

# CHLORINE AND BROMINE IN THE NUTRITION OF HIGHER PLANTS

By P. G. OZANNE,\* J. T. WOOLLEY,† and T. C. BROYER†

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## Summary

Plants were cultured in purified inorganic salt solutions and with filtered glass-house air to reduce contamination by chlorine. Leaf and top yields of tomato and clover respectively were reduced by over 80 per cent. when insufficient chlorine was supplied.

Supplying bromine in place of chlorine gave yields to 75–95 per cent. of the yields with chlorine. However, plants receiving bromine always extracted more chlorine from their environment. In some instances, five times as much chlorine appeared in plants treated with bromine as in “no bromine” controls. In all cases the yields with bromine applications were less than those with applied chlorine. Bromine does not appear to completely substitute for chlorine in growth and development of plants, as evidenced by weight and wilt differences. Part of the apparent effect of bromine was due to chlorine arising from extraneous contamination, e.g. the atmosphere. When plants were given ample chlorine, no yield differences were obtained by additions of bromine.

Without added halide, foliar concentrations of chlorine were as low as 1.0  $\mu\text{g-atom/g}$  dry weight for tomatoes, and 0.8  $\mu\text{g-atom/g}$  for clover. With ample bromine supplied, these values fell only to 0.7 and 0.6 respectively. Large decreases in tomato yields were found when leaf chlorine concentrations fell below 2.0  $\mu\text{g-atoms/g}$  dry weight.

The addition of bromine to roots of intact plants in a “minus chloride” culture solution caused loss of chlorine from the roots. Similarly, with decapitated plants, chlorine was found to be displaced, moving upwards in the exuding sap. It is inferred that the effect of bromine supplied to chlorine-deficient plants is due to displacement of the chlorine differentially from non-effective places to more essential loci.

## I. INTRODUCTION

The recent demonstration (Broyer *et al.* 1954) that chlorine is necessary for growth of higher plants has opened up a number of questions with respect to this element in nature. From one point of view, since chlorine is a plant micronutrient, it becomes of interest to learn more of its occurrence and availability to plants in their natural settings. There are also questions of its quantitative requirements for different plant species and of the functions of chlorine in plant metabolism including possible interrelationships with other plant nutrients.

A finding of immediate interest is the degree to which bromine can substitute in plant functions normally dependent upon chlorine. Perhaps only potassium, among the presently recognized essential elements for higher plants, can go as far toward partially sharing functions with another element—as, for example, with sodium.

\* Division of Plant Industry, C.S.I.R.O., Western Australia Regional Laboratory, University Grounds, Nedlands, W.A.

† Department of Soils and Plant Nutrition, University of California, Berkeley, California, U.S.A.

Tomato plants cultured in rigorously purified salt solutions provided the first clear-cut demonstration of the requirement for chlorine (Broyer *et al.* 1954) and similar cultural techniques were successfully extended to a number of other species (Johnson *et al.* 1957). Chlorine deficiency is characterized by leaflet wilting, followed progressively by chlorosis, bronzing, and finally, necrosis. These symptoms could be prevented by providing the culture solutions with small amounts of chloride ion. It was also noted that additions of bromide delayed the onset of chlorine deficiency, but the efficiency of bromine was less. Consequently, it was tentatively concluded that bromine exhibited a "sparing effect" for at least some functions normally met by chlorine. The effect of bromine in alleviating chlorine deficiency has been confirmed by Ulrich and Ohki (1956).

Thus, when the studies to be described here were commenced, it appeared evident that chlorine activates some essential function in plants and that bromine exercised a sparing effect for chlorine. It was not clear how far bromine could serve as a substitute for chlorine. Also, it remained to be shown whether or not bromine played some necessary role distinct from that of chlorine. In the six experiments to be described, both highly purified salt solutions and filtered air were used during plant cultivation, with the result that concentrations of chlorine in plant tissues were reduced well below those reported earlier. New evidence on the relative effectiveness of chlorine and bromine is presented.

## II. MATERIALS AND METHODS

### (a) Culture Technique

The method used in these studies was to culture four tomato plants (*Lycopersicon esculentum* Mill. var. Marglobe) or six clover plants (*Trifolium subterraneum* L.) per 4 l. of inorganic salt solution. For further details of containers etc. see Broyer *et al.* (1954). Seeds were germinated on cheesecloth over dilute culture solution "free" from chlorine and bromine, then transplanted to 4-l. beakers when the seedlings had roots 2-3 in. long. The culture solutions were aerated continuously during plant growth.

All experiments were carried out in a glass-house fitted with charcoal and granular limestone filters through which much of the air was drawn into the glass-house. At harvest, rubber gloves were used in the separation of the plant material. Roots were rinsed in distilled water and spun in a centrifuge. All material was dried for 48 hr in a forced draught oven at 90°C. Dry weights were taken and the samples ground to pass a 40-mesh sieve prior to analysis.

In these experiments, two types of contamination are recognized. The first, *intrinsic* to the plant-culture system, includes known contamination from seed, in the repurified salts and water, and from aeration. The second, *extrinsic* to the system, includes that from airborne or other sources unknown or uncontrolled.

### (b) Culture Solutions

The culture solutions were all compounded on the chemical equivalency basis as proposed by Johnson *et al.* (1957). The M6 fraction contained the six macro-nutrient ions in m-moles/l as follows:  $\text{Ca}^{++}$ , 4;  $\text{Mg}^{++}$ , 1;  $\text{K}^+$ , 6;  $\text{NH}_4^+$ , 2;  $\text{PO}_4^{=}$ , 2;

$\text{SO}_4^{=}$ , 1; and  $\text{NO}_3^-$ , 14. For culturing clover, the ammonium and phosphate ions were each reduced to a concentration of 1 m-mole/l. The (m7-Cl) micronutrient fraction (Johnson *et al.* (1957), Table 1), providing six micronutrient elements, was added in the following microgram-atoms/l quantities: B, 25; Mn, 5; Fe, 4; Zn, 2; Cu, 0.5; and Mo, 0.1. The iron was added as  $\text{FeSO}_4$  at 8  $\mu\text{g}$ -atoms per culture each week. Only analytical reagent grade salts, which had been purified by recrystallizing twice, were used in preparation of the cultures. Solutions so prepared contained less than 2  $\mu\text{g}$ -atoms Cl per culture. During growth, the plants obtained a continual small supply of chlorine apparently from contamination from the atmosphere, and from the glass-redistilled water. This contamination increased with plant size. Contamination with bromine during growth appeared to be negligible. Where chlorine and bromine were added to the solutions, twice recrystallized reagent grade potassium salts were used. Maximum chlorine in the recrystallized potassium bromide was less than 1  $\mu\text{g}$ -atom per culture in the highest bromine level supplied (2000  $\mu\text{g}$ -atoms per culture).

The plants cultured without added chlorine or bromine are referred to as M6+(m7-Cl) controls and received only the "chlorine" intrinsically derived from the purified solutions and extrinsically derived from atmosphere sources. In 6-week-old plants this contamination chlorine varied from 10 to 30  $\mu\text{g}$ -atoms per culture.

### (c) Analytical Methods

Chlorine and bromine in the plant material were measured by modifications of the Conway (1947) microdiffusion technique, after incomplete ashing at 550°C in the presence of potassium carbonate.

When "tracer" amounts of radiochlorine were used as treatments, they were applied as  $^{36}\text{Cl}$ . This was measured by use of a thin end-window Geiger tube (1.7 mg/cm<sup>2</sup>).

## III. OBSERVATIONS AND RESULTS

### (a) Comparative Plant Growth Effects of Chlorine and Bromine

In experiment 1, tomato plants were cultured over a wide range of halide supply levels—one series having 0, 2, 8, 30, 100, 300, and 1000  $\mu\text{g}$ -atoms Cl applied per culture, and the other series 0, 10, 30, 100, 1000, and 2000  $\mu\text{g}$ -atoms Br applied per culture. Three replicates of each treatment level were prepared. Acute chlorine-deficiency symptoms as described by Broyer *et al.* (1954) were observed in the lower halide cultures. Symptoms of leaf wilting and bronzing did not appear however at the higher levels of chlorine, i.e. 30  $\mu\text{g}$ -atoms per culture and above. On the bromine treatments of 100  $\mu\text{g}$ -atoms per culture or more, no bronzing occurred, although occasionally, under conditions of enhanced water stress, wilting occurred even at the highest level of bromine. After 6 weeks growth the trial was terminated and the plant material separated into roots, stems, and leaves.

Data showing yields of roots, stems, and leaves at the various levels of each applied halide are presented in Figure 1. Marked effects of treatment were obtained in all plant parts, although the leaf weights were affected more than the stems from

applied halide, while the root weights increased least of all. Both chlorine and bromine application gave effectively the same general yield pattern. However, the

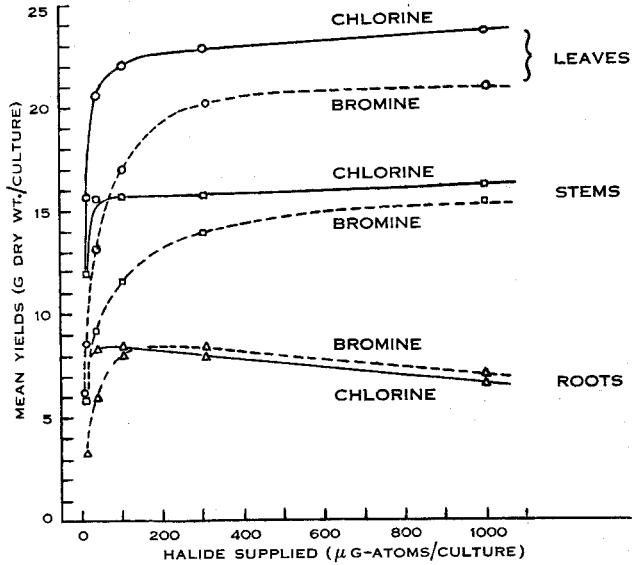


Fig. 1.—Yields of tomato plants when chlorine or bromine is added to the culture solutions in increasing amounts.

effect of added chlorine on the leaves and stems was greater than that of bromine and was obtained at a much lower level of application. Also, the maximum leaf

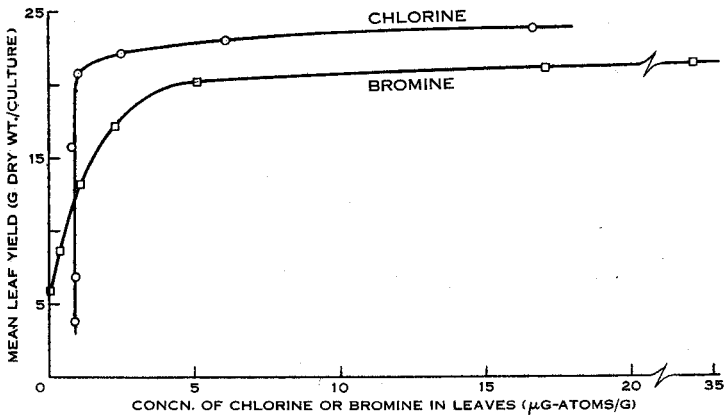


Fig. 2.—Relationship in tomato plants between concentration of chlorine or bromine present in the leaves (dry basis) and the mean leaf yield per culture.

yields with adequate chlorine were significantly greater than those obtained with ample bromine.

The concentrations of halide in the leaves, together with the related yields from experiment 1, are shown in Figure 2. Leaf yields increased rapidly up to bromine concentrations of  $5 \mu\text{g-atoms/g}$  tissue. Further increases of bromine to  $34 \mu\text{g-atoms/g}$  tissue showed no toxic effect. The growth effects at deficiency levels of chlorine and bromine are shown in more detail in Figure 3. Yields were not markedly affected

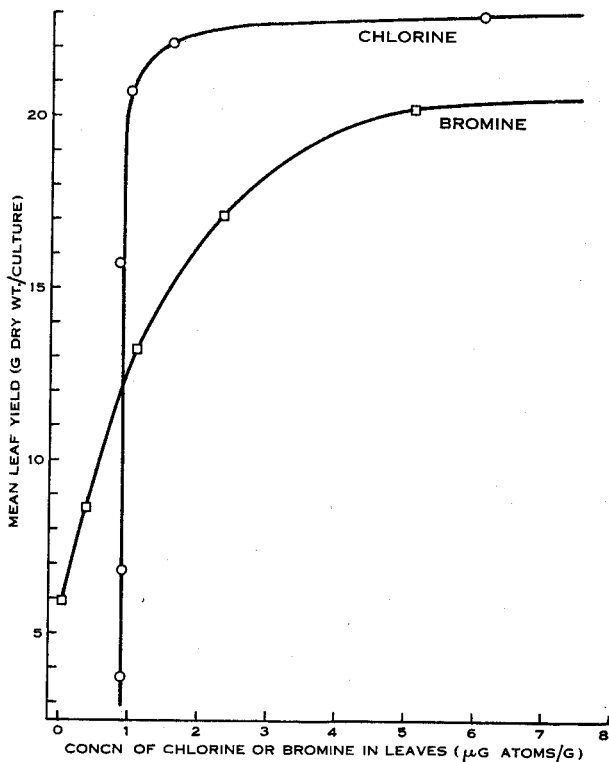


Fig. 3.—Enlarged view of relationship between concentration of chlorine or bromine in leaves and leaf yield at chlorine-deficiency levels (see Fig. 2).

until the leaf-chlorine level became less than  $2 \mu\text{g-atoms/g}$  tissue. Then, yields decreased sharply to a minimum at concentrations of  $0.9 \mu\text{g-atom/g}$ . Further reductions in chlorine supply merely decreased leaf yield without decreasing leaf chlorine concentrations. In the absence of applied bromine, it has not been possible to obtain tomato plants containing chlorine concentrations lower than this value in leaves.

In contrast to the minimal critical threshold of chlorine in the chlorine-yield curves, withholding bromine from plants reduced their bromine concentration to the limit of chemical detection ( $0.1 \mu\text{g-atom/g}$  dry weight). However, the concentration of chlorine in the leaves of plants supplied with bromine did not fall below  $0.5 \mu\text{g-atom/g}$ . The contribution to growth from this contamination chlorine will be discussed later.

It remained to be shown whether additions of bromine to plants which were supplied adequately with chlorine could give further growth effects. To examine this, in experiment 2, 10 cultures of tomato plants were prepared. To all of these, an ample supply (400  $\mu\text{g}$ -atoms per culture) of chlorine was added. In addition, a high level of bromine (1000  $\mu\text{g}$ -atoms per culture) was added to each of five of the beakers. All the plants made healthy growth without deficiency or toxicity symptoms, and were harvested after 6 weeks. The dry weight yields of the plant parts are shown in Table 1. It may be seen that the plants cultured with bromine in addition to chlorine were equal in yield to those supplied chlorine alone. Thus, in the presence of adequate chlorine, bromine neither increased growth through some beneficial function, nor decreased yields by hindering chlorine activity or by some other form of toxicity.

TABLE 1  
FAILURE OF BROMINE TO ENHANCE GROWTH OF TOMATOES GIVEN AMPLE CHLORINE  
All values are the means of six replicates

Treatment	Yield—Dry Weight (g)			
	Leaves	Stems	Roots	Total
400 $\mu\text{g}$ -atoms chlorine	8.2	5.9	2.4	16.5
400 $\mu\text{g}$ -atoms chlorine + 1000 $\mu\text{g}$ -atoms bromine	8.1	5.9	2.5	16.5
Least significant difference ( $P = 0.01$ )	1.0	0.9	0.3	2.0

In experiment 3, the relative effects of bromine and chlorine were examined with a different plant species, viz. subterranean clover. In addition to six control cultures without added chlorine, four replications of each of the following treatments were planted: plus chlorine only at 400  $\mu\text{g}$ -atoms per culture; plus bromine only at 400  $\mu\text{g}$ -atoms per culture; and plus both chlorine and bromine, at 400  $\mu\text{g}$ -atoms each per culture. This trial was commenced on November 11, 1955 at Berkeley. Due to slow plant growth in winter, harvest was delayed until February 6, 1956. Chlorine-deficiency symptoms developed on the control plants after 5 weeks, and became acute before the plants were harvested. Shortly after deficiency symptoms developed on the controls, the plus bromine plants became slightly retarded in growth and showed a tendency to wilt on sunny days. The appearance of the respective treatment effects are shown in Plate 1, Figures 1 and 2. The control plants showed marked stunting of roots as well as reduced top growth. Although somewhat larger, the plus bromine plants also had stubby, short, thick roots. The relative root development in the various treatments is shown better in Plate 1, Figure 2. Despite their poor elongation, however, the dry weights of the plus bromine roots were not significantly less than the root weights from the two treatments given chlorine.

This trial was repeated the following summer under more favourable growth conditions. Planted on May 28, 1956, the plants made more growth in 6 weeks than in the 12 weeks in winter. To ensure ample halide supplies, the levels were increased

TABLE 2  
 HALOGEN CONTENTS AND YIELDS OF CLOVER TOPS WITH CHLORINE AND BROMINE ADDED TO THE CULTURE SOLUTION  
 All values are the means of four replicates

Treatment	Concentrations		Totals		Total Yield (dry wt.) (g)	"Chlorine Yield" (dry wt.) (g)	"Bromine Yield"* (dry wt.) (g)	"Bromine Yield" (% of total)
	Bromine ( $\mu\text{g-atoms/g}$ )	Chlorine ( $\mu\text{g-atoms/g}$ )	Bromine ( $\mu\text{g-atoms}$ )	Chlorine ( $\mu\text{g-atoms}$ )				
Winter								
Control	<0.1	1.42†	—	21.8†	15.5	15.5	Nil	115
Chlorine added	<0.1	13.4‡	—	372‡	27.9	27.9	Nil	
Bromine added	15.4†	1.08†	325	22.7†	21.0	21.0	5.0	24
Chlorine and bromine added	13.1†	13.8†	345	363‡	26.5	26.5		
Least significant difference at $P = 0.01$	2.7†	0.25† 2.7‡	41	8.0† 41‡	3.9			
Summer								
Control	<0.1	0.77†	—	2.7†	3.3†	3.3	Nil	
Chlorine added	<0.1	13.6‡	—	408‡	30.0‡	30.0‡	Nil	
Bromine added	14.5†	0.54†	389	14.5†	26.8‡	26.8‡	16.0	60
Chlorine and bromine added	12.8†	14.6†	389	444‡	30.4‡	30.4‡		
Least significant difference at $P = 0.01$	2.0†	0.21† 2.0‡	31	1.8† 31‡	1.6† 3.1‡			

\* "Bromine yield" by difference: total yield minus "chlorine yield".

‡‡ In some instances two values are given for least significant differences. The treatments to which these values refer are shown by the daggers.

from 400 to 500  $\mu\text{g}$ -atoms per culture. In general, results were similar to those of the winter trial, but the control cultures showed acute chlorine deficiency after only 10 days, and made little growth thereafter. Yields at harvest, for both trials, are given in Table 2, together with the chlorine and bromine concentrations present in the clover tops from the various treatments.

With subterranean clover, the addition of chlorine or bromine or both gave significant increases in plant yields. As was expected from experiment 2, the chlorine and chlorine plus bromine treatments gave similar yields; however, the addition of bromine alone gave plants having significantly smaller tops than did the two treat-

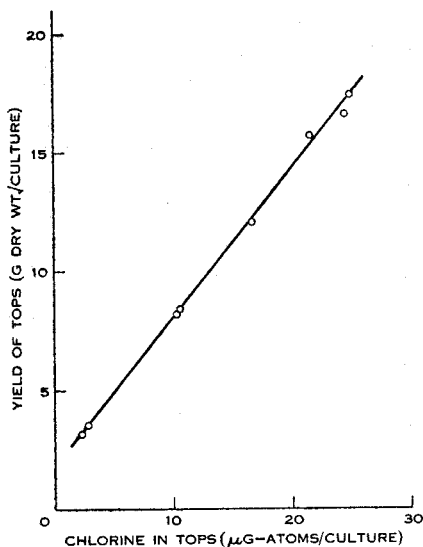


Fig. 4

Fig. 4.—Effect of chlorine content on yield of tops with subterranean clover cultured under very low chlorine-supply conditions.

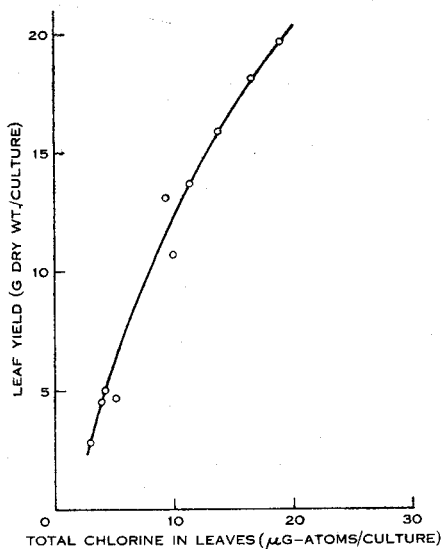


Fig. 5

Fig. 5.—Effect of chlorine content on yield of leaves of tomato plants cultured under very low chlorine-supply conditions.

ments containing added chlorine. Thus, even though bromine caused a great increase in yield in the summer trials, it did not completely substitute for chlorine.

In these trials where chlorine was added, the concentration of chlorine in the plant tops was not decreased by the further addition of bromine. Similarly, the addition of chlorine did not depress bromine uptake.

The control cultures without added chlorine contained 1.42  $\mu\text{g}$ -atoms/g dry weight in winter and 0.77  $\mu\text{g}$ -atom in summer, but the tops of plants supplied bromine still contained appreciable amounts of chlorine, e.g. 1.08  $\mu\text{g}$ -atoms/g in winter, and 0.54  $\mu\text{g}$ -atom in summer. When the total chlorine present in the tops was plotted against top yield for control cultures (without added halide), as in Figure 4, a close linear relationship was found. Each increment in total chlorine from contamination produced an equivalent increase in top weight, so that given a chlorine content it is possible to calculate the approximate "chlorine yield", or dry weight



of tops, which that amount of chlorine would be expected to produce. Assuming this relationship between chlorine and yield holds also for the contamination chlorine in the plants to which only bromine was applied, then in the winter trial it appears that for the plus bromine treatment, 16 of the 21 g top weight could have been produced by the contamination chlorine, leaving only about 24 per cent. of the total yield as the effect of bromine. Similarly, in Table 2, of the 26.8 g yield with applied bromine, only 60 per cent. can be attributed to the bromine present.

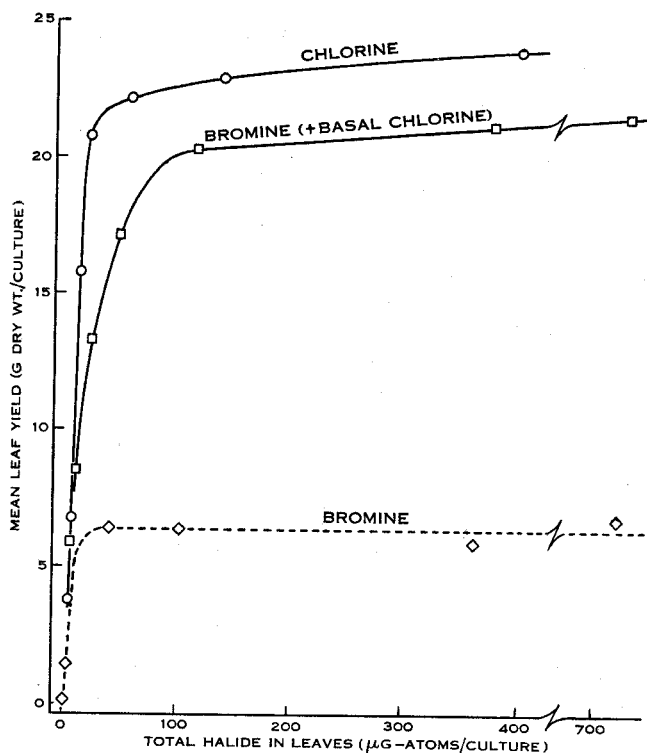


Fig. 6.—Mean leaf yields of tomato plants as modified by halide content. The upper two curves were drawn from experimentally observed values, whilst the lowest was derived from the “bromine (+ basal chlorine)” curve by subtracting the “chlorine yield” as found from Figure 5.

It was decided to use this same method to ascertain how much of the leaf yield from the tomato plants in experiment 1 was caused by the applied bromine. In Figure 5, the leaf yields from the lower chlorine treatments of this experiment are plotted against leaf chlorine content. As with the clover tops, the relationship of leaf weight to chlorine content is very close. After determining the chlorine content of the leaves cultured at different levels of applied bromine, then the yield which this amount of chlorine could have allowed was derived from the graph in Figure 5. The difference between this “chlorine yield” and the total yield was assumed to be the dry weight produced by the bromine present.

The leaf yields plotted against their halide contents are shown in Figure 6. The calculated "bromide yield" curve, shown as a broken line, indicates that the applied bromine did not account for more than about one-third of the total yield—the remainder being produced by the "basal chlorine" received by the plant from the atmosphere and other contamination sources.

(b) *The Differential Displacement of Chlorine by Bromine*

In the trials already described, bromine increased the growth of plants having a limited chlorine supply. It was considered possible that this effect of bromine might

TABLE 3

EFFECT OF BROMINE IN DISPLACING RADIOACTIVE CHLORINE FROM THE ROOTS OF TOMATO PLANTS  
Plants were cultured with radiochlorine in the culture solution for 4 weeks, then transferred to "chloride-free" solution and the bromine added. Roots were harvested 2 weeks later

Set	<sup>36</sup> Cl Pretreatment ( $\mu\text{g}$ -atoms)	Bromine Added ( $\mu\text{g}$ -atoms)	Roots	
			Dry Wt.* (g)	Radioactivity* (counts/min)
A	13†	—	3.40	4,350
	13†	400	3.43	3,940
B	80	—	3.83	25,100
	80	400	3.83	23,300
C	154	—	4.17	42,200
	154	400	4.03	36,400
D	154	—	3.80	42,200
	154	400	3.77	33,400

\* All values are means of three replicates.

† Chloride level so low that plants developed symptoms of chlorine deficiency.

result from the displacement of chlorine from non-active sites thus making the chlorine available elsewhere for the essential function which it performs. To discover whether bromine is sufficiently similar in its biological activity actually to replace chlorine in some functions, two experiments were conducted.

In experiment 4, four pairs of cultures, each containing three tomato plants, were prepared. To the first pair (set A) 13  $\mu\text{g}$ -atoms per culture of radioactive chlorine were added; to the second pair (set B) 80  $\mu\text{g}$ -atoms per culture were added; to sets C and D 154  $\mu\text{g}$ -atoms per culture. After growing for 4 weeks, the plants in set A had developed symptoms of chlorine deficiency. At this time the plants were removed from the radioactive culture solutions, the roots rinsed in distilled water, and the plants then transferred to fresh control M6+(m7-Cl) nutrient solutions. To one beaker of each of pairs A-D, 400  $\mu\text{g}$ -atoms per culture of bromine were now

added. In set A, the chlorine-deficient plants which received bromine soon showed recovery, and the new growth was healthy and free from deficiency symptoms. A comparison of the new terminal growth on the two cultures in set A, after addition of bromine to one, is shown in Plate 1, Figure 3.

Two weeks after the addition of bromine, all plants were harvested, and dry weights and total radioactivity in the roots were measured. Harvest data are presented in Table 3. It may be seen that the added bromine caused an appreciable amount of chlorine to be displaced from the plant roots, concurrent with the growth recovery of the tops in the lowest chlorine-level plants of set A.

TABLE 4  
EFFECT OF BROMINE ON EXUDATION OF  $^{36}\text{Cl}$  FROM DECAPITATED TOMATO PLANTS IN PRESENCE OF RELATIVELY LARGE AMOUNTS OF OTHER ANIONS

Set	$^{36}\text{Cl}$ before Cutting ( $\mu\text{g}$ -atoms)	Chlorine- deficiency Symptoms on Leaves	Bromine Added* ( $\mu\text{g}$ -atoms)	Sap Exuded in 24 Hr		Roots		
				Radioactivity (counts/min)		Volume (ml)	Dry Wt. (g)	Residual Activity (counts/min)
				10 MI	Total			
A	13	Bronzing	—	28	137	49	2.03	4,390
	13	Bronzing	400	122	634	52	1.70	3,740
B	13	Bronzing	—	33	221	67	1.73	5,320
	13	Bronzing	400	84	900	107	2.13	4,150
C	40	None	—	104	593	57	2.50	15,060
	40	None	400	522	3290	63	2.30	12,300
D	40	None	—	58	284	49	2.70	15,500
	40	None	400	566	4410	78	2.40	11,360

\* Ratio of added bromide ions to total (nitrate+sulphate+phosphate) ions present in culture solution was approximately 1 : 160.

This trial did not show whether the chlorine was merely exchanged to the culture solution or whether it was displaced upward into the plant; nor did it demonstrate whether the bromine was much more effective in displacing chlorine than were the other anions present in the culture solution. To clarify these points, this trial was repeated in experiment 5, with some modifications. Four pairs of cultures were again transplanted. This time, two pairs (sets A and B) were given 13  $\mu\text{g}$ -atoms of  $^{36}\text{Cl}$  per culture, and sets B and C 40  $\mu\text{g}$ -atoms of  $^{36}\text{Cl}$  per culture. After 4 weeks growth, the plants were again transferred to M6+(m7-Cl) solutions, but this time the tops were cut off before the addition of 400  $\mu\text{g}$ -atoms Br to one culture from each pair. Rubber tubes were immediately slipped over the cut stems and the exuding sap caught in conical flasks. This exudate was later made slightly alkaline with NaOH and

evaporated to dryness in nickel-plated planchets. The  $^{36}\text{Cl}$  displaced from the roots and present in the exuded sap was then counted. The results of this experiment are recorded in Table 4.

Although the added bromine comprised less than 1 per cent. of the total anions present in the culture solution, nevertheless, it increased the concentration of displaced chlorine in the exuding sap by 3–9 times. As in the previous trial with entire plants, the residual activity in the roots was decreased by the addition of bromine to the culture. This decrease, at least at the  $40\ \mu\text{g-atoms } ^{36}\text{Cl}$  level, was approximately equal to the additional chlorine displaced to the exudate by the bromine. Hence it seems that bromine has a differential power of replacement for chlorine in plant roots and that the displaced chlorine moves upwards through the plant rather than merely being exchanged for bromine in the culture solution.

#### IV. DISCUSSION

##### (a) *Yield Effects*

The results from the foregoing experiments demonstrate that chlorine participates in some function essential to the growth of plants. In this way they confirm the results of Broyer *et al.* (1954), Ulrich and Ohki (1956), and Johnson *et al.* (1957). Where the supply of chlorine is severely limiting bromine is able to substitute partially for chlorine although it cannot completely replace it in the tomato plant or subterranean clover, as evidenced by yields being smaller from the cultures receiving added bromine than were obtained by supplying ample chlorine. Also, plants cultured with bromine added instead of chlorine may appear somewhat less healthy, may wilt under conditions of water stress, and may have modified root development. Even when the yield of low-chlorine plants was increased several times by the addition of bromine it was still not possible to show that the added bromine accounted for more than 30–60 per cent. of the total plant yield. The remainder could have been produced by the chlorine acquired during growth from contamination sources.

The possibility that the bromine effects were due to some essential function performed only by bromine, and not chlorine, could not be substantiated. In the three trials in which bromine was supplied to plants having adequate chlorine, no yield increase from the addition of bromine was obtained.

##### (b) *Ion Relationships*

The finding that chlorine can be largely replaced by another element is reminiscent of the sparing effects of sodium for potassium and strontium for calcium. Chlorine and bromine are alike in many properties as is shown by their similar chemistry and their ionic radii (1.31 and 1.95Å respectively). The bonds retaining chlorine in plant tissue are apparently only of a loose nature and easily broken. Such loose bonds may be much less specific in their requirements than the strong intimate linkages formed by say, nitrogen or carbon in plant tissue.

When bromine does replace chlorine in a complex normally formed with the chloride ion, then, due to the greater mass, larger size, and less intense surface charge of the bromide ion, the similar complex formed with bromine might be expected to form less readily and be less active. In Figure 3, the concentrations of bromine in

plant tissues required to give the maximum growth effect are 2-3 times as great as the concentrations of chlorine required to give equivalent yields thereof. Biological systems are often very specific with regard to the elements which serve particular chemical reactions and, at least in the animal body, the function of chlorine does not appear to be replaceable by bromine.

In the trial with decapitated plants, the roots containing  $^{36}\text{Cl}$ , a direct demonstration was given of the ability of bromine to replace chlorine in living tissue. As relatively small numbers of bromide ions displaced far more  $^{36}\text{Cl}$  than did larger numbers of nitrate, sulphate, and phosphate ions, it seems that chlorine is held by bonds with specific requirements only satisfied by an ion very similar to the chloride ion. Chlorine displaced from plant roots by bromine apparently moves upwards into the shoots and becomes available to other tissues. Thus the recovery of chlorine-deficient plants when they were given bromine may be largely through the displacement of chlorine from non-essential locations to more vital sites: either at the plant level, as from roots to the young growing shoots; or at the cellular level, as from inactive adsorption sites in the protoplasm to active complexes in the cytoplasm.

An interesting comparison may be drawn between the effects of bromine in displacing chlorine, and the effects of sodium in displacing potassium. The sparing effect of sodium on potassium is well recognized. Several workers, such as Holt and Volk (1945), have obtained large growth effects by applying sodium to potassium-deficient plants. A displacement of potassium by sodium from petioles to leaf blades of potassium-deficient sugar-beets may be inferred from the recent work of Ulrich (1956). Presumably, in the laminae the potassium performs a more specific role. Potassium, like chlorine, apparently forms only loose bonds in plant tissues.

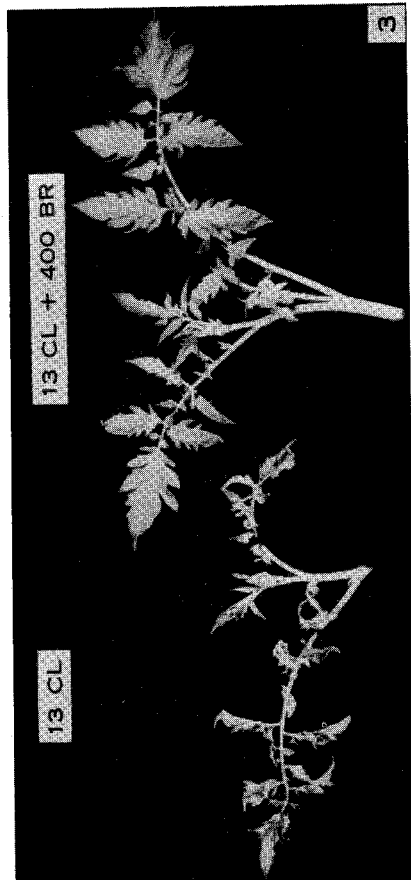
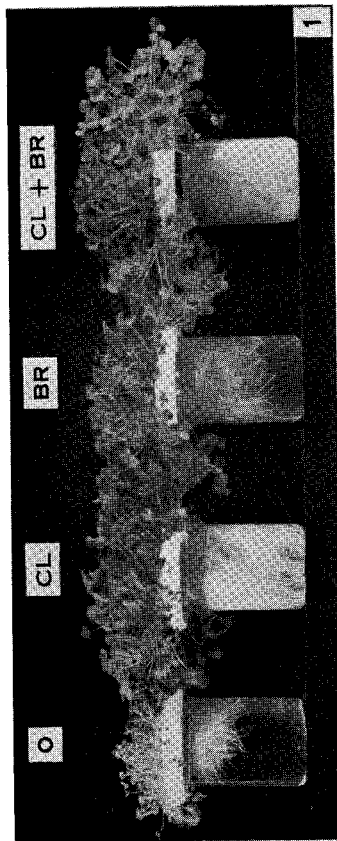
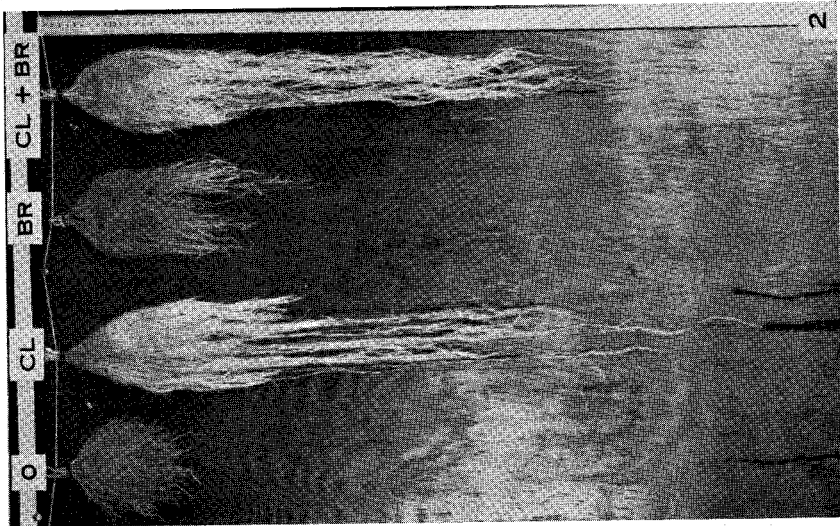
### (c) *Plant Functions*

Chlorine has been suggested as necessary for at least two plant processes. Warburg and Luttgens (1946) proposed chlorine as a coenzyme in photosynthesis. In similar experiments of Arnon and Whatley (1949) on the photochemical activity of isolated chloroplasts, bromine was found to exert the same sort of activating effect as chlorine, but to a somewhat lesser extent. A second function for chlorine is suggested in the earlier physiological work on chlorine deficiency (Broyer *et al.* 1954; Johnson *et al.* 1957). Here, localized wilting in leaf blades has been observed as a general symptom of this deficiency in most species studied. The wilted marginal areas of the leaves showed a characteristic recovery when the transpiration stress was removed. From this it may be inferred that chlorine plays some role in the water relations of leaves. Struckmeyer and Wedin (1955) found poor differentiation of xylem in low-chlorine tobacco plants. However, Johnson *et al.* (1957) mention that chlorine-deficient and chlorine-sufficient tomato leaves of the same age, size, and position had the same general histological structure at the time of onset of chlorine-deficiency symptoms in the low-chlorine leaves. There was no obvious difference in xylem development.

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## VI. REFERENCES

- ARNON, D. I., and WHATLEY, F. R. (1949).—Is chloride a coenzyme of photosynthesis? *Science* **110**: 554–6.
- BROYER, T. C., CARLTON, A. B., JOHNSON, C. M., and STOUT, P. R. (1954).—Chlorine—a micro-nutrient element for higher plants. *Plant Physiol.* **29**: 526–32.
- CONWAY, E. J. (1947).—“Micro-diffusion Analysis and Volumetric Error.” Ch. 21, 22. (Rev. Ed.) (Crosley Lockwood: London.)
- HOLT, M. E., and VOLK, M. J. (1945).—Sodium as a plant nutrient and substitute for potassium. *J. Amer. Soc. Agron.* **37**: 821–7.
- JOHNSON, C. M., STOUT, P. R., BROYER, T. C., and CARLTON, A. B. (1957).—Comparative chlorine requirements of different plant species. *Plant & Soil* **9**: (in press).
- STRUCKMEYER, B. E., and WEDIN, W. F. (1955).—Some effects of sulfate and chlorine levels on the anatomical structure of the stems and leaves of tobacco. *Plant Physiol.* **30** (suppl.): xxxiii.
- ULRICH, A. (1956).—Plant analysis as a guide to the fertilization of crops. *Bett. Crops* **40**: 6.
- ULRICH, A., and OHKI, K. (1956).—Chlorine, bromine and sodium as nutrients for sugar beet plants. *Plant Physiol.* **31**: 171–81.
- WARBURG, O., and LUTTGENS, W. (1946).—Photochemical reduction of quinone in green cells and granules. *Biochimia* **11**: 303–22.

## EXPLANATION OF PLATE I

- Fig. 1.—Comparative growth of subterranean clover plants cultivated under different halide-supply conditions. 0, no added halide; *CL*, 400  $\mu\text{g}$ -atoms of added chlorine per culture; *BR*, 400  $\mu\text{g}$ -atoms of added bromine per culture; *CL+BR*, 400  $\mu\text{g}$ -atoms each of added chlorine and bromine per culture.
- Fig. 2.—Comparative development of roots from the subterranean clover plants illustrated in Plate 1, Figure 1.
- Fig. 3.—Effect of adding bromine to the culture solution on new growth of tomato shoots, following chlorine deficiency. 400  $\mu\text{g}$ -atoms of bromine were added to the culture represented on the right, following transfer of each plant from deficiency levels of 13  $\mu\text{g}$ -atoms of chlorine per culture to “chlorine-free” solutions.