96·2	100·9
Bordeaux mixture .. .. $\left(\frac{CuSO_4.5H_2O}{Ca\,(OH)_2} = \frac{2}{1}\right)$	133·2	106·0	97·2	112·2
Copper oxychloride (spray) .	101·5	112·8	127·8	114·0
Copper oxychloride (dust) ..	99·0	107·2	101·0	102·4
Means	110·4	106·1	105·6	107·4

(*i.*) *Experiment* 9.

Objective.—To investigate the phytotoxic effects of (i) high lime content of Bordeaux mixture, (ii) total spray load, and (iii) copper dusts.

Materials, methods and layout.—This was a field experiment using Break o' Day tomato grown unpruned on the ground. Twelve spray treatments were used—viz., four different forms of copper fungicide, each at three levels of copper content, which are detailed in Table 13. Four replications were used in the form of randomized blocks. There were 20 plants per plot. Six applications of the fungicides were made over a period of 10 weeks, commencing on 2/5/47.

Summary of results.—The mean yields per treatment are given in Table 13, and the differences necessary for significance in Table 14.

Table 14.

DIFFERENCES NECESSARY FOR SIGNIFICANCE.

—	s.e.	Necessary difference for significance.	
		5 % level.	1 % level..
Spray means	3·48	10·0	13·5
Strength means	3·01	8·7	11·7
Individual means	6·02	17·3	23·3

(*a*) *Spray means.*—Generally Bordeaux 4-2-40 significantly exceeded Bordeaux 4-4-40 and copper oxychloride spray significantly exceeded copper oxychloride dusts and Bordeaux 4-4-40 at the 5% level. The difference between Bordeaux 4-2-40 and copper oxychloride dust was just below the value necessary at this level.

(*b*) *Strength means.*—Considering the mean of all four fungicides, there were no significant differences between spray strengths. Differences between strengths of the one treatment are discussed below with the interactions.

(*c*) *Interaction.*—At the highest strength Bordeaux 4-2-40 significantly exceeded all other treatments at the 1% level. At the weakest strength, copper oxychloride spray significantly exceeded all other treatments. Considering Bordeaux 4-2-40 alone, the highest concentration of copper significantly exceeded the other two at the 1% level. In the case of copper oxychloride spray treatment, the lowest concentration exceeded the other two concentrations at the 1% level. This interaction is striking and is considered as possibly due to a copper stimulation by the higher concentrations of Bordeaux 4-2-40, together with a reaction of the plant to spray-load in the case of the copper oxychloride. These points are further considered later in the discussion on copper stimulation (pages 32-34).

(*ii.*) *Experiment* 10.

Objective.—To investigate the effect of various proportions of hydrated lime and copper sulphate on the phytotoxicity of Bordeaux mixture.

Materials, methods and layout.—The plants were grown in pots under conditions previously described. The total amount of solids—i.e., of calcium hydroxide and dehydrated copper sulphate—was kept constant at 16·4 grams per litre and was equal to that for Bordeaux 4-4-40. Six treatments (see Table 15) were used and the trial was laid down as four randomized blocks. The date of commencement of the experiment was 7/10/48.

Summary of results.—The results are summarized in Table 15.

Table 15.

COMPARISON OF EFFECT OF VARIOUS RATIOS OF COPPER SULPHATE AND LIME ON AVERAGE RELATIVE GROWTH RATES (EXPERIMENT 10).

Treatment. Grams per litre. $CuSO_4$	$Ca(OH)_2$	Equivalent field spray.	Growth rate ratio (*a*).	
16·4	0·0	8–0–40	70·0	(0·0) (*b*)
13·4	3·0	7–1–40	70·7	72·1
10·8	5·6	6–2–40	119·1	110·5
6·4	10·0	4–4–40	88·9	88·2
2·9	13·5	2–6–40	112·2	100·3
0·0	16·4	0–8–40	88·4	76·0
Control		..	94·0	104·0
Necessary difference for significance—				
at 5 % level		..	29·1	No signif.
at 1 % level		..	40·3	diffs.

(*a*) The left hand column gives the ratios for the first two days after treatment, while the right hand column gives the figures for the third to fifth days.

(*b*) Only two replications.

Variability was relatively high in this trial but for the first two days Bordeaux 6-2-40 significantly exceeded Bordeaux 4-4-40. The former treatment gave the highest growth rate ratio for all treatments but did not significantly exceed the 2-6-40 mixture. Due to the rapid drying of the spray, the copper sulphate solution over this period produced only a general bronzing with pin-point size necrotic spots. With the advent of dews overnight the necrosis became general.

(*iii.*) *Experiment* 11.

Objective.—To investigate (i) the effect of various proportions of copper sulphate and lime in Bordeaux mixture, and (ii) the effect of varying the spray load.

Materials, methods and layout.—The experiment was carried out in pots. Five ratios of copper sulphate to lime were used, each at four different concentrations, making 20 treatments in all, which were laid down as three randomized blocks. The date of commencement was 12/11/48.

Summary of results.—The results are summarized in Table 16.

Table 16.

THE EFFECT OF VARIOUS BORDEAUX MIXTURES AND TOTAL LOAD OF SOLIDS ON THE GROWTH RATE RATIO (EXPERIMENT 11).

$\frac{CuSO_4}{Ca(OH)_2}$ Ratio.	Equivalent ratio $\frac{CuSO_4.5H_2O}{Ca(OH)_2}$	Load. (lb. of solid per 40 gall.)				
		1	$3\frac{1}{2}$	6	10	Means.
2 : 1	3 : 1	74·1	75·7	76·1	74·0	74·7
4 : 3	2 : 1	78·8	63·6	73·4	81·0	74·2
2 : 3	1 : 1	83·1	60·1	61·5	68·3	68·2
1 : 3	1 : 2	68·2	62·7	61·6	76·4	67·2
0 : 3	0 : 3	65·7	63·8	87·0	65·1	70·4
Means		74·0	65·2	71·9	72·9	..

The two low-lime mixtures had the highest means but the differences were not significant at the 5% level. The differences between the loads were insignificant and showed no suggestive trends.

(*iv.*) *Experiment* 12.

Objective.—To repeat the interactions between spray strengths and spray materials obtained in Experiment 9, using relative growth rates instead of fruit yields.

Materials, methods and layout.—The experiment was carried out in pots. The spray treatments applied were as follows:—(i) Bordeaux 4-4-40; (ii) Bordeaux 1-$\frac{1}{2}$-40; (iii) copper oxychloride (2 lb. to 40 gall.); (iv) copper oxychloride ($\frac{1}{2}$ lb. to 40 gall.); (v) control (no spray treatment). In addition, the following soil treatments were used:—(i) copper sulphate was added to soil at the rate of 1·875 mgm. per 5 in. pot; (ii) no copper sulphate added.

The spray treatments were laid out as five randomized blocks and the plots split for the soil treatment. Each sub-plot consisted of one plant in a 5 in. diameter container. The experiment began on 11/11/48.

Summary of results.—The resultant growth rate ratios are listed in Table 17 with the means for each treatment.

The average difference due to the addition of copper to the soil was not significant. Considering the individual sprays the plants treated with copper oxychloride (2:40) alone showed a reduced growth rate on addition of the copper sulphate which was statistically significant for this experiment at the 1% level. No deductions can be drawn from this single apparent interaction without further experimental evidence.

Table 17.

VARIATION IN GROWTH RATE RATIO WITH SPRAY TREATMENTS (EXPERIMENT 12).

Soil treatment.	Plant treatment.					
	Copper oxychloride.		Bordeaux mixture.		Control. No treatment.	Means.
—	½–40.	2–40.	1–½–40.	4–2–40.		
Copper sulphate ..	100·4	81·6	96·0	108·6	90·2	95·4
No copper	93·2	96·4	104·6	105·0	87·0	97·2
Means	96·8	89·0	100·3	106·8	88·6	96·3

Table 18.

NECESSARY DIFFERENCES FOR SIGNIFICANCE BETWEEN MEAN VALUES (EXPERIMENT 12).

Means of—	s.e.	Necessary difference for significance.	
		5% level.	1% level.
5	3·68	10·6	14·2
10	2·61	7·5	10·0
20	1·84	5·3	7·1
25	1·65	4·7	6·4

On the basis of no significant differences between treated and untreated soils, the following differences between mean values of the sprays were significant:—

At 1% level: Bordeaux 1-½-40 exceeded copper oxychloride (2:40) and the control; Bordeaux 4-2-40 exceeded both copper oxychlorides and the control.

At 5% level: Copper oxychloride (½:40) exceeded copper oxychloride (2:40) and the control.

As the strengths of metallic copper are the same for the two sprays, the eight treatments, excluding the no sprays, form a $2 \times 2 \times 2$ factorial system. The comparisons in Table 19 refer to these eight treatments.

Table 19.

SUMMARY OF GROWTH RATE RATIOS (EXPERIMENT 12).

Metallic copper in 40 gall. (lb.)	Copper oxychloride.	Bordeaux mixture.	Means.
¼	96·8	100·3	98·6
1	89·0	106·8	97·9
Means	92·9	103·6	98·2

Bordeaux mixture was significantly greater than copper oxychloride at the 1% level, the difference being significantly more marked at the higher concentration of copper. The weaker copper oxychloride significantly exceeded the stronger mixture at the 5% level and the stronger Bordeaux exceeded the weaker by 6·5. This interaction parallels the field results in Experiment 9, though the difference between the Bordeaux mixtures in this pot trial was not quite significant at the 5% level, which requires 7·5.

To summarize, this experiment together with Experiment 9 gave significant results contrary to the majority of the trials. These results indicate that stimulation by Bordeaux mixture on this particular soil is greater than any toxic effect due to lime or increased spray loads. A fuller discussion of this point appears later on pages 32-34.

(*v.*) *Experiment No.* 13.

Objective.—To compare the effect of Bordeaux 4-4-40 on the transpiration of mature and immature leaves.

Materials, methods and layout.—Individual leaves from a plant grown in the field were used. For the period of the trial they were maintained with their cut ends in water and kept in the shade with ample indirect light and free movement of the surrounding air. The loss of water by transpiration was estimated by weighing the container and leaf twice daily. Following a pre-treatment recording for three days the treatments were applied and the weighings continued for seven days. The two treatments (see Table 20) were applied to both mature and immature leaves, the leaf groups being split for the treatments. Five replications were used. The experiment commenced on 16/10/48.

Summary of results.—Table 20 lists the comparative rates of transpiration subsequent to treatment. The ratio listed in each case is obtained from—

$$\frac{\text{Average daily loss for post-treatment period} \times 100}{\text{Average daily loss for pre-treatment period}}$$

Table 20.

COMPARATIVE TRANSPIRATION PER 24 HOURS (EXPERIMENT 13).

Treatment.	Ratios.		Means.
	Mature leaves.	Immature leaves.	
Bordeaux 4–4–40	70·4	100·7	85·6
Water	51·1	79·6	65·3
Means	60·8	90·2	75·5

	s.e.	Nec. diff. (1% level).
Marginal means	2·78	12·0
Individual means	3·93	17·0

Bordeaux 4–4–40 exceeds water at the 1% level.

Young leaves (immature) exceed old leaves (mature) at the 1% level.

(*vi.*) *Experiment* 14.

Objective.—To ascertain the influence of Bordeaux mixture on photosynthesis and translocation.

Materials, methods and layout.—The underlying principles are the same as demonstrated by Goodall (1945, 1946) in his detailed work on photosynthesis and translocation in the tomato seedling. A comparison was made of the changes in weight of sprayed and unsprayed pairs of leaflets. Entire plants and detached leaves with petioles immersed in water were sprayed and compared with unsprayed plants and leaves. A pair of opposite leaflets on a number of leaves in each group were selected and immediately after treatment one member of each pair was detached, measured, dried and weighed. Twenty-four hours afterwards the second member of the pair was detached, measured, dried and weighed. Leaflets from leaves still attached to the plants were compared with those from detached leaves, and mature leaves were also compared with very young leaves. Ten replications were used and the two treatments randomized within each of these blocks.

Summary of results.—The data given in Tables 21 and 22 are the weights of the leaflets 24 hours after treatment expressed as percentages of the weights before treatment. Those referring to mature and immature leaves were analysed separately.

Table 21.

MEAN VALUES OF LEAFLET WEIGHTS FOR MATURE LEAVES (EXPERIMENT 14).

—	Sprayed.	Unsprayed.	Means.
Detached leaves	94·8	101·9	98·3
Attached leaves	91·9	90·2	91·0
Means	93·3	96·0	94·7

Differences necessary for significance—
Marginal means .. at 5% level 5·5
at 1% level 7·5
Individual means .. at 5% level 7·8
at 1% level 10·6

Table 22.

MEAN VALUES OF LEAFLET WEIGHTS FOR IMMATURE LEAVES (EXPERIMENT 14).

—	Sprayed.	Unsprayed.	Means.
Detached leaves	97·6	99·0	98·3
Attached leaves	99·6	103·8	101·7
Means	98·6	101·4	100·0

No significant differences.
Standard error—marginal means .. .. 2·58
individual means .. .. 3·65

At the 5% level, the weights of detached leaves significantly exceeded those of the attached, the difference being accentuated and significant at the 1% level in the case of the unsprayed leaves, but not significant for the sprayed leaves.

These results are considered in more detail in the discussion on the plant processes affected (pages 36-38).

2. Discussion of the Results of Analytical Experiments.

The previous set of experiments (9-14) were designed with two goals in view:—(1) to separate the factors responsible for the phytotoxicity of Bordeaux mixture, and (2) to ascertain the plant processes affected. In the following discussion each factor will be discussed separately, the relevant data being drawn from the various experiments.

(i.) The Factors Responsible for Phytotoxicity.

(*a*) *Lime content of spray.*—The lime content of Bordeaux 4-4-40 has been demonstrated as an important factor in the phytotoxicity of this mixture by several experimenters and their results have been summarized by Horsfall and Harrison (1939) and Horsfall (1945). The results of the field experiments reported in this paper are consistent with the findings of these other workers.

To check the matter further Experiment 9 was designed to compare copper sulphate-hydrated lime mixtures at the ratios of 1:1 and 2:1 and at three different spray loads. Tomatoes treated with the lower lime mixture significantly exceeded in yield of fruit those treated with the spray containing equal proportions of the constituents. This was particularly noticeable at the greater spray loads, the difference between the 1-1-40 and 1-½-40 mixtures alone not being significant.

The same point was checked again in Experiments 10 and 11 on plants grown in pots. In the first case the 6-2-40 mixture significantly exceeded the 4-4-40 and 0-8-40 mixtures but not the 2-6-40. In Experiment 11 the difference between the treatments was not statistically significant but showed the two low-lime mixtures exceeding the high-lime sprays. The absence of significance in this latter experiment is attributed to the vigorous growth of the experimental plants.

The 2-6-40 mixture involves a ratio of dehydrated copper sulphate to calcium hydroxide of nearly 1 to 5. A factor tending to reduce the toxic effect of the extremely high lime mixtures below that expected is possibly the difference in physical condition of these mixtures compared with the others, since it is accepted that the better the spreading and suspension properties of the spray the greater the phytotoxic effects (Wilson and Runnels, 1934; Horsfall and Harrison, 1939). It is generally considered that the high-lime mixtures do lose some of the gelatinous properties associated with the other mixtures.

(*b*) *Spray load.*—This factor has been listed as contributing to the phytotoxicity of fungicides (Horsfall, Hervey and Suit, 1939). Two of the experiments in this series (9 and 11) were designed to check this point. In the firstmentioned, copper oxychloride showed an appreciable increase in yield with reduced spray load. The two Bordeaux mixtures (4-4-40 and 4-2-40), however, showed the opposite tendency—i.e., an increase in yield with higher spray loads. On the other hand, the phytotoxic effect of excess lime in Bordeaux 4-4-40 as compared with Bordeaux 4-2-40 is emphasized as the total load of the sprays is increased. If the Bordeaux mixture with 1:1 ratio is taken as 100 at each level of spray concentration, the yields of Bordeaux mixtures with 1:$\frac{1}{2}$ ratio are 101, 108 and 121. The anomaly of the two Bordeaux mixtures increasing the yield in spite of increased spray load is possibly bound up with a copper stimulation, and the apparent greater stimulation with higher concentrations (e.g., 4-2-40 mixture exceeds 1-$\frac{1}{2}$-40 mixture) could be taken to support this. Copper stimulation by copper oxychloride is relatively negligible, due presumably to its relative insolubility.

Experiment 11, with Bordeaux mixtures of varying loads, did not give any appreciable differences in growth rates between the loads. On the other hand, within the same trial there was a definite interveinal necrosis, principally with the high concentrations, which would have undoubtedly affected growth rates later.

The evidence presented from these experiments supports the view that spray load has a tendency to increase phytotoxicity, but also indicates that it may be appreciably modified by other factors.

The data discussed later under seasonal and cultural conditions (page 34) also suggest extra phytotoxicity from heavier spray loads.

(*c*) *Copper stimulation of Bordeaux mixture.*—Throughout these field trials there have been certain inconsistencies—viz., in Experiments 1, 4 and 9, in which Bordeaux 4-4-40 gave practically equivalent yields to cuprous oxide and copper carbonate dusts and sprays. Moreover, Experiment Number 9 had been laid down on an area adjacent to an earlier trial (not mentioned in this series) in which Bordeaux mixture had given a high yield relative to a copper carbonate dust. It was thought that the common locality (i.e., soil type) might explain the unexpectedly good performance of Bordeaux mixture. Experiment 6 had also been conducted on this farm and had included Bordeaux 4-4-40 and copper oxychloride spray (2 lb. to 40 gall.), but overall had shown the yield from Bordeaux treated plants to be significantly less than that for those treated with copper oxychloride. A detailed examination of the individual plot yields, however, gave interesting indications. To clarify the discussion of these data the relative positions of Experiments 6 and 9 are indicated in Fig. 8. A characteristic of this farm is the obvious change in the nature of the soil in colour and texture from one side to the other. This is also indicated in Fig. 8. Experiment 6 was in the form of a Latin square and therefore a comparison of the yields from these two treatments could be made progressively across the property.

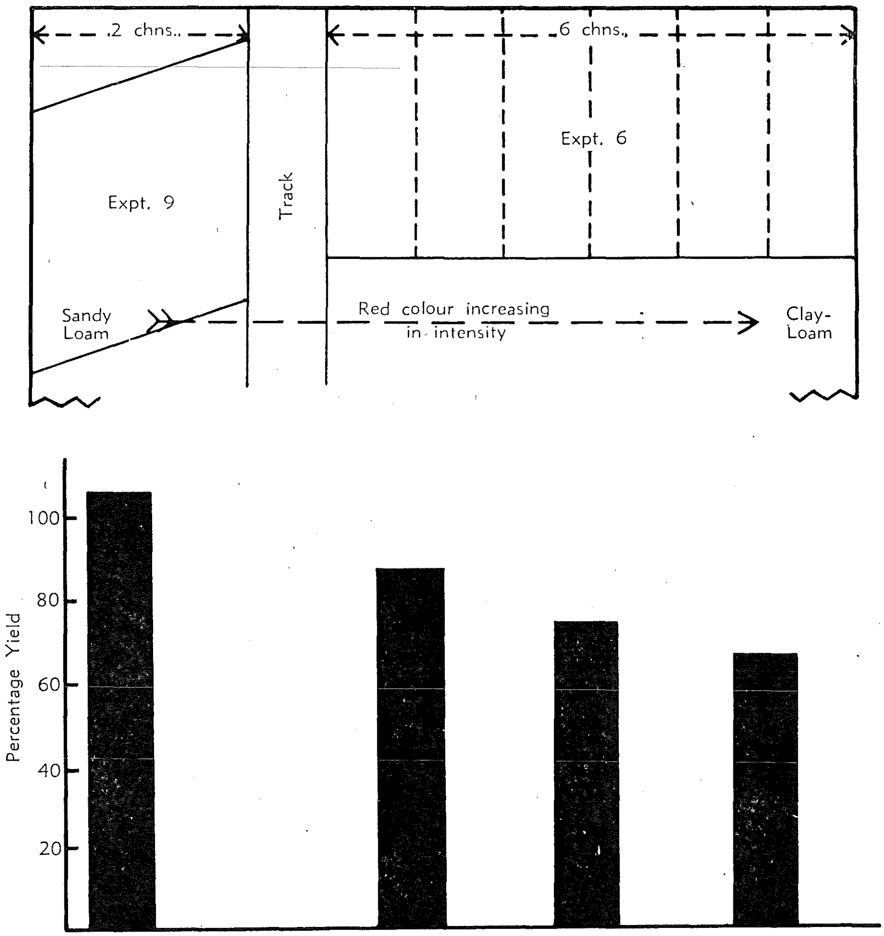

Fig. 8.

Diagram Showing the Relative Positions of Experiments 6 and 9 in the Field and the Yield of Bordeaux 4-4-40 relative to Copper Oxychloride (2:40) Treatment.

On the assumption that copper oxychloride (2 lb. to 40 gall.) equals 100 for each column, the comparative yields of Bordeaux mixture were 74, 56, 56, 92, 74 and 100, proceeding from the heavier soil to the lighter. In Experiment 9 the relative yield figure was 106. This figure is the average of two replications and if the other six yields are averaged in pairs it would give samples from four approximately equal areas across the property. The sequence then runs 65, 74, 87 and 106. This is shown diagrammatically in Fig. 6. This evidence thus further suggested a direct relationship between the soil type and the response of the tomato plants to Bordeaux mixture.

Experiment 12 was designed to investigate this stimulus further. Soil from the location of Experiment 9 was used, and relative growth rates estimated. Stimulation due to Bordeaux mixture, in comparison with copper oxychloride, was again present, and the interaction which shows the stronger Bordeaux exceeding the weaker while the weaker copper oxychloride exceeded the stronger was also present. However, the endeavour to ascertain whether this difference was due to deficiency of copper in the soil was not successful. There was no disease present in this experiment, and in Experiment 9 it was negligible.

In summarizing the evidence from the field and pot experiments it can be said that (i) Bordeaux mixture significantly stimulated growth in comparison with copper oxychloride in both the field and pot experiments; (ii) the higher concentration of Bordeaux mixture stimulated growth more than the weaker spray in both the field experiment and the pot experiment and *vice versa* for copper oxychloride; and (iii) the soil treatment with copper sulphate did not alter the relationships between the two spray treatments.

The results may possibly be explained on the basis that the plants were slightly copper deficient when grown on this particular soil, but that there is no response to the soil treatment with copper sulphate, as this is made unavailable to the plants.

(*d*) *Influence of seasons and cultural conditions.*—On close examination of the yields from Experiments 1-6, it will be noticed that the differential effect on total yield of the various treatments is emphasized more in some experiments than in others, particularly when considering the differences between Bordeaux 4-4-40 and cuprous oxide mixture (home-made). These differences may be grouped as follows:—(i) comparing crops grown in the spring season, the trellised experiments (2 and 5) show greater differences between treatments than crops grown unpruned on the ground (1 and 4), and (ii) comparing unpruned crops, those grown in autumn (3 and 6) show a greater differential effect between treatments than those grown in spring (1 and 4). A comparison of dust treatments common to the experiments also illustrates this point. It is suggested that those trials showing the greatest differences received a greater quantity of fungicide per unit leaf area, thus tending to accentuate any phytotoxic differences. This is readily appreciated in the spring crop, because with pruned and trellised plants the majority of the leaf surfaces are easily accessible and both the upper and lower leaf surfaces are more readily sprayed, while in the case of the unpruned plant on the ground the outside canopy of leaves tends to shelter the inner leaves from heavy deposits of fungicide. The greater differences shown in the autumn crop, in comparison with the spring crop, would be accounted for by the relatively slower growth and more frequent application of fungicides in the autumn and winter, thus resulting in a heavier cover of fungicide per unit of leaf surface owing to the relatively smaller amount of fresh growth between applications of the fungicide (i.e., the heavier cover has a cumulative effect).

(*e*) *Vigour of plants at time of spraying.*—The comparison of spring and autumn crops in the previous section brings to light another important factor in the phytotoxicity of fungicides—viz., the vigour of the plant at the time of spraying. The previous experiments of Martin (1916) and Wilson and Runnels (1935 a and b) pointed out the declining effect of the spray over the days following the application. It is also discussed by Horsfall (1945, p. 179). Thus it is reasonable to expect that under good growing conditions the effect of the spray would pass away more rapidly as the new growth is made than in those circumstances when the rate of growth is decreased.

Additional support for this comes from a comparison of the results of Experiments 10 and 11. In the one case significant differences were obtained between Bordeaux 4-4-40 and Bordeaux 6-2-40; in the other case, however, the differences were not significant. The average relative growth rate in the second case was exceptionally high, being approximately 50 per cent. per day for a $2\frac{1}{2}$ in. leaf as compared with 12 per cent. in the other experiment.

(*f*) *Method of application.*—It was noticed during these experiments that there was a greater tendency for necrosis of the tips to appear if the plants were "washed" with a coarse spray than if one of fine drops was used. This damage is possibly due to an accumulation in the leaves not yet fully opened. No attempt was made to confirm this statistically.

(*g*) *Copper content of the spray.*—Since copper toxicity was the obvious danger to avoid in past years and is still a difficulty with the "copper sensitive" fruit crops, no discussion of Bordeaux mixture toxicity would be complete without mention of this factor. Tomatoes appear to possess some degree of tolerance. Shutak and Christopher (1939) found that the 12-4-40 mixture which they used contained free copper by the ferrocyanide test but it did not injure the tomatoes in any way. The 6-2-40 mixture in Experiment 10 produced no toxic effects and actually gave the greatest growth rate. This mixture is approximately neutral when made with hydrated lime. In this particular experiment, even the 7-1-40 mixture did not produce necrosis though it definitely retarded growth. While care must always be exercised, it does appear that the amount of lime used in tomato sprays could be safely reduced to about half the weight of bluestone so long as good quality hydrated lime is available.

(*ii.*) *Physiological Processes Affected.*

The next point of interest is to ascertain which of the physiological processes of the plant is affected. This aspect has been summarized by Horsfall (1945) under the headings:—(i) transpiration, (ii) photosynthesis, (iii) translocation, and (iv) direct retardation of cell growth by hardening of the middle lamella. The relevance of the results of these experiments to three of these points and to pollen poisoning is discussed below.

(*a*) *Transpiration.*—The evidence for an increased transpiration due to treatment with Bordeaux 4-4-40 is convincing but is not entirely consistent, and has been discussed in some detail by Horsfall and Harrison (1939), Wagner (1939) and Horsfall (1945). The results from Experiment 13 show an increase of 31 per cent. for Bordeaux mixture as compared with treatment with water and this is in accord with the general conclusion arrived at by these writers.

However, results have been published at intervals indicating that Bordeaux mixture does not increase the transpiration rate of the plant (Childers, 1935; Miller, 1938; Foster and Tatman, 1940). The results obtained by this group of experimenters can be summarized as follows:—(*a*) young greenhouse grown plants and detached leaves have uniformly increased loss of moisture, and (*b*) mature plants grown either in the field or in the glasshouse have not shown significant increases in moisture loss. Horsfall suggested that the differences can be explained on the basis of varying thicknesses of cuticle and that glasshouse grown plants were thus more sensitive. Foster and Tatman, using mature plants grown in the glasshouse, did not find any significant increase in transpiration due to Bordeaux mixture, though some of the other copper sprays showed such differences. A better general explanation of the results of these previous workers may be that the sensitivity to Bordeaux spray increases with the proportion of young foliage on the plant (i.e., the difference between young and mature plants, which is still correlated with the relative thickness of the cuticle). Further, on examining all the results published at various times it appears that, given optimum moisture conditions, accelerated transpiration losses due to the action of Bordeaux mixture on mature plants are not sufficient to reduce yield to the extent shown in the experiments in this paper. Horsfall, Hervey and Suit (1939) were also of this opinion for cucumbers. The extra loss is significant, however, under the following conditions:—(i) heavy applications of spray to young plants, which may also cause necrosis of the growing points, (ii) spraying the young plants just prior to or subsequent to transplanting (Wilson and Runnels, 1937), and (iii) for mature plants in time of moisture shortage (Childers, 1935).

(*b*) *Photosynthesis and translocation.*—Very little evidence on the effect of Bordeaux mixture on the photosynthesis and translocation of tomatoes is available. Christopher (1937) found that neither Bordeaux mixture nor lime had any significant effect on carbon dioxide assimilation of treated tomato leaves. Horsfall (1945, p. 181) pointed out the indirect evidence that if growth is ultimately reduced the photosynthetic rate must have been reduced. In the same publication an observation by Ewart (1905) is quoted stating that Bordeaux-sprayed leaves were still heavily loaded with starch in the morning while the starch in check leaves was removed during the night.

Experiment 14 was designed to obtain some information on these points, and was based on the fundamental work of Goodall (1945, 1946) on the part played by the various parts of the young tomato plant in the manufacture and distribution of the photosynthate. In the case of the unsprayed and sprayed mature detached leaves, differences in dry weight are taken to be entirely due to the reduced photosynthetic rate. This difference was 7·1 per cent. in favour of the unsprayed leaf. Goodall's work indicates that the photosynthate prepared by the young immature leaves is very small, so the relatively small differences between these are to be expected. The difference of 7·1 per cent. is just under that required for significance at the 5% level in Experiment 14.

Some observations on the effect on translocation are also possible. On considering again the results of Experiment 14, and comparing the results for the sprayed and unsprayed mature attached leaves, the sprayed ones average 1·7 per cent. greater than the unsprayed. One would expect that these mature leaves would be both approximately equal before and after any period of investigation, as both are at a stage when all the material photosynthesized is used to support other parts of the plant, and irrespective of the differential rate of photosynthesis one would expect the total product to be translocated. The difference obtained is not statistically significant for this experiment, but in the light of the general figures for both immature and mature leaves showing differences as one would theoretically expect, it suggests that the figure of 1·7 may be a true one and due to an interference with the translocation of the sprayed plant.

On comparing the immature leaves still attached to the plant, the unsprayed leaf shows an increase of 4·2 per cent. over the sprayed. The difference due to photosynthesis alone was 1·4 per cent (calculated from a comparison of detached leaves), thus suggesting that the unsprayed attached immature leaf has received 2·8 per cent. more than the sprayed attached immature leaf. This difference may be due either to the reduced amount of photosynthate available or to impaired translocation, and it does not seem possible to separate these two factors in this case.

Another comparison of interest contributing to this point of translocation is the differences between the mature attached leaves and the immature attached leaves of the same plant. The unsprayed immature show a 13·6 per cent. higher figure than the unsprayed mature leaves. This is the greatest difference in the experiment and is to be expected from theoretical considerations. On comparing the immature and mature sprayed leaves the difference is reduced to 7·7 per cent. If this increase in the immature leaves is all due to translocated material received, then the unsprayed immature ones have received approximately 76 per cent. more translocated material than the others. If some of the increases in the young leaves is due to their own photosynthate, the proportion of translocated material received by the immature unsprayed leaf over the immature sprayed leaf is even greater than 76 per cent. because of the greater efficiency of the unsprayed leaf in photosynthesis. The inference that the unsprayed immature leaf receives a greater proportion of translocate than its sprayed partner is strong; but as before it cannot be proved whether this extra amount is due to the extra photosynthate from the unsprayed mature leaf, to the unimpaired translocation of the unsprayed plant, or to the combination of both factors.

In the discussion of the results of this experiment so far no comparisons between detached and attached leaves have been made. This has been avoided owing to the doubt as to whether the rate of photosynthesis is increased or decreased for detached leaves. Assuming, however, a comparable rate of photosynthesis, which is not unreasonable from Goodall's work, comparison of unsprayed mature leaves shows a significant increase in weight (11·7 per cent.) for the detached over the attached at the 1 per cent. level. Comparing the sprayed mature leaves, the difference is definitely not significant and reduced to 2·9 per cent. This reduction of a highly significant difference to non-significance can be explained on the basis that the total effect of both photosynthesis and translocation has been significantly reduced. It can be analysed further as follows:—a mature leaf (unsprayed) can photosynthesize 11·7 per cent. of its weight, which is all translocated to other parts of the plant, and a mature sprayed leaf photosynthesizes 4·6 per cent. of its weight but only translocates 2·9 per cent. of its weight. On this basis the spray reduces both photosynthesis and translocation.

Making a similar assumption for the immature leaves—i.e., that detachment does not change appreciably the rate of photosynthesis—the increase in weight of immature unsprayed leaves due to translocated material received is 4·8 per cent. of its weight and for sprayed leaves it is 2·0 per cent. of its weight. Consideration of the immature leaves alone, however, cannot demonstrate whether the difference is due to the smaller amounts of photosynthate available from sprayed mature leaves or to a reduced rate of translocation.

(*c*) *Toxicity to pollen.*—Horsfall and Harrison (1939) demonstrated that the differences in yield were due principally to defloration, and also showed that defloration is correlated with dwarfing of the plant.

The correlation of flower setting with total yield and the absence of any significant variation in the size of the fruit in these experiments (Table 6) supports this conclusion.

The question then arises as to whether this defloration is due to impaired pollination due to fungicide, or to the reduced vigour of the host. MacDaniels and Hildebrand (1938 and 1940), investigating the effect of various bactericides on pollen germination, found that copper sprays did not reduce fruit setting by flowers specially sprayed with the various compounds, though in laboratory tests the materials were definitely toxic. The explanation was that the stigma is not easily wetted and apparently the pollen grains on the stigma were unaffected. It is considered that the effect of the fungicides on tomato pollen would probably be even less as the flowers of most of the common varieties have short stigmas (i.e., they do not emerge from the staminal tube.) In addition, the normal pendulous position of the tomato flower makes it even less likely that the fungicide would reach the stigma.

It appears reasonable to assume then that defloration in the tomato is a reflection of general physiological upset rather than a specific poisoning of the pollen grains.

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