ORGAN REGENERATION ON EXCISED ROOTS OF *CHONDRILLA JUNCEA* AND ITS CHEMICAL REGULATION*

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Abstract

The effects of endogenous factors (plant age, section length, and section location) and environmental factors (temperature and mineral nutrition) upon organ regeneration on isolated root sections of *Chondrilla juncea* L. were used to develop a standard assay system for the study of the chemical regulation of regeneration. Bud and root formation and its polarity in the presence of a variety of regulators alone and in combinations were observed quantitatively. Bud numbers were increased by auxin (low concentrations), cytokinin, and gibberellin treatments. High concentrations of auxin inhibited bud formation and this effect was reversed by antiauxin, cytokinin, or gibberellin. Adenine did not counteract auxin-induced bud inhibition but adenine and *N*-6-benzyladenine did counteract inhibition induced by the purine antagonist 2,6-diaminopurine. Numbers of regenerated roots were increased by auxin treatment and reduced by cytokinin and gibberellin treatment. On control and auxin-treated sections, bud formation was strongly polar and proximal and cytokinin and gibberellin treatments lessened the polarity. Growth retardants inhibited regeneration. Of a number of synthetic auxins tested, 2,4-dichlorophenoxyacet-*O*-methylhydroxamic acid and 4-amino-3,5,6-trichloropicolinic acid were the most effective inhibitors of bud formation.

Mechanisms for the regulation of regeneration are inferred from the results.

I. INTRODUCTION

It is a common characteristic of plant development that a disruption of the developmental pattern of a whole plant is followed by regeneration leading to the restoration of the whole. If plant development is a manifestation of a pattern of chemical regulators, the regulator pattern of an intact plant, once disrupted, should react by establishing a pattern which could recreate an entire plant. A study of the regulation of regeneration provides an opportunity for the examination of these concepts. The present work investigates regeneration as it occurs on root sections of *Chondrilla juncea* L. ("skeleton weed"), a member of the family Compositae of Mediterranean origin. Under Australian conditions, this plant is a relatively long-lived

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perennial and during its life cycle a vegetative, rosette stage is followed in late spring or early summer by the bolting of flowering stems.

This investigation is of agricultural interest because, like many other weed species resistant to current weed-control practices, *C. juncea* plants have deep tap roots with potent regenerative capacities. Most investigations of *C. juncea* have been directly concerned with its control as a weed and few have been related to its physiology (see Caso and Kefford 1968).

In the present study, a system for the observation of regeneration on root sections of *C. juncea* is established. Then the effects upon regeneration of treatments with regulators and other chemicals are observed. From such effects, mechanisms for the regulation of regeneration and leads to control measures have been proposed. Particular attention has been given to bud regeneration on roots because control of this phenomenon appears to hold most promise for weed control. Existing control measures can readily kill the shoot and the upper portions of the root, but the remaining portion of the root regenerates a number of shoots to replace the one destroyed. Also, during cultivation roots can be broken into many pieces, each of which can produce a number of buds.

II. Materials and Methods

Chondrilla juncea plants were grown under natural photoperiod in a glasshouse with the temperature regulated at roughly 25/20°C. Seeds were sown in sandy soil contained in wood-veneer or galvanized steel sheet tubes, 25 cm long and 5 cm in diameter. Following germination, plants were thinned to one per tube and at harvest only plants with a single, non-ramified tap root were used for experimentation. The shoot and the top 2 cm of root were discarded and the remainder of the root was sectioned into pieces 5 cm long, usually three pieces being taken from each root. Sections were pooled and between 10 and 18 were used for each treatment.

Unless indicated otherwise, treatments consisted of soaking root sections overnight in 20 ml of a solution or water in a 10-cm Petri dish—the sections were thereby half-submerged in solution. After the treatment period, sections were drained and washed several times in distilled water.

For regeneration studies, treated or untreated root sections were planted at 2 cm depth in a perlite–vermiculite mixture (50 : 50) in 7·5 cm diameter plastic pots. The perlite–vermiculite was watered with a fifth-strength, complete, Hoagland solution, except in an experiment which tested the effects of mineral nutrition. Regeneration occurred during 3 weeks at 25°C in darkness except for occasional exposure to weak light for watering. At the end of this period, the numbers of buds and roots and their positions on the root section were recorded. Usually the results are expressed as the average number of regenerated buds or roots per root section or as the number of sections on which buds or roots were observed.

III. Results

(a) *Endogenous and Environmental Factors Affecting Regeneration on Root Sections*

(i) *Age of Plant and Regeneration Capability of Root Sections*

The capability of roots from plants of increasing age to regenerate buds was studied using root sections cut from plants from staggered sowings and set to regenerate at the same time. This experiment was repeated at different times during the year with similar results. The data in Table 1 are from an experiment done in the autumn of 1965 and, in this particular instance, plants were grown in tubes 37·5 cm long and the long roots so obtained were cut into five 5-cm sections. For plants about 13 weeks old or older, the age of the plant did not affect the number of buds regenerated.

Thus for the standard regeneration procedure, plants 12–24 weeks old were used. Results from this and a number of similar experiments showed no significant differences between the numbers of buds formed on root sections from different parts of the root, thus it was possible to pool sections from all parts of the root as a regular procedure.

TABLE 1

EFFECT OF PLANT AGE ON BUD REGENERATION ON ROOT SECTIONS

The average number of buds per 5-cm root section formed on sections taken from different locations along roots of plants of increasing age. Twelve sections were used to obtain each mean

Plant age (weeks)	Location of section in root				
	Proximal	Second	Third	Fourth	Distal
8	1·0*	3·4*	2·0*	2·3*	2·3*
13	5·5	5·4	5·0	3·7	3·8
18	5·0	4·5	3·4	3·5	3·5
22	4·2	4·0	3·6	3·9	3·3
26	5·2	4·2	3·8	3·4	4·0

* Some sections were dead at the end of the 3-week period of regeneration.

(ii) *Effects of Root Section Length and Location in the Parent Root upon Bud Formation and Polarity*

The effects of section length and the location of the section in the root upon the number of buds and the polarity of organ regeneration were studied. From 20-cm long portions of roots, including the proximal 2 cm that was excised in the standard procedure, fractions of the length were excised and the remainders were set to regenerate. The portions of root used in each treatment are shown in Figure 1.

Typical root sections, photographed in Figure 1 after regeneration, show the strong polarity of bud formation in all root sections—all buds formed very near the proximal ends of all types of section. The numbers of buds formed on each section are given in Table 2 and, with the exception of section type I, which was only 2 cm long, the average number of buds was independent of section length and location in the parent root. These results supported the pooling of root sections from along the length of roots in the standard regeneration procedure.

(iii) *Effect of Nutrient Concentration on the Number and Growth Rate of Regenerated Buds*

Root sections were placed to regenerate in pots of perlite–vermiculite mixture and watered with nutrient solutions of full, one-half, one-quarter, or one-eighth strength or with water alone. The nutrient solution was a standard Hoagland formulation with half the normal phosphate concentration. The application of nutrient solution during regeneration did not affect the numbers of buds but their growth rate was increased by a weak nutrient solution. In the standard procedure, one-fifth strength nutrient solution was used.

(iv) *Effect of Temperature on Bud Regeneration from Root Sections*

Root sections, 5 cm long, from plants 22 weeks old were placed to regenerate under the standard conditions except that the day/night temperature regimes of

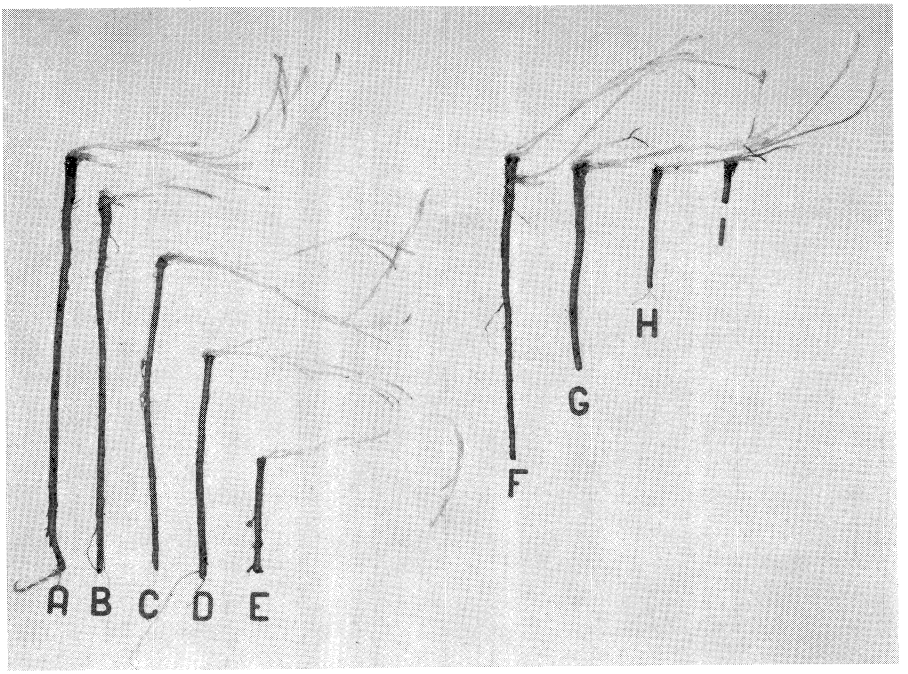

Fig. 1.—Regeneration of buds from the proximal ends of *C. juncea* root sections of lengths ranging from 2 to 20 cm and taken from various locations in the parent root. Beginning with proximal, 20-cm lengths of root, the portions *excised* in each treatment were: A, none; B, proximal 2 cm; C, proximal 5 cm; D, proximal 10 cm; E, proximal 15 cm; F, distal 5 cm; G, distal 10 cm; H, distal 15 cm; I, distal 18 cm.

TABLE 2

EFFECTS OF SECTION LENGTH AND LOCATION ON BUD REGENERATION

Numbers of buds formed on sections of *C. juncea* roots of the lengths shown and from the locations described in Figure 1

Type of section	Length of section (cm)	Average number of buds per section	Type of section	Length of section (cm)	Average number of buds per section
A	20	5·8±1·1	F	15	6·1±0·3
B	18	6·7±1·1	G	10	6·6±0·3
C	15	6·9±0·3	H	5	7·3±1·3
D	10	5·3±0·2	I	2	4·1±1·3*
E	5	6·0±1·0			

* Significantly different from values for section types C and H at 5% level.

10/5, 15/10, 18/13, 21/16, 24/19, 27/22, and 30/25°C were used. These temperatures were obtained in the controlled conditions of the CERES phytotron at Canberra

(Morse and Evans 1962). There were 12 sections per temperature regime and bud formation was observed after 2 and 5 weeks.

The relationship between temperature during the regeneration periods and the numbers of buds formed is shown in Figure 2. At 30/25°C some root sections died. Regeneration occurred at all temperatures with a broad optimum from 21/16 to 27/22°C. The temperature used normally in the regeneration tests was 25°C, about the middle of the optimal range. The *elongation* of the buds was fastest at 27/22°C.

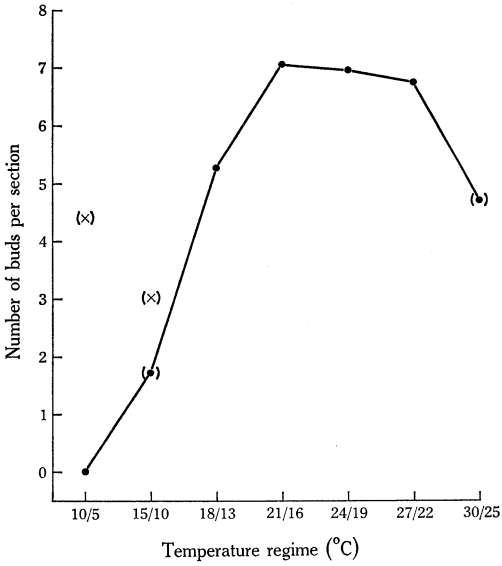

Fig. 2.—Effect of temperature regime during the regeneration period on bud regeneration upon root sections held in moist perlite–vermiculite mixture for 2 weeks (●) or 5 weeks (×). Symbols in parentheses indicate that some sections did not form buds. The temperatures indicate the day/night regimes.

(*b*) *Chemical Regulation of Organ Regeneration on Root Sections*

(i) *Effects of Auxins and Antiauxins*

The effects on regeneration of soaking root sections from 24-week-old plants in a series of concentrations of 1-naphthaleneacetic acid (NAA) are shown in Figure 3(*a*). As the concentration increased, the number of buds formed decreased while the number of roots increased. Not all the roots formed after auxin treatment arose directly from tissues of the parent root section. Some roots arose from the stems of adventitious buds and at the highest auxin concentration some roots arose in callus that proliferated at the distal end of the root section.

Treatment of root sections with a broad range of concentrations of the auxins 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-amino-3,5,6-trichloropicolinic acid (Picloram, Dow Chemical Australia Pty. Ltd.) produced an increase in bud number at low auxin concentrations [Figs. 3(*b*), 3(*c*)] and a similar effect was observed when NAA was used at lower concentrations than those shown in Figure 3(*a*). In higher concentrations of these auxins, bud regeneration was inhibited.

Effects on bud regeneration of auxins of a variety of structures are shown in Table 3. The general effect at the concentrations used was inhibitory; 3-indoleacetic acid (IAA) and 5-carboxymethyl-*N*,*N*-dimethyldithiocarbamate did not inhibit and the latter increased the number of buds. These compounds could have been broken down in the tissue.

As the regulation of bud regeneration on root sections has implications in weed control, sections treated with a series of auxin herbicides were observed. The effects

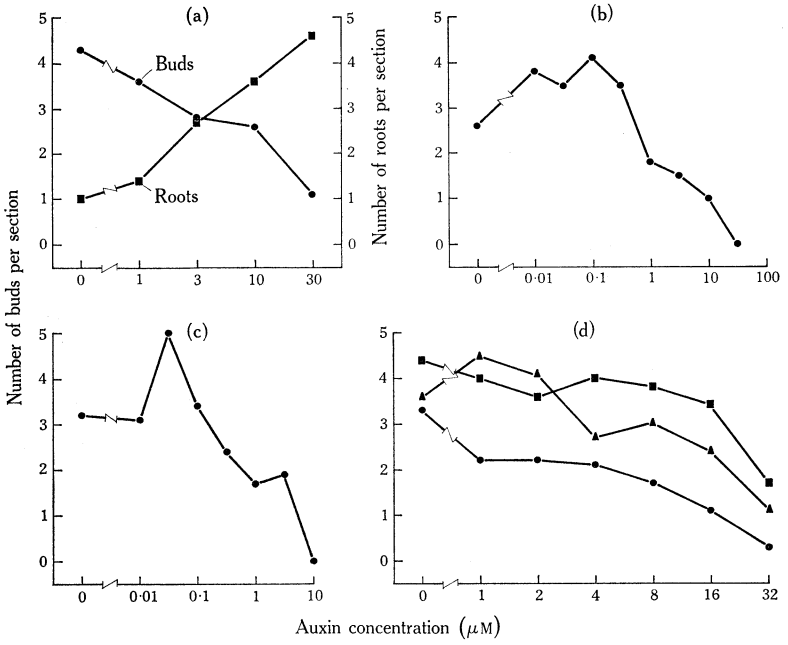

Fig. 3.—Effects of auxins and antiauxin upon regeneration of root sections. (*a*) Promotion of root formation and inhibition of bud formation by increasing concentrations of 1-naphthaleneacetic acid; (*b*) effects of 2,4-dichlorophenoxyacetic acid (2,4-D) concentrations on bud formation; (*c*) effects of 4-amino-3,5,6-trichloropicolinic acid concentrations on bud formation; (*d*) interaction of 2,4-D alone (●) and in mixtures with *p*-chlorophenoxyisobutyric acid at concentrations of 5×10^{-5}M (▲) and 10^{-4}M (■).

TABLE 3

EFFECT OF A VARIETY OF AUXINS ON BUD REGENERATION

The numbers of sections out of 12 that produced buds and, in parentheses, the mean number of buds per section, for root sections treated with the concentrations of the auxins shown. Results for water-treated control sections were 11 (2·9)

Auxin concn. (M)	Auxin*					
	A	B	C	D	E	F
3×10^{-6}	11 (3·0)	11 (2·8)	11 (2·8)	12 (4·5)	12 (3·4)	12 (3·1)
10^{-5}	11 (2·9)	8 (2·0)	9 (1·4)	12 (4·3)	11 (3·5)	9 (1·7)
3×10^{-5}	12 (3·8)	4 (0·6)	5 (0·9)	10 (3·6)	12 (2·4)	3 (0·4)
10^{-4}	10 (2·2)	0 (0·0)	0 (0·0)	11 (2·9)	7 (1·7)	0 (0·0)

* A, 3-indoleacetic acid (IAA); B, 1-naphthaleneacetic acid (NAA); C, sodium salt of 2,4-dichlorophenoxyacetic acid (2,4-D); D, 5-carboxymethyl-*N*,*N*-dimethyldithiocarbamate; E, 2-oxobenzothiazolin-3-ylacetic acid; F, potassium salt of 4-chloro-2-oxobenzothiazolin-3-ylacetic acid.

of derivatives of 2,4-D and of Picloram may be compared in Table 4, experiment I, and those of other commercial herbicides in Table 4, experiment II. In the latter case, NAA is included in the comparison.

TABLE 4

EFFECTS OF AUXIN HERBICIDES ON BUD REGENERATION

Experiment I: results for water-treated, control sections 12 (5·6). Other conventions as in Table 3

Auxin concn. (M)	Auxin*							
	A	B	C	D	E	F	G	H
10^{-7}	10 (5·2)	10 (3·7)	8 (3·1)	8 (1·5)	7 (3·3)	9 (4·1)	4 (1·5)	8 (4·5)
3×10^{-7}	11 (6·7)	7 (2·0)	4 (2·6)	6 (1·7)	6 (2·4)	11 (6·1)	6 (2·1)	11 (3·7)
10^{-6}	9 (4·6)	8 (3·0)	8 (3·1)	2 (1·4)	7 (3·0)	9 (4·2)	7 (2·1)	8 (3·2)
3×10^{-6}	8 (3·3)	6 (2·5)	6 (2·3)	1 (0·1)	3 (0·9)	4 (1·5)	4 (1·0)	4 (0·7)
10^{-5}	8 (2·8)	0 (0·0)	5 (1·3)	0 (0·0)	3 (0·5)	0 (0·0)	1 (0·2)	0 (0·0)
3×10^{-5}	1 (0·1)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	1 (0·2)	0 (0·0)

Experiment II: results for water-treated control sections 10 (2·9). Other conventions as in Table 3

Auxin concn. (M)	Auxin†			
	I	J	K	L
10^{-7}	8 (2·8)	9 (3·0)	10 (2·3)	9 (2·5)
3×10^{-7}	7 (1·4)	7 (1·4)	10 (1·8)	8 (2·0)
10^{-6}	9 (1·4)	4 (2·0)	3 (0·9)	9 (2·8)
3×10^{-6}	7 (2·0)	10 (4·4)	9 (1·6)	8 (1·7)
10^{-5}	0 (0·0)	6 (1·9)	0 (0·0)	4 (0·9)
3×10^{-5}	0 (0·0)	4 (0·6)	0 (0·0)	0 (0·0)

* A, 2,4-D; B, 2,4-dichlorophenoxyacethydroxamic acid; C, 2,4-dichlorophenoxyacet-*N*-methylhydroxamic acid; D, 2,4-dichlorophenoxyacet-*O*-methylhydroxamic acid; E, 2,4-dichlorophenoxyacet-*N*,*O*-dimethylhydroxamic acid; F, *N*-(diethylaminomethyl)-2,4-dichlorophenoxyacetamide; G, sodium salt of 2,4,5-trichlorophenoxyacetic acid; H, 4-amino-3,5,6-trichloropicolinic acid (Picloram).

† I, NAA; J, *N*-cyclohexyl-2,4-dichlorophenoxyacetamide; K, *N*,*N'*-di(2,4-dichlorophenoxyacetyl)thiourea; L, potassium salt of 2-(2,4-dichlorophenoxy)propionic acid.

The antiauxin *p*-chlorophenoxyisobutyric acid (PCPIB) counteracted the inhibition of bud formation by the auxin 2,4-D [Fig. 3(*d*)]. The compound 2,3,5-triiodobenzoic acid, which may act as an antiauxin or an antagonist of auxin transport, promoted bud formation (Table 5).

(ii) *Effects of Cytokinins on Regeneration and Interactions between Cytokinins and Auxins*

The treatment of root sections with the cytokinin *N*-6-furfuryladenine (kinetin) had three effects. The numbers of buds increased with increasing kinetin concentration up to at least 300 μM [Fig. 4(*a*)] while roots were not formed on any of the sections treated with kinetin. Kinetin not only increased bud numbers but changed the polarity of their formation. In water-treated sections, buds form predominantly at

the proximal end with few forming on the flanks of the sections. Kinetin treatment changed this pattern of bud formation [Fig. 4(*a*)].

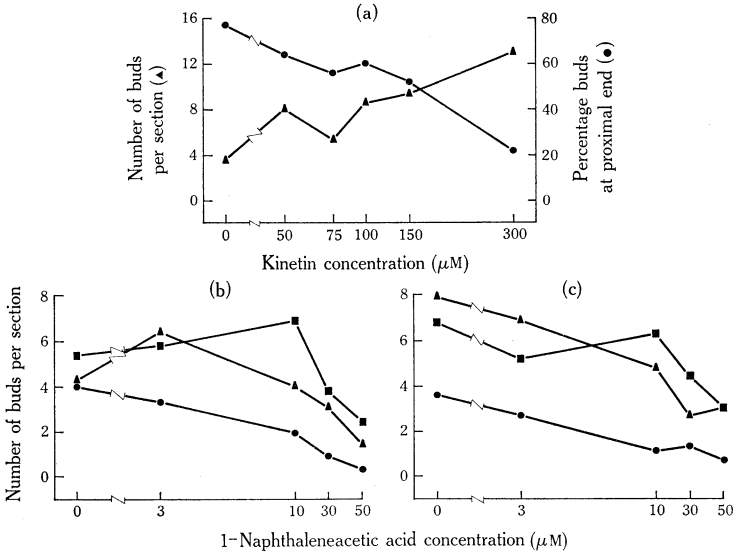

Fig. 4.—Bud formation on root sections treated with cytokinins alone and in mixtures with auxin. (*a*) Number of buds formed (▲) and the percentage of buds formed at the proximal end (●) of sections treated with kinetin (*N*-6-furfuryladenine); (*b*) number of buds formed on root sections treated with 1-naphthaleneacetic acid alone (●) and in mixtures with kinetin at 3×10^{-5}M (▲) and 10^{-4}M (■); (*c*) number of buds formed on root sections treated with 1-naphthaleneacetic acid alone (●) and in mixtures with *N*-6-benzyladenine at 10^{-4}M (▲) and 2×10^{-4}M (■).

TABLE 5

INTERACTION OF AUXIN AND ANTIAUXIN IN REGULATING BUD REGENERATION

The number of sections out of 12 that produced buds and, in parentheses, the mean number of buds per section, for root sections treated with the concentrations of 1-naphthaleneacetic acid (NAA) or 2,3,5-triiodobenzoic acid (TIBA) shown

TIBA conen. (M)	NAA concentration (M)		
	0	10^{-5}M	3×10^{-5}M
0	12 (2·9)	10 (2·5)	7 (1·4)
10^{-5}	12 (6·7)	9 (1·4)	10 (1·9)
3×10^{-5}	12 (5·6)	10 (2·7)	9 (1·2)
10^{-4}	12 (8·2)	11 (3·9)	11 (2·8)
3×10^{-4}	12 (5·5)	12 (5·2)	9 (2·7)

Cytokinins and auxins were observed to interact in their effects on bud regeneration, in that cytokinin treatment countered auxin inhibition of bud development and vice versa. In Figures 4(*b*) and 4(*c*) are the results of experiments involving kinetin and *N*-6-benzyladenine as cytokinins and NAA as auxin. Similar results were obtained when 2,4-D or Picloram were the auxins.

To test whether the effect of kinetin and *N*-6-benzyladenine in counteracting auxin inhibited bud development was merely an effect common to purine or adenine derivatives, the interaction between adenine at concentrations from 10^{-6} to 3×10^{-4}M and NAA at 3×10^{-5}M was observed. Adenine had no effect on the inhibition of bud formation by auxin nor did it have an influence in the absence of auxin.

TABLE 6

EFFECT OF GIBBERELLIC ACID ON THE REGENERATION OF BUDS AND ROOTS ON ROOT SECTIONS

The number of sections out of 12 that produced buds or roots and, in parentheses, the mean number of buds or roots per section, for root sections treated with the concentrations of gibberellic acid (GA_3) shown

GA_3 concn. (M)	Buds	Roots
0	12 (5·2)	8 (1·3)
3×10^{-5}	12 (5·4)	6 (0·8)
10^{-4}	12 (5·8)	6 (1·5)
3×10^{-4}	12 (6·6)	3 (0·2)
10^{-3}	12 (9·7)	3 (0·3)
3×10^{-3}	12 (8·4)	3 (0·2)

(iii) *Effect of Gibberellin on Regeneration*

Treatment of root sections with gibberellic acid (GA_3) produced an increase in the numbers of buds when concentrations of 10^{-3} and 3×10^{-3}M were used (Table 6). These are relatively high concentrations but the sections received gibberellin only during the period of overnight treatment then were left for 3 weeks to regenerate. Gibberellic acid treatment resulted in a small change in the polarity of bud production but most of the buds were still produced within 2 cm from the proximal ends of the sections. Undifferentiated outgrowths, up to 10 mm long and 4 mm diameter, occurred along the sides of gibberellin-treated sections. The buds formed on gibberellin-treated sections were no longer, but were thinner, than those from water-treated sections. The number of sections bearing roots and the average number of roots per section was reduced by gibberellic acid (Table 6).

In an experiment using mixtures of GA_3 and NAA, GA_3 at 3×10^{-5}M counteracted the inhibition of bud formation by NAA at 10^{-5}M.

(iv) *Effect of Growth Retardants on Regeneration*

From the class of compounds known as growth retardants the following compounds have been used: β-chloroethyltrimethylammonium chloride (Cycocel, American Cyanamid Co.); 2′-isopropyl-4′-(trimethylammonium chloride)-5′-methylphenyl piperidine carboxylate (Amo 1618, Rainbow Color and Chemical Co.); tributyl-2,4-dichlorobenzylphosphonium chloride (Phosphon D, Virginia–Carolina Chemical Co.); *N*-dimethylaminosuccinamic acid (B-995, Naugatuck Chemical Co.); and tris(2-aziridinyl)phosphine oxide (APO, Dow Chemical Co.).

Cycocel in a range of concentrations from 3×10^{-3} to 10^{-1}M did not affect the numbers of buds formed or their length, except at the highest concentration which

was toxic. Buds that formed on sections treated with 3×10^{-4} and 10^{-3}M Amo 1618 were shorter than controls but the numbers of buds were unchanged.

Phosphon D inhibited bud formation at concentrations up to 5×10^{-5}M (Table 7) and at higher concentrations was toxic to the root sections. Similarly, B-995 was toxic at concentrations higher than 6×10^{-2}M and lower concentrations inhibited the appearance of buds (Table 7). Observations on sections treated with B-995 over a 6-week regeneration period indicated that the effect of this compound was on bud elongation rather than bud initiation. The compound APO was inhibitory from 4×10^{-5} to 5×10^{-3}M and at higher concentrations was toxic (Table 7).

TABLE 7

EFFECTS OF GROWTH RETARDANTS ON BUD REGENERATION

Number of sections out of 12 which produced buds and the mean number of buds per section for root sections treated with the concentrations of growth retardants shown. The tests with each growth retardant were done on different occasions hence each has its control

Growth retardant	Concn. (M)	Sections with buds	Buds per section
Phosphon D	0	12	5·2
	2×10^{-5}	12	4·0
	3×10^{-5}	12	4·1
	4×10^{-5}	12	3·2
	5×10^{-5}	12	3·3
B-995	0	12	3·7
	3×10^{-2}	5	0·9
	6×10^{-2}	0	—
	10^{-1}	dead	—
APO	0	12	3·2
	4×10^{-5}	10	3·1
	2×10^{-4}	7	1·2
	10^{-3}	1	0·1
	5×10^{-3}	0	—
	$2{\cdot}5\times10^{-2}$	dead	—

There is evidence that some growth retardants act by inhibiting the synthesis of native gibberellin or auxin (Lang 1970). Thus interactions between the growth retardants and 10^{-5}M GA_3 and 10^{-5}M NAA were studied. At this concentration, GA_3 slightly promoted bud formation while NAA slightly inhibited. NAA and GA_3 were used without or with 3×10^{-2}M Cycocel, 10^{-3}M Amo 1618, 3×10^{-5}M Phosphon D, and 3×10^{-2}M B-995, and some results are shown in Table 8. The inhibition of buds by B-995 and Amo 1618 increased in the presence of NAA and was not diminished by GA_3. A reversal of the inhibition of bud development by Phosphon D occurred in the presence of gibberellin; auxin treatment in this case produced no change. Cycocel was without effect, alone or in mixtures.

(v) *Effects of Maleimides on Regeneration*

Another chemical class known to inhibit growth is the maleimides and two compounds from this class, *N*-(2,4-dichlorophenyl)maleimide and *N*-(1-naphthyl)-

maleimide were tested for effects on bud regeneration. The compounds were poorly soluble in water so were dissolved in 2% isopropanol solution which was also used

TABLE 8

INTERACTIONS BETWEEN GROWTH RETARDANTS AND AUXIN AND GIBBERELLIN

Numbers of sections out of 12 producing buds, the average number of buds produced per section, and the average length of the longest bud on each section for root sections treated as shown

Treatment	Sections with buds	Buds per section	Length of longest bud (mm)
Water control	12	3·4	189±15
NAA, 10^{-5}M	10	2·3	135±14
GA_3, 10^{-5}M	12	4·3	184±6
B-995, 3×10^{-2}M	9	1·8	7±5
B-995+NAA	3	0·4	5±2
B-995+GA_3	11	1·8	12±4
Phosphon D 3×10^{-5}M	8	2·0	6±3
Phosphon D+NAA	7	1·7	9±4
Phosphon D+GA_3	10	4·0	23±4
Amo 1618, 10^{-3}M	10	4·2	12±4
Amo 1618+NAA	6	2·3	6±3
Amo 1618+GA_3	11	5·1	21±9

for the control. At 3×10^{-4} and 10^{-3}M, both compounds inhibited bud formation while lower concentrations slightly increased the number of buds produced on each section (Table 9).

TABLE 9

EFFECT OF MALEIMIDES ON BUD REGENERATION

The two maleimides were dissolved in 2% aqueous isopropanol and the results for control sections treated with the solvent alone were 12 (6·2). Other conventions as in Table 3

Concn. (M)	*N*-(2,4-dichlorophenyl)-maleimide	*N*-(1-naphthyl)-maleimide
10^{-5}	12 (7·4)	12 (7·1)
3×10^{-5}	12 (7·7)	12 (8·7)
10^{-4}	11 (7·5)	11 (6·7)
3×10^{-4}	4 (1·8)	4 (2·2)
10^{-3}	2 (1·1)	2 (1·1)

(vi) *Effects of Purine Antimetabolites on Bud Regeneration*

Three compounds which may act as antagonists of purine metabolism, 2,6-diaminopurine, 3-amino-1,2,4-triazole (Amitrole, American Chemical Paint Co., USA), and maleic hydrazide have been tested.

Concentrations of 2,6-diaminopurine of 2×10^{-4}M or greater inhibited bud regeneration, but inhibition was not permanent and was shown to be partly relieved during a 6-week regeneration period (Table 10). The inhibition of buds by 2,6-di-

aminopurine could be largely relieved by the addition of kinetin and partly relieved by the addition of adenine (Table 10).

TABLE 10

INHIBITION OF BUD REGENERATION BY 2,6-DIAMINOPURINE AND INTERACTIONS WITH KINETIN AND ADENINE

The number of sections bearing buds and, in parentheses, the average number of buds per section for root sections treated with 2,6-diaminopurine and left to regenerate for 3 or 6 weeks (expt. I) or treated with 2,6-diaminopurine alone and mixtures with kinetin or adenine (expt. II)

Experiment I		
2,6-Diaminopurine concn. (M)	Buds after 3 weeks	Buds after 6 weeks
0	10 (2·9)	10 (2·9)
2×10^{-4}	7 (3·0)	8 (3·0)
4×10^{-4}	3 (1·2)	5 (3·3)
6×10^{-4}	4 (1·5)	5 (2·6)
8×10^{-4}	0 (0·0)	3 (0·9)

Experiment II			
2,6-Diaminopurine concn. (M)	Control	Kinetin, 5×10^{-5}M	Adenine, 10^{-5}M
0	9 (2·3)	11 (3·8)	10 (3·0)
5×10^{-5}	1 (0·9)	9 (4·6)	4 (2·5)

Amitrole in concentrations from 10^{-5} to 3×10^{-4}M increased the numbers of buds formed on sections and at 10^{-2} and 3×10^{-2}M the compound was inhibitory

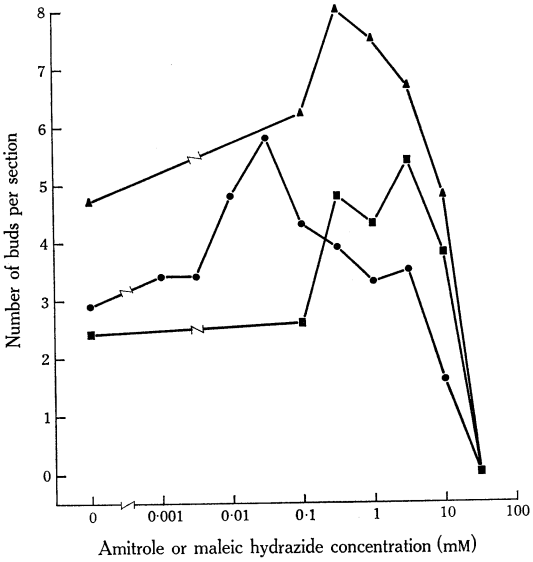

Fig. 5.—Effect of Amitrole (3-amino-1,2,4-triazole) (●) and maleic hydrazide (■) upon bud number on regenerating root sections. Maleic hydrazide was also tested in mixtures with 10^{-4}M kinetin (▲).

(Fig. 5). In the absence and presence of 10^{-4}M kinetin, maleic hydrazide promoted bud formation at concentrations from 3×10^{-4} to 10^{-2}M, and was toxic at 3×10^{-2}M (Fig. 5).

IV. Discussion

Provided roots older than about 12 weeks were used and sections were at least approximately 5 cm long, there was little variation in the pattern of regeneration with age of plant (Table 1), length of the section or its position in the root (Table 2; Fig. 1), and the developmental phase of the plant up to flowering (Caso and Kefford, unpublished data). These findings permitted much flexibility in producing root sections that acted uniformly in the standard assay procedure for regeneration. The findings also indicate that regeneration in *C. juncea* root sections is a phenomenon determined within the section—the status of the whole plant and the precise position of the section in the root is not of consequence to regeneration. The extent of the regenerating system of concern is therefore a root section with two cut surfaces and whatever properties can be carried over from the parent root and can operate upon the system. In this case of regeneration, particular cells do not have a role that is predetermined while they are within the parent root; how a cell will react is determined by its location in the section which in turn is determined by chance during excision. These generalities, which investigators over many years have shown to apply to various species (Dore 1965), are important in defining the regenerating system and the possibilities for its regulation.

Treatment of root sections with the auxin NAA inhibited bud formation [Fig. 3(*a*)], but there was not a general inhibition of meristematic activity because, as bud formation decreased with rising concentration, root initiation increased—the total number of new organs remaining relatively constant. PCPIB, which exhibits antiauxin properties toward cell expansion (McRae, Foster, and Bonner 1953), promoted bud formation and also counteracted auxin-induced inhibition [Fig. 3(*d*)]. Thus overall, a root section appears to have an endogenous auxin concentration inhibitory to bud formation and the reduction of its auxin status with antiauxin treatment produces conditions less inhibitory to bud regeneration. The auxins NAA, 2,4-D, and Picloram (Kefford and Caso 1966), in low concentrations, increased bud numbers on root sections [Figs. 3(*b*), 3(*c*)] and only as the concentration was increased did inhibition occur. Two explanations are possible for the promotion of bud formation by low concentrations of these auxins. Either they are acting as antiauxins towards the endogenous auxin, as low concentrations of NAA and 2,4-D have been postulated to do in elongating *Avena* coleoptiles (McRae, Foster, and Bonner 1953), or the endogenous auxin concentration, in at least part of a root section, is less than optimal for bud formation and the addition of low concentrations of these auxins brings it closer to the optimal. The former explanation is favoured because the results of a number of experiments with low and high concentrations of IAA, the presumed native auxin, failed to show promotions of bud numbers.

Because the practical aim of the investigation was to seek means of controlling bud regeneration as a lead to weed control measures and because auxin may provide such a means, a variety of auxins were tried for their efficiency as bud inhibitors (Tables 3 and 4). The most effective compounds were 2,4-dichlorophenoxyacet-*O*-methylhydroxamic acid followed by Picloram and other derivatives of 2,4-D. Most of the 2,4-D derivatives tested were synthesized with the aim of increasing their rate of translocation in roots (J. N. Phillips, personal communication). This study of the effects of various auxins on regeneration when applied directly to the root sections has

been used as a guide for treatments applied through the shoot and the performances of auxins under the two modes of application have been compared (Caso and Kefford, unpublished data).

On water-treated sections, buds formed almost exclusively at the proximal end, independent of the length of the section and its position in the parent root. This fact and the other evidence discussed so far can be explained by the following regulatory system operating in roots. The endogenous auxin concentration in roots is inhibitory to bud initiation; thus, on intact roots, buds do not form. On excision, however, the auxin source is cut off and, as a result of the acropetal auxin transport system of roots (Kirk and Jacobs 1968), the auxin concentration at the proximal end of a section is reduced to a level not inhibitory to bud regeneration. The number of buds produced is limited and their location on a section is circumscribed, independent of section length and hence food reserves. Therefore the development of first-formed buds would appear to inhibit subsequent development presumably due to their synthesis of auxin. Treatments applied to sections could, of course, influence their auxin status through effects occurring before or after bud initiation or both. This concept of bud regeneration on roots occurring as a result of a release from auxin inhibition was developed from earlier investigations of regulatory mechanisms (reviewed by Dore 1965). The investigation of auxin effects on *C. juncea* root sections confirms the earlier phenomena but experiments with other regulators suggest other facets to the mechanism for the regulation of regeneration.

Cytokinin treatment (Fig. 4) increased bud numbers, counteracted bud inhibition by auxin treatment, and extended the zone over which buds were formed on a root section. Cytokinin treatment also inhibited root formation. In producing these effects, the cytokinins used, although adenine derivatives, were not acting merely as sources of adenine because treatment with adenine itself, alone or in the presence of auxin, had no effect upon bud formation. The observed response of root sections to cytokinin treatment is the one that would be anticipated if bud formation on sections of *C. juncea* was regulated by an auxin–cytokinin ratio as was originally proposed for tobacco stem callus (Skoog and Miller 1956) and appears to apply to other tissues (Miller 1961). According to the theory of the auxin–cytokinin ratio determining bud formation, auxin inhibition may be interpreted in terms of the ratio being too high. Then cytokinin treatment would lower the ratio towards values more appropriate for bud initiation and bud numbers would rise. As increasing cytokinin concentrations were applied in the current experiments, the zone of bud formation moved from the proximal toward the basal ends of sections [Fig. 4(*a*)] which would be expected if there was an increasing gradient of auxin concentration in that direction and only higher concentrations of cytokinin could give the auxin–cytokinin ratios necessary for bud initiation. Torrey (1958) and Bonnett and Torrey (1965) found cytokinin treatment to increase bud formation on root sections of *Convolvulus arvensis* with no effect noted on polarity; however, this and other evidence suggests that such root sections may have a lower auxin status and less strict polarity than *C. juncea* root sections. A role for cytokinin in addition to auxin in the regulation of regeneration must be considered, as cytokinin is known to occur in roots (Miller 1961). If cytokinin has a role in regeneration, the current evidence gives no indication of particular zones of high concentration, such as the cut ends, in *C. juncea* root sections.

The effects of gibberellin treatment upon bud and root regeneration on *C. juncea* sections, like those of cytokinin, were a promotion of buds and an inhibition of roots (Table 6), with some shift in the polarity of bud formation. If auxin concentration is determining bud and root number in untreated root sections, an increase in buds and a decrease in roots by treatment with gibberellin suggests that this regulator is reducing the auxin status of the root section with respect to meristematic activity. It has been proposed (Kefford and Goldacre 1961) that gibberellin can divert auxin activity from meristematic to expansionary in a tissue or organ where these two phases of development can be manifest. An indication that a diversion of auxin activity to cell expansion was occurring in *C. juncea* root sections was the expansion of cells along the flanks of gibberellin-treated sections. An increase in meristematic activity by gibberellin treatment is not a general phenomenon, indeed there are more instances of the reverse and the auxin–gibberellin ratio has not been proposed as a determinant of meristem patterns. Paulet and Nitsch (1959), however, found that gibberellic acid promoted bud formation on leaf segments of *Cardamine pratensis* and a callus from sycamore poplar showed increased cell division in the presence of gibberellic acid (Digby and Wareing 1966). Clearly gibberellin, which occurs in roots (Lang 1970), has a potential role in the regulation of regeneration.

The effects of growth retardants on bud initiation (Tables 7 and 8) suggest that gibberellin *synthesis* is not a determinant because CCC and Amo 1618, which are proposed as inhibitors of gibberellin synthesis, did not affect bud number. Amo 1618 did, however, inhibit bud elongation. The compounds Phosphon D, B-995, and APO were toxic at the higher concentrations and therefore the possibility that their effects on development at lower concentrations were due to unspecific inhibition cannot be excluded. However, their potential in regeneration control should be noted, particularly in conjunction with auxin treatment. The maleimides, another class of plant inhibitor of unknown mechanism of action, inhibited bud formation.

Purine or pyrimidine antimetabolites have a general potential for inhibiting processes of development; this was the case for 2,6-diaminopurine and the inhibition could be relieved by adenine or kinetin (Table 10). Amitrole and maleic hydrazide, two compounds with structural analogies with pyrimidines and for which one proposed mechanism of action is pyrimidine antagonism (Moreland 1967), promoted bud formation in low concentrations and inhibited at higher (Fig. 5). No explanation of such effects can be proffered.

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