THE DEVELOPMENT IN TIME OF STRESS EFFECTS IN TWO SPECIES OF *GLYCINE* DIFFERING IN SENSITIVITY TO SALT

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[*Manuscript received March* 3, 1970]

Summary

For two *Glycine* species (*wightii*‡ and *tomentella*) varying in sensitivity to salt the development of stress over a range of salinities was traced through changes in growth, water content, and chemical composition of tissues of different type and age.

The general picture of salt stress appears to be one of immediate growth reduction through an initial water stress approximately proportional to the concentration of salt applied. At 40 m-equiv/l of sodium chloride osmotic adjustment apparently occurs, control growth rate is regained, and, the data suggest, is likely to be maintained without significant tissue injury developing. At 80 m-equiv/l of sodium chloride, the trends in tissue water content indicate that some osmotic adjustment may occur, particularly in the older leaves, but at this level of salinity and especially at 160 m-equiv/l of sodium chloride the rapid and excessive chloride accumulation injures the leaves and growth rate falls progressively in relation to that of the control. The detrimental effects of salt on nutrient and water content are more pronounced in the young leaf tissue developing during salt treatment than for the older leaves.

Species difference in sensitivity appears associated with the leaf injury phase of salt stress. It is thought that leaf injury is greater in *tomentella* than *wightii* because of the faster initial increase in chloride concentration in *tomentella*, particularly in the younger leaf tissue, and also that a reduced potential for protein synthesis in these leaves in *tomentella* may have aggravated the situation.

Analysis of time trends in growth rate and other plant attributes, a technique largely ignored in salt resistance studies, helps to distinguish between the osmotic and toxic effects of ions.

I. INTRODUCTION

Assessment of the reasons for differences in reaction to salt between plants closely related taxonomically is a valuable approach to the problem of understanding resistance to salinity, a field in which it has proved difficult to generalize (Gale, Kohl, and Hagan 1967).

In an examination of species within the genus *Glycine*, Wilson (1967) and Wilson, Haydock, and Robins (1970) established that *G. wightii* was less sensitive to salt than *G. tomentella* and suggested that this difference was associated with the damaging effects of the higher accumulations of sodium and chloride recorded in the latter species. Also, reductions in nutrient uptake per gram of root suggested that, in addition to obvious damage to leaf tissue, salt may have also impaired root function.

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These conclusions were based on data from harvests taken before and after salt treatment. However, as pointed out by Petrie (1942), when treatments alter both plant growth and metabolism, sounder interpretation of the effects are obtained from measurements of the trends with time. In the present context, the determination of the factors important in the primary effects of salt and the development of the species differences requires more detailed analysis of plant response with time during the actual salt stress. Despite the voluminous literature on salinity in relation to plant growth this form of analysis has been used only rarely to assess the basis for difference in plant response.

Accordingly, the above two species were chosen for further study and this paper describes the time trends in dry matter accumulation of the whole plant and its parts and the changes in chemical composition during treatment with a range of sodium chloride concentrations. Recovery after removal of salt serves as a further assessment of the severity of treatment effect.

These data complement the work of Gates, Haydock, and Robins (1970) who concluded that the ability to regulate the ion content of the tissues was an important factor in determining salt tolerance in a range of 22 *G. wightii* cultivars. *G. wightii* cv. Cooper (cv. 20, loc. cit.) is in the least sensitive group of these cultivars, and *G. tomentella* may be regarded as comparable to the more sensitive cultivars. The time trends during treatment for these species thus represent two extremes of the range which should reveal differences between species, salt levels, and plant parts, and aid the understanding of the development of salt stress.

Growth analysis, considering the exponential or logarithmic nature of plant growth, is also used in this paper to examine the capacity of plants to adjust osmotically to salinity. The value of such an analysis is evident using the data of Wadleigh, Gauch, and Davies (1943); their growth trends if plotted on a logarithmic scale are almost parallel for control and saline treatments although the absolute yields are quite different. This suggests that before the first growth measurements were taken there was a strong, presumably osmotic, salt effect resulting from the initial salinization, followed by a substantial adjustment to salinity. This paper presents data which elaborate on this point.

II. Methods

The species used, *G. wightii* cv. Cooper and *G. tomentella*, are henceforth referred to as *wightii* and *tomentella*.

The plants were grown in continuously aerated culture solution (Wilson, Haydock, and Robins 1970), in a glasshouse heated to a minimum of 25°C at night and humidified to a minimum of approximately 50% R.H. during the day.

The experimental procedure was as follows. Seeds were scarified, soaked overnight in 1% thiourea, rinsed in demineralized water, and germinated at 32°C on filter pads moistened with dilute calcium sulphate solution (≡ 8 p.p.m. Ca). On day 3, when the radicles were 1–3 cm long, the seedlings were transferred to the glasshouse into shallow trays filled with half-strength nutrient solution. The seedlings were transferred again on day 11 to pots containing 2·4 litres of full-strength nutrient solution; each pot held two seedlings of the same species.

Harvests were taken on days 25, 29, 33, 37, 41, 45, and 52, referred to as harvest 1, 2, . . . , 7, respectively. The salt treatments were imposed after harvest 2 by additions of sodium chloride to the basal nutrient to achieve concentrations of 0·5 (S_0—control), 40 (S_1), 80 (S_2), and 160 (S_4) m-equiv. per litre of nutrient solution, and were removed after harvest 6 by returning the plants to basal nutrient. The sodium chloride was initially added in increments of 40 m-equiv/l/day in late

afternoon up to the S_2 level, and then a further increment of 80 m-equiv/l/day to the S_4 level. At the start of salt treatment both species had six expanded trifoliate leaves on the main stem and only rudimentary axillary shoots.

The experiment was a randomized block design with three replicate pots per treatment grown on each of two occasions, commencing September 9, 1964, and October 31, 1964, giving six replicates for each treatment–harvest combination. Pots were re-randomized twice weekly.

The original nutrient solution in the pots was changed on day 20 after 9 days growth and then again on day 29 (harvest 2), 37 (harvest 4), and 45 (harvest 6). The solutions were topped up to volume as required.

At harvest the plants were separated into roots, stems, and groups of leaves of varying age; the cotyledons, two unifoliate leaves, and first three trifoliate leaves as group A, trifoliate leaves 4–6 as group B, trifoliate leaves appearing between harvests 2 and 6 (i.e. during salt treatment) as group C, and leaves appearing over the recovery period (harvest 6–7) as group D. Data for the mature leaf groups A and B have been combined to simplify presentation in the figures for dry weight and ion content. Material from the two plants in each pot was bulked to give a single pot estimate. The roots were rinsed twice in demineralized water and blotted dry. The samples were weighed immediately for fresh weight (except for roots), and dry weights were recorded after oven drying for 24 hr at 80°C.

For chemical analysis, material was bulked over the three replicates for each experimental occasion. Nitrogen and phosphorus were estimated on Kjeldahl digests, and sodium, potassium, and chloride on distilled water extracts by flame photometry for cations and colorimetry for chloride.

The experimental design was orthogonal so that although pots were assigned to the four salt levels for harvests 1 and 2 they all in fact had received only the basal control nutrient solution before harvest. Values for these pots (24 for each harvest) have thus been averaged in the presentation of data for harvests 1 and 2. Data for dry weight yield, and sodium and chloride concentrations, were transformed logarithmically for analysis of variance.

III. Results

(a) *Dry Weight*

(i) *Whole Plant*

Salinity reduced growth of both species (Fig. 1) significantly from the control for all three levels of salt (S_1, $P<0{\cdot}05$; S_2 and S_4, $P<0{\cdot}001$).

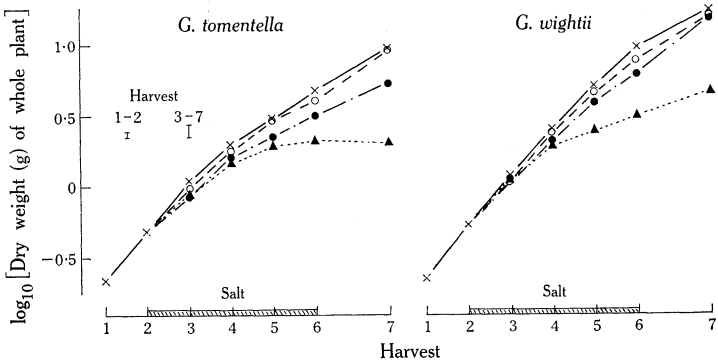

Fig. 1.—Effect of salinity on the whole plant dry weight of *G. tomentella* and *G. wightii*, at salinity levels S_0 (×), S_1 (○), S_2 (●), and S_4 (▲). Vertical lines indicate least significant differences at 0·05 level.

There was no visible leaf injury in the S_1 treatment, and at the S_2 and S_4 levels marginal necrosis and yellowing of leaves were not generally noticeable until after

8–12 days of salt. In the S_4 plants these burning symptoms appeared sooner than in the S_2 plants and rapidly led to the death of many leaves, particularly for *tomentella*.

Analysis of trends over harvests 3–6 revealed that the effect of the S_2 and S_4 treatments increased in severity the longer the period in salt (S_2, $P<0\cdot05$; S_4, $P<0\cdot001$). For the S_1 treatment the divergence from the control curve was not significant; after the initial lag between harvests 2 and 3 the dry weight curve paralleled that of the control.

Species differences in response during actual salt treatment could be detected only at the S_4 level and the lower resistance of *tomentella* is reflected in the greater negative curvilinearity of the S_4 curve by comparison with that for *wightii* ($P<0\cdot05$).

After the removal of salt the severity of the treatments in causing tissue damage, and the greater resistance of *wightii*, are clearly seen in the relative growth rates of the S_2 and S_4 plants (*wightii* 0·133 and 0·060, *tomentella* 0·074 and −0·006 g/g/day, respectively).

TABLE 1

DRY WEIGHT, CHLORIDE, AND SODIUM CONCENTRATIONS FOR LEAVES PRODUCED AFTER THE REMOVAL OF SALT FROM THE CULTURE SOLUTION (i.e. GROUP D LEAVES, HARVEST 7)

Logarithmic values for ion concentrations in brackets

	G. tomentella	*G. wightii*	L.S.D. ($P = 0\cdot05$)
	$\log_{10}$ [Dry weight (g)]		
S_0	0·42	0·76	0·33
S_1	0·36	0·71	
S_2	0·03	0·70	
S_4	−0·89	−0·21	
	Chloride (m-equiv/100 g)		
S_0	1·7 (0·22)	1·5 (0·18)	0·09*
S_1	3·4 (0·53)	1·9 (0·28)	
S_2	7·3 (0·86)	4·0 (0·61)	
S_4	30·8 (1·49)	12·5 (1·10)	
	Sodium (m-equiv/100 g)		
S_0	0·4 (−0·44)	0·5 (−0·33)	0·65*
S_1	1·1 (0·03)	1·4 (0·13)	
S_2	1·3 (0·12)	1·2 (0·09)	
S_4	4·3 (0·63)	3·4 (0·53)	

* Applicable to logarithmic values only.

(ii) *Plant Parts*

The species differences elaborated for the whole plant dry weight are clearly seen in the growing tissues (Fig. 2), namely, the root, stem, and especially the group C leaves, which were those formed after the commencement of salt treatment. The dry weight of leaves in the A and B group which were either mature or approaching maturity at the start of salt was not affected by treatment.

The effect of salt on the growth of group C leaves was significant in both species (*tomentella*, $P<0\cdot001$; *wightii*, $P<0\cdot05$) at the first harvest after commencement of

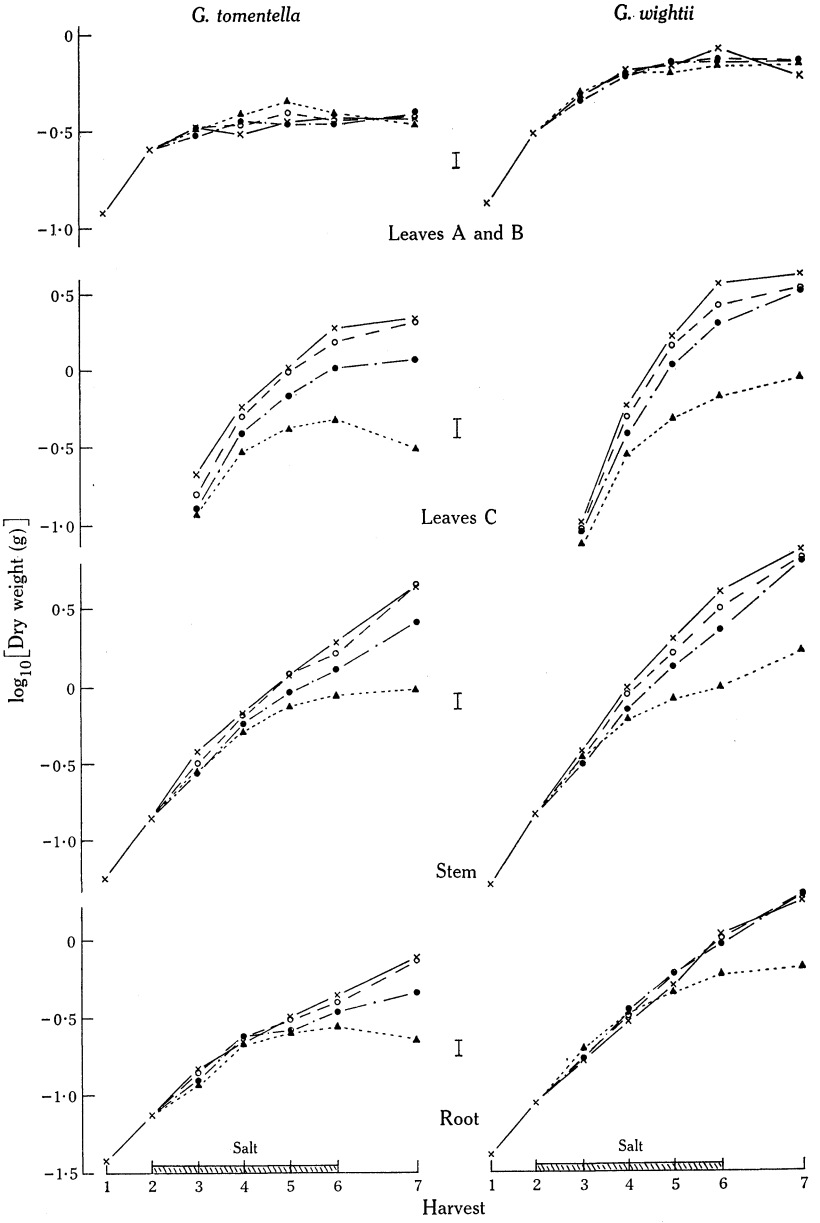

Fig. 2.—Dry weight trends in response to salt for leaves expanded before salt treatment (A and B), leaves formed during treatment (C), stems, and roots of *G. tomentella* and *G. wightii*. Salinity levels S_0 (×), S_1 (○), S_2 (●), and S_4 (▲). Vertical lines indicate least significant differences at 0·05 level.

treatment. The production of new leaves during salt was inhibited relatively more for *tomentella* (52, 44, 31, and 17 for S_0 to S_4, respectively) than for *wightii* (46, 37, 34, and

19). Root growth on the other hand was not significantly reduced by salt even at the highest level until after 12–16 days of treatment.

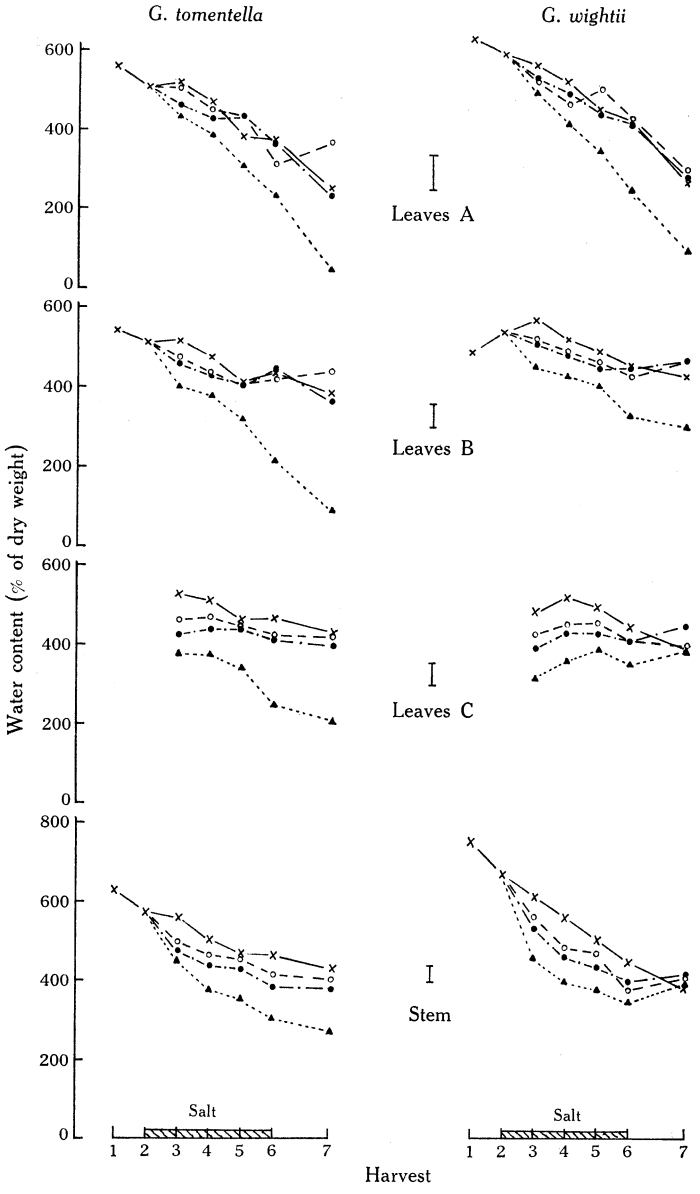

Fig. 3.—Changes in water content with salinity in leaves expanded before salt treatment (A and B), leaves formed during treatment (*C*), and stems of *G. tomentella* and *G. wightii*. Salinity levels S_0 (×), S_1 (○), S_2 (●), and S_4 (▲). Vertical lines indicate least significant differences at 0·05 level.

The dry weights of the group D leaves (Table 1) also demonstrate the slower recovery of the *tomentella* S_2 and S_4 plants.

(*b*) *Water Content*

Since all dead tissue was collected the dry weight data do not fully reflect the severity of the salt treatments, especially for the older leaves. However, the very low water contents of these tissues toward the end of salt treatment and at harvest 7 (Fig. 3) reflect a high proportion of dead tissue. It is thus apparent that in the S_4 treatment the older (group A and B) leaves of *tomentella* were dead by the end of the experiment and that there was also a considerable proportion of dead material in the younger (group C) leaves. The weight of leaves in this latter group actually declined after salt was removed (Fig. 2). In *wightii* on the other hand, whilst a high proportion of the three oldest trifoliate leaves died, the mortality in the other leaf groups was much less than in *tomentella*, as seen in the higher leaf water contents at harvest 7.

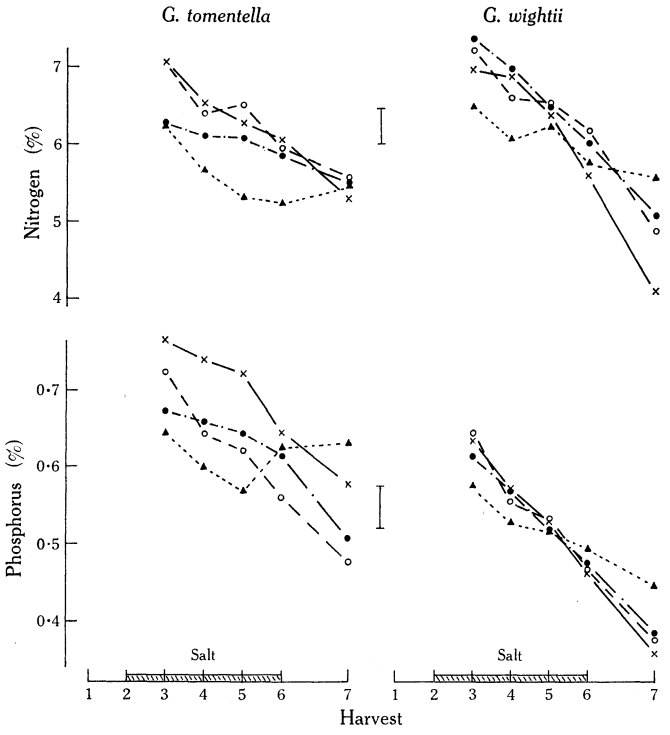

Fig. 4.—Changes in nitrogen and phosphorus concentration in leaves (group C) of *G. tomentella* and *G. wightii* formed during salt treatment. Salinity levels S_0 (×), S_1 (○), S_2 (●), and S_4 (▲). Vertical lines indicate least significant differences at 0·05 level.

During the initial stages of salt treatment there was a consistent decline in the water content of all plant parts with increasing salt level; the regression coefficients at harvest 3 were 0·467 for leaf A ($P<0·05$), 0·687 for B, 0·954 for C, and 0·806 for stem (all $P<0·001$). The effect was more severe the younger the leaf tissue. This is a direct effect on water content and is not at this stage at all confounded with death of tissue. During the latter part of the salt treatment there was partial or complete

re-adjustment in water content of the S_1 and S_2 plants towards the control level, especially in the older leaves. In *wightii* even the group C leaves in the S_4 treatment increased in water content after the initial effect of salt.

At all levels of salinity the water content of *wightii* was higher ($P < 0{\cdot}001$) during treatment than that of *tomentella* in leaf A, leaf B, and stem tissue.

(c) *Nitrogen and Phosphorus*

There was a greater adverse effect of salt on the nitrogen and phosphorus concentration in the leaves and stem of *tomentella* than of *wightii*. This was most pronounced for the young (group C) leaves and data for this plant part only are shown (Fig. 4). For the roots, both species responded similarly to salt with nitrogen concentration showing little change, and phosphorus concentration increasing markedly, as in previous trials.

(d) *Potassium*

The effects of salt on potassium concentration were generally similar for both species with a decrease in both stem and root ($P < 0{\cdot}001$) and an increase at the S_4 level in the leaves. The response was consistent throughout salt treatment and thus only data for harvest 6 are presented (in m-equiv/100 g dry weight) in the following tabulation:

Salt Level	Leaves A and B		Leaves C		Stem		Root	
	G. tomentella	*G. wightii*	*G. tomentella*	*G. wightii*	*G. tomentella*	*G. wightii*	*G. tomentella*	*G. wightii*
S_0	102	109	135	126	143	135	199	192
S_1	96	101	116	124	120	118	171	168
S_2	121	109	114	121	101	113	130	122
S_4	163	150	152	140	71	84	114	112
L.S.D. ($P = 0{\cdot}05$)	22		18		22		29	

(e) *Chloride*

In the S_2 and S_4 treatments, chloride concentration (Fig. 5) increased progressively with time in salt in all tissues but most rapidly in the older leaves and least rapidly in the roots and stem. For the latter parts, increase in concentration was very rapid only during the first four days in salt. In the S_1 treatment, chloride concentration increased initially but then reached a stable level in the rapidly growing stem and leaf C tissue.

As seen in a comparison of distribution indices for dry weight, nitrogen, chloride, and sodium in S_4 plants (Table 2), chloride accumulated preferentially in the older (group A and B) leaves rather than younger (group C) leaves. This was especially apparent for harvest interval 2–3 when group C comprised only very immature leaves, but with later harvests a range of maturities was represented and proportionately more chloride went to this plant fraction. Data for the other salt levels were similar.

The small differences in root chloride concentration between S_1, S_2, and S_4 treatments suggest that a ceiling concentration was approached, which was similar for both species.

There were significant ($P < 0{\cdot}05$–$0{\cdot}001$) species by salt level interactions in all parts of the plant tops, with chloride concentration generally lower in *wightii* at the S_1 level but accumulating to higher concentrations toward the end of salt treatment at the S_4 level than in *tomentella*. Another species difference of major interest is the lower chloride concentrations in the young, developing, group C leaves of *wightii* by

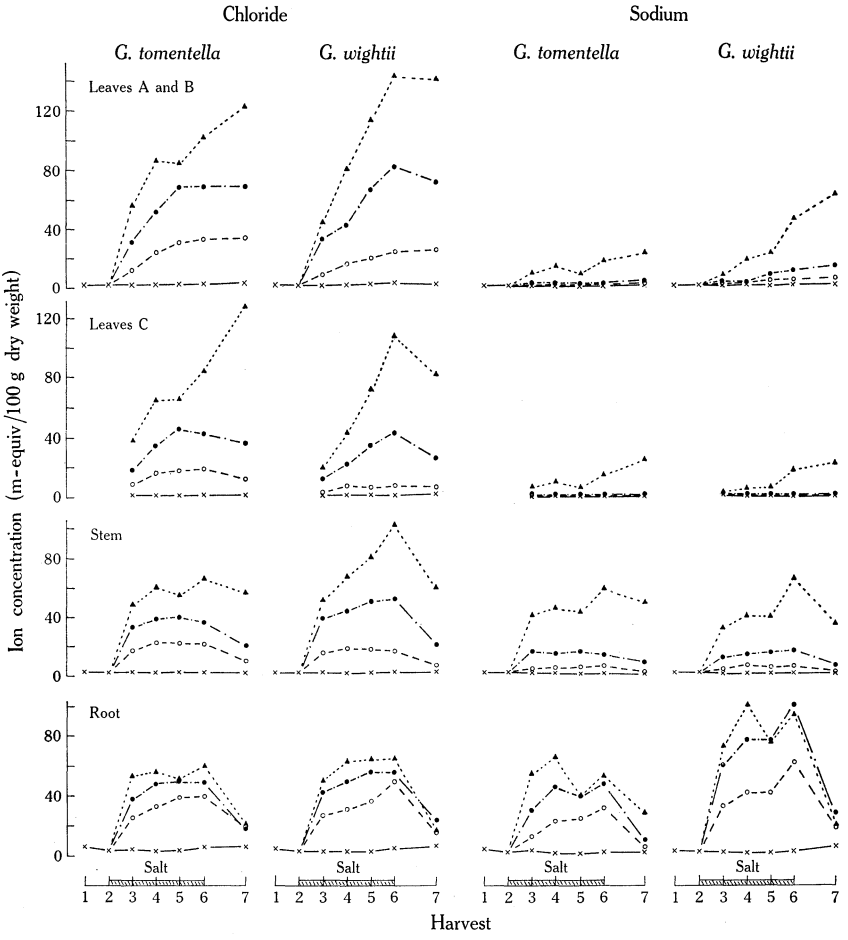

Fig. 5.—Changes in chloride and sodium concentration in response to salinity of leaves expanded before salt treatment (A and B), leaves formed during treatment (C), stems, and roots of *G. tomentella* and *G. wightii*. Salinity levels S_0 (×), S_1 (○), S_2 (●), and S_4 (▲).

comparison with that of *tomentella* at all levels of added salt ($P < 0{\cdot}05$–$0{\cdot}001$) during the first 8 days of treatment. The difference was as great as 100% in the S_4 treatment at harvest 3. Using the formula of Williams (1948), the initial rate of chloride uptake per gram of root in *tomentella* was 25% higher ($P < 0{\cdot}05$) than for *wightii*. When concentration is expressed on a water content basis (Table 3) the chloride concentrations in *wightii* were consistently lower than in *tomentella* throughout the full period

of salt treatment. Even after the removal of salt the chloride concentration of the young, developing, group D leaves of *wightii* was considerably less than that of *tomentella* (Table 1).

TABLE 2

INDICES OF DISTRIBUTION, WHILST IN SALT, OF CHLORIDE AND SODIUM BETWEEN PLANT PARTS COMPARED WITH THOSE FOR DRY WEIGHT AND NITROGEN

Increment increase or decrease in content between harvests expressed as a percentage of the incremental change for the plant as a whole: data for S_4 treatment only

Plant Part	Harvest Interval	*G. tomentella*				*G. wightii*			
		Dry Wt.	Nitrogen	Chloride	Sodium	Dry Wt.	Nitrogen	Chloride	Sodium
Leaves A	2–3	3	−3	9	2	4	1	14	11
	3–4	1	0	6	3	2	−2	10	9
	4–5	−2	−5	2	−6	−2	−4	7	18
	5–6	−1	−6	0	1	−1	−5	3	5
Leaves B	2–3	16	10	33	8	29	37	28	6
	3–4	11	10	22	10	15	19	27	3
	4–5	16	15	22	−41	3	−5	21	11
	5–6	−43	−180	1	8	10	9	20	15
Leaves C	2–3	31	51	12	3	13	19	3	1
	3–4	30	38	27	13	28	44	15	4
	4–5	26	30	33	−7	37	53	29	12
	5–6	41	75	37	13	34	53	36	12
Stem	2–3	39	30	32	65	35	21	35	36
	3–4	40	29	33	30	36	20	33	35
	4–5	51	51	39	298	41	20	33	60
	5–6	92	179	52	62	30	16	31	40
Root	2–3	11	12	14	22	19	22	20	46
	3–4	18	23	12	44	19	19	15	49
	4–5	9	9	4	−144	21	36	10	−1
	5–6	11	32	10	16	27	27	10	28

(*f*) *Sodium*

There was a rapid accumulation of sodium in the roots, and to a lesser extent the stems, of both species (Fig. 5), but generally only a very small increase with time in salt in the sodium concentration of the leaves. The exception is the high sodium concentration in the mature leaves in *wightii* at the S_4 level. In the younger (group C) leaves sodium concentration in the S_1 and S_2 treatments did not increase above the control level.

Although there were substantial differences between sodium and chloride accumulation in the leaves, the pattern of sodium accumulation in stems and roots was similar to that described for chloride. Sodium in the roots approached a maximum concentration which is in agreement with Jacoby's conclusion (1964) that bean roots accumulate large amounts of sodium from the xylem sap until the binding sites approach a saturated state.

Species differences were significant ($P < 0 \cdot 001$) only for the higher concentrations of sodium in the root and group A leaves of *wightii* than *tomentella*. The trend towards lower sodium concentration in group C leaves of *wightii* during the early stages of salinity was similar to that for chloride but did not attain significance. The initial rate of sodium uptake per gram of root in *tomentella* was 35% higher ($P < 0 \cdot 01$) than *wightii*. On a plant water basis (Table 3) sodium concentration of *wightii* was lower ($P < 0 \cdot 05$) in the group C leaves of the S_4 plants throughout salt treatment. Sodium concentration in group D leaves was low even in S_4 plants, and similar for both species (Table 1).

TABLE 3

CONCENTRATION OF CHLORIDE AND SODIUM IONS IN GROUP C LEAVES DURING SALT TREATMENT (HARVESTS 3–6), AND AFTER REMOVAL OF SALT FROM THE CULTURE SOLUTION (HARVEST 7)

Harvest	S_0		S_1		S_2		S_4	
	G. tomentella	*G. wightii*	*G. tomentella*	*G. wightii*	*G. tomentella*	*G. wightii*	*G. tomentella*	*G. wightii*
				Chloride (m-equiv/l of plant sap)*				
3	4	3	20	9	44	34	98	61
4	3	3	35	19	80	51	172	119
5	4	4	41	16	104	82	190	182
6	4	3	46	20	105	105	369	307
7	5	6	31	16	92	59	866	203
				Sodium (m-equiv/l of plant sap)†				
3	1	2	4	4	8	4	19	12
4	2	1	5	4	5	6	28	18
5	1	1	4	4	4	4	20	18
6	1	2	4	4	4	5	66	53
7	2	2	4	4	5	5	173	57

* Significance of differences between species (on analysis of $\log_{10}$ transform data) as follows: S_1, $P < 0 \cdot 001$; S_2, $P < 0 \cdot 05$; S_4, $P < 0 \cdot 001$.

† Significance of differences between species (on analysis of $\log_{10}$ transform data) as follows: S_4, $P < 0 \cdot 05$.

IV. DISCUSSION

(*a*) *General Nature of the Response to Salt*

The response to salinity was rapid for all plant attributes and was greatest in the stems and leaves growing actively at the commencement of salt treatment. However, these plant fractions comprised material of different ages and the reduction in growth rate and water content for the youngest tissue of each fraction would probably be very great indeed.

The initial reduction in tissue water content is as would be expected for water-stressed plants (Gates 1955). It is associated with a reduced rate of dry matter accumulation and thus is a real reduction in rate of water uptake, as Hayward and Spurr (1943) measured directly for corn under saline treatment. It is likely that even lower water contents would be recorded if plants were harvested sooner than 4 days from the start of treatment, since Slatyer (1961) and Bernstein (1963) report osmotic adjustment within 24 hr.

Thus, in support of Brouwer (1963) who measured reduced leaf growth in beans only 24 hr after addition of sodium chloride, the immediate effect of salt is on the water balance within the plants, with a larger effect in the young than in the old leaves.

However, with time in salt there is, particularly in the older leaves, re-adjustment of water content at the lower salt levels towards the control, presumably following an osmotic adjustment associated with accumulation of salts. This adjustment is not complete in the group C leaves or stem but this may reflect the delay in accumulation of salt, and hence adjustment, in new tissue arising during treatment. Since growth rate of the S_1 plants regains that of the control it may be presumed that much of the plant tissue recovered to a favourable water balance.

This adjustment back to normal growth rate may perhaps explain why in some plants later stages of development such as grain formation are apparently little affected by salt (Bernstein and Hayward 1958; Greenway 1965).

Sodium and chloride in the growing tissues of the S_1 plants reached a stable concentration but, again, this probably represented a balance between the higher concentrations in the relatively older tissue, where these ions tend to accumulate, and the slower increase in concentration in the very young tissues. Rate of salt uptake fell with time so that it would presumably require a prolonged salt treatment before injurious concentrations are reached even in old leaves, and young actively growing tissue of the S_1 plants would always be low in salt.

Gates, Haydock, and Robins (1970), and Wilson, Haydock, and Robins (1970) have found that within *Glycine* the less resistant varieties and species are those with high concentrations of sodium and chloride in their plant tops. In this experiment sodium concentrations in the leaves, before salt injury became apparent, were very low at all salt levels and in *tomentella* the maximum concentration during exposure to salt did not exceed 20 m-equiv/100 g dry matter. It therefore seems unlikely that sodium accumulation was responsible for the severe leaf injury.

The data suggest strongly that leaf injury results from excessive chloride, and on a plant sap basis the lowering of tissue water content aggravates the build-up of chloride concentration. Since S_2 salinity resulted in injury, during the latter part of treatment, to some older leaves of some plants, mostly of *tomentella*, the data in Figure 5 suggest a chloride concentration above about 60 m-equiv/100 g dry weight (approximately equivalent to 150 m-equiv. chloride per litre of plant sap) as critical for producing salt injury. This estimate is slightly lower than the 85 m-equiv. chloride/100 g dry matter associated with leaf burn in *Glycine max* (Abel and MacKenzie 1964). The experiments of Gates, Haydock, and Little (1966) on *G. wightii* cv. Tinaroo further suggest that it may well be the rapidity with which such a concentration is reached that determines the severity of plant injury.

The detrimental effects of high salt concentration in the roots are difficult to assess; their growth was much less affected than that of young leaves and stem. Also, the differences in chloride and sodium concentration between salt levels, especially S_2 and S_4, were relatively small compared with those in the plant tops and were not closely correlated with the effects of these treatments on plant growth.

The apparent "saturation" level for sodium and chloride in the roots is relevant to osmotic adjustment. Oertli (1966) suggested that root xylem sap cannot adjust to high salinity and that rate of water uptake will thus be lowered, and later (1967)

measured a positive differential between root xylem sap and external solution only for osmotic increases up to 2 bars; equivalent to the S_1 level in this experiment. The differential then became negative because rate of salt entry approached a maximum value as external salt concentration increased; the present situation is similar.

Estimates of the maximum concentrations of sodium and chloride in the root cell sap, in m-equiv/l, are compared with the external solution concentrations in the following tabulation:

		External Solution	Root Cell Sap	
			G. tomentella	*G. wightii*
S_1	Na	40	50	86
	Cl	40	57	71
S_2	Na	80	71	121
	Cl	80	71	79
S_4	Na	160	79	129
	Cl	160	79	93

The estimates were calculated using the maximum salt concentrations from Figure 5 and the lowest root water content (1000% of dry weight) recorded in a previous trial (Wilson, unpublished data) with the same species, salt level, culture treatment, nutrient solution, and growth stage. Since the roots were rinsed in distilled water at harvest a generous correction of 30% for salt displacement in the apparent free space (Bernstein 1963; Hendricks 1966) was made. Despite this attempt to maximize the root cell sap concentration the possibility of full osmotic adjustment through salt accumulation in the S_4 roots appears remote, especially as there is a marked fall in potassium concentration. Calcium and magnesium do not appear to change significantly (Bower and Wadleigh 1948; Bernstein 1963) and thus osmotic adjustment could only be achieved by a large increase in organic solutes.

The picture of salt stress evolved in this experiment is of an immediate reduction in growth rate through osmotically induced water stress. At low salinity there is then adjustment through salt accumulation in the tissues, control growth rate is regained and, since tissue chloride and sodium remains below the injurious level, is likely to be maintained during subsequent growth. At high salinity, osmotic adjustment by the roots appears unlikely, whilst the rapid and excessive chloride accumulation in the plant tops causes obvious leaf injury which increases with higher solution salinity and duration of treatment. The plants cannot adjust successfully and growth rate falls progressively in relation to that of the control plants.

(*b*) *Species Differences*

Within the above framework, the development of species differences in sensitivity seems to be associated with the capacity to resist tissue injury. Leaf injury, from observations supported by the water content data, was more severe in *tomentella* than *wightii*, especially for group B and C leaves. The initial increase in chloride in these leaves was greater for *tomentella*, particularly in the S_4 plants, with a difference in concentration of 100% for group C leaves at harvest 3. Whilst this species difference in chloride concentration disappears with time in salt or as the leaves age, it probably

continues to be expressed in new leaves that are produced, and this may account for the greater degree of leaf injury in this species. The reduced potential for protein synthesis and hence growth in the young leaves of *tomentella* following their reduction in nitrogen and phosphorus content may aggravate the salt effect.

After the removal of salt from the culture solutions, continued accumulation and increase in chloride concentration was particularly pronounced in the older leaves of the S_4 plants of *tomentella*; this probably also further aggravated injury, and thereby contributed to the poor recovery of these plants. On the other hand, the concentration of chloride and sodium in the new leaves produced over this period was low and their function is thus unlikely to have been significantly impaired by the redistribution of ions from root to tops.

The slower initial increase in chloride concentration in S_4 plants of *wightii* was largely a consequence of both a slower initial rate of ion uptake and a greater relative growth rate (0·185 and 0·149 g/g/day for *wightii* and *tomentella*, respectively) expressed particularly in the faster growth of new leaves contributing to group C. The relation between relative growth rate and ion concentration has been discussed by Greenway and Thomas (1965) and the relation between high yielding ability and low ion concentration in the plant tops has been clearly demonstrated for 22 *G. wightii* cultivars by Gates, Haydock, and Robins (1970). Also, the higher water content of *wightii* leaves and stem results in lower chloride concentrations on a plant sap basis.

The time sequence of salt accumulation, and hence presumably osmotic adjustment, obviously differs for leaves of different age and also between major plant parts. It is greatly influenced by the level of salinity and more than likely also by the rate of increase of salinity in the substrate (Gates, Haydock, and Little 1966). By manipulation of these factors and the balance of ions in the substrate, measurements of changes in plant performance, e.g. rate of growth or, perhaps more sensitively, changes in rate of photosynthesis for individual leaves, should enable clearer delineation of osmotic effects from those of specific ion toxicities. It is apparent that the time sequences in response cannot be ignored in salt studies, even those using the most refined techniques of water potential, enzyme analysis, etc. As pointed out by Gates (1964) ". . . the conflicting nature of the data (on plant response to water stress) might be reconciled by . . . the study of growth trends in time". With these points in view, the difficulties of generalization with respect to salt response (Gale, Kohl, and Hagan 1967) might be resolved.

V. Acknowledgments

We should like to convey thanks to Mr. C. T. Gates, Division of Tropical Pastures, CSIRO, for helpful advice. The technical assistance of Mr. G. Paynter, Mr. J. G. Simons, and Mrs. Patricia Gaunson is much appreciated.

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