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SOME RESPONSES OF PLANTS TO 2,3,5-TRIIODOBENZOIC ACID

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(WITH ONE FIGURE)

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Introduction

Several investigators have recently reported certain plant responses to 2,3,5-triiodobenzoic acid (hereafter referred to as TIBA). ZIMMERMAN and HITCHCOCK (5) reported that TIBA was inactive with regard to cell elongation but had formative effects on tomato. Although TIBA did not cause epinasty of leaves, soil application of TIBA in solution resulted in an odd bending of stems as subsequent growth occurred. These investigators also reported a tendency for an increase in number as well as amount of axillary shoot growth and the production of flower clusters from buds which normally developed vegetative shoots. GALSTON (1) was unable to induce flowering of vegetative Peking soybeans by application of TIBA but obtained an increased floral response of photoinduced plants. Morphological responses of soybean to TIBA included shortening of internodes, loss of apical dominance, epinasty of young leaves, and premature abscission of apical leaves and buds.

Telemorphic changes of vegetative structures of TIBA-treated bean plants have been reported by KRAUS and MITCHELL (2). Elongation of the internodes was checked, terminal and axillary bud growth affected, and leaves showed characteristic dwarfing and curling. Neither tumor formation nor root initiation was reported.

WHITING and MURRAY (4) applied TIBA in lanolin and in aqueous and emulsion sprays to red kidney bean. Application of TIBA in lanolin to decapitated surfaces of the second internode resulted in discoloration of the tissue, necrosis of the treated tissue, and formation of small tumors in the stem immediately below the treated area. Telemorphic effects were noted on the first trifoliate leaves of the developing axillary shoots, howover, subsequent growth was normal. The responses to lanolin ringing of the internode were similar to those noted as the result of spray applica-. tions. Telemorphic responses induced in leaves included downward curvature of tips, inward curvature of margins, and dwarfing of leaflets. A silver-hairiness, discoloration, and abscission, was apparent on severely af-Inhibition of elongation of the internode occurred below fected leaves. the terminal bud. Severely affected buds showed discoloration and abscission, whereas, buds less severely affected showed recovery. As the

plants lost apical dominance strong development of axillary shoots occurred. Axillary shoots above the lanolin ring showed the same symptoms as the apical bud, and those shoots below the application frequently developed the symptoms. Histological examination showed insignificant tumor development with applications to the cut surface, but no tumor formation resulted from the ringing treatment. Leaf and bud abscission was caused by the formation of well-defined separation layers.

On the basis of the Avena coleoptile curvature test. GALSTON (1) has reported inhibition of the effectiveness of indoleacetic acid (IAA) by low concentrations of TIBA and a complete negation of the effect by higher concentrations. THIMANN and BONNER (3) obtained similar results with high mol ratios of TIBA/IAA, however lower mol ratios (up to 8.7) resulted in an increase in the effectiveness of IAA. They have suggested that if auxin brings about its growth-promoting activities by combining with a special substrate, then, because of the similarity between the molecules of TIBA and IAA there may be competition to occupy these posi-The combining of TIBA with the substrate does not result in tions. growth. If the concentration of TIBA is not too high, there are still left open a small number of spaces or active groups on the substrate with which auxin combines. The result might then be that auxin causes growth with the expenditure of a smaller number of auxin molecules than are normally required.

It is evident that the responses brought about by TIBA are significantly different from those of growth-regulators of the auxin-type. To determine whether or not certain responses of plants to naturally occurring and applied growth-regulators would be altered by applications of TIBA, the present study was initiated and is presented in three sections:

- a) Effect on rooting of cuttings
- b) Effect on development of axillary buds
- c) Formative and injurious effects

The effect of TIBA on rooting of cuttings

MATERIALS AND METHODS.—Clonal stock of Coleus Blumei Benth. was used for all experiments. Terminal cuttings five to six inches in length were made with the basal cut immediately below a node. The solutions for the treatment of the cuttings were prepared by dissolving TIBA or indolebutyric acid (IBA) in 10 cc. of 95% ethyl alcohol and diluting with distilled water. Four hundred cc. of solution were used for each lot of 20 cuttings. The bases of the cuttings were submerged to a depth of one and one-half inches in the various solutions for 24 hours. At the end of the soaking period, the cuttings were removed from the solutions. After the excess solution was allowed to run off, they were stuck in medium grade quartz sand to a depth of two inches. Except as otherwise noted the cuttings remained in the rooting medium for 12 days before removal for examination. During the experimental periods, the temperature varied from 75° to 80° F. and the relative humidity was never below 60%. Fluorescent light (daylight tubes) of approximately 600 f. c. was supplied from 8 A.M. to 5 P.M.

RESULTS AND DISCUSSION .--- In one experiment, conducted in June, 1947, Coleus cuttings were subjected to a 24-hour soak in distilled water and in solutions containing concentrations of TIBA ranging from 1 to 250 p.p.m. After 12 days the cuttings were examined for the number rooting and the number of roots formed. The results, recorded in table I, show that concentrations of five p.p.m. or more TIBA inhibited the rooting of cuttings as well as reduced the average number of roots formed. These results were substantiated by similar experiments conducted in July and

Observation	Rep-	WATER	P.P.M. TIBA OF SOAK						
	CATE	TROL	1	5	10	25	50	100	250
Total number of	1	19	17	15	9	3	0	1	0
cuttings rooted	2	20	20	12	14	1	1	0	0
0	3	18	17	13	9	2	2	0	0
	4	20	18	13	11	1	0	0	0
	Ave*	19.25	18.00	13.25	10.75	1.75	0.75	0.25	0.0
Average number	1	9.0	9.5	6.4	3.9	0.4	0.0	0.2	0.0
roots/ cutting/	2	12.0	14.2	6.4	5.2	0.1	0.1	0.0	0.0
treatment	3	7.3	9.2	8.4	4.8	0.7	0.2	0.0	0.0
	4	19.4	15.2	6.4	3.1	0.1	0.0	0.0	0.0
	Ave**	11.92	12.02	6.90	4.20	0.32	0.08	0.05	0.0

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ROOTING RESPONSE OF Coleus Blumei CUTTINGS FOLLOWING 24-HOUR SOAKING OF BASE IN 2,3,5-TRIIODOBENZOIC ACID PRIOR TO STICKING. EACH TREATMENT INCLUDED 20 CUTTINGS. DATA TAKEN 12 DAYS AFTER COMPLETION OF

THE SOAKING TREATMENT

* Least significant difference: 1%-2.59; 5%-1.90. ** Least significant difference: 1%-4.71; 5%-3.48.

September, 1947, and again in April, 1948. The slight stimulation of the average number of roots of the one p.p.m. TIBA treatment, though not significant in any given experiment, was observed in all trials.

In a second experiment one series of cuttings was given only an initial soaking in the various solutions, while a second series received the initial soaking plus additional soaking treatments every fourth day. The data. presented in table II, show that the inhibitory effect of the single treatment was gradually lost, while with repeated treatment with TIBA, continued inhibition occurred with concentrations of five or more p.p.m. After 20 days those cuttings which had received repetitions of the higher concentrations of TIBA were so severely injured that most of the treated portion of the stem was either rotting or dead. These treatments were discontinued at this time. However, where only a single soaking treatment was given, 96 to 100% rooting was obtained after 45 days.

Since it had been shown that TIBA may inhibit the rooting of Coleus cuttings, a third experiment was conducted to ascertain whether or not soaking with IBA prior to or subsequent to the TIBA treatment would alter the inhibitory effect of the TIBA. In one instance, lots of uniform cuttings were subjected to a 24-hour soak of TIBA ranging in concentration from one to 100 p.p.m. followed by a 24-hour soak in IBA solutions ranging from one to 50 p.p.m. In a second series of treatments the soaking

TABLE II	T.	A]	ΒL	Έ	II
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PER CENT. ROOTING OF Coleus Blumei CUTTINGS FOLLOWING VARIOUS 24-HOUR SOAKING TREATMENTS OF BASE OF CUTTINGS IN SOLUTIONS CONTAINING 2,3,5-TRIIODOBENZOIC ACID AS INDICATED. EACH SAMPLE CONSISTED OF 20 CUTTINGS.

Concentration		NO. DAYS AFTER			INITIAL SOAKING		
TIBA IN P.P.M.	TREATMENT -	4	8	12	16	20	
0	Initial soaking only Initial soaking plus			100		100	
	soaking every 4th day	100	100	100	100	100	
1	Initial soaking only Initial soaking plus			95		100	
	soaking every 4th day	50	90	90	100	100	
5	Initial soaking only Initial soaking plus			75		95	
-	soaking every 4th day	20	55	65	75	75*	
10	Initial soaking only Initial soaking plus			60		90	
	soaking every 4th day	5	15	35	55	55*	
25	Initial soaking only Initial soaking plus			10		80	
	soaking every 4th day	0	0	10	15	20*	
50	Initial soaking only Initial soaking plus			5		65	
	soaking every 4th day	0	0	0	0*	0*	
100	Initial soaking only Initial soaking plus			0		50	
200	soaking every 4th day	0	0	0*	0*	0*	

* Severe injury to basal end of many cuttings which had not rooted and slight injury to a few rooted cuttings.

sequence was reversed. Control treatments consisted of a TIBA series, an IBA series, and distilled water. Table III records the average number of roots produced by the various groups.

Regardless of the sequence of treatment, for any given concentration of TIBA an increase in the average number of roots occurred with increased concentration of IBA. Within a given concentration of IBA, treatment with 10 to 100 p.p.m. TIBA decreased the effectiveness of the IBA treatment. An increased effectiveness of IBA when accompanied by a low concentration of TIBA is again indicated by these results, since, in general, soaking of the cuttings in one p.p.m. TIBA solution resulted in an increased number of roots.

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A reduction in the percentage of cuttings forming roots was obtained with treatments of 10 or more p.p.m. TIBA alone or 25 to 100 p.p.m. TIBA followed by one p.p.m. IBA.

A comparison of the results shows that soaking of the cuttings in solutions containing TIBA prior to solutions containing IBA results in a lower average number of roots than by the cuttings of comparable treatments of the reversed soaking series. Two exceptions to this are the treatments which received 50 and 100 p.p.m. TIBA followed by one p.p.m. IBA.

On the basis of the explanation of the competitive action between molecules for positions on a substrate, it is suggested that positions of the substrate may be occupied by the most available kind of molecule (TIBA or IBA, whichever is available first). Thus the molecules of the material of the second soaking may occupy only the positions that remain. If a suffi-

TABLE III

Average number of roots formed per cutting of *Coleus Blumei* when treated with 24-hour soaking of base of cuttings in 2,3,5-triiodobenzoic acid and indolebutyric acid as indicated. Ten cuttings per treatment. Data taken 12 days after completion of soaking treatment.

0	TIBA	SOAK	FIRST;	IBA, s	ECOND	IBA	SOAK 1	FIRST;	TIBA, s	ECOND
CONC. TIBA IN	Conce	ENTRATI	ON OF 1	BA IN	Р.Р.М.	Conc	ENTRAT	ION OF	IBA IN	P.P.M.
Р.Р.М.	0	1	10	25	50	0	1	10	25	50
0	20.5	28.5	57.4	88.0	90.1	20.5	28.5	57.4	88.0	90.1
1	32.1	35.8	37.5	77.5	96.5	32.1	44.5	75.3	105.0	108.9
10	11.1	17.1	35.4	75.6	97.5	11.1				
25	10.2	12.6	30.0	52.8	79.8	10.2	19.3	55.7	93.6	108.5
50	0.7	8.4	26.1	51.9	75.4	0.7	1.4	39.0	77.5	84.8
100	0.5	6.7	16.9	25.7	77.6	0.5	0.1	22.1	41.0	42.3

cient number of positions are occupied by molecules of TIBA, then auxin is not effective in causing rooting. However, if relatively few substrate positions are occupied by molecules of TIBA, then, there may remain sufficient positions for auxin occupation to bring about the growth response. The stimulatory effect of one p.p.m. TIBA on rooting of Coleus cuttings is in accord with results obtained by THIMANN and BONNER (3) with the pea and Avena tests. If the concentration of TIBA is not too high or if there is available a large number of positions on the substrate, it is quite possible that a fewer number of auxin molecules might occupy positions and bring about the increased response.

Since the inhibitory effect of TIBA may eventually disappear or at least be appreciably decreased, auxin molecules must eventually occupy sufficient positions to bring about the growth response. Whether this is brought about by replacement of the TIBA of the TIBA-substrate union, by destruction of the TIBA molecule, by the occupation of new positions on the substrate, or by some other process is not definitely indicated by the results so far obtained. However, if the TIBA in union with the substrate is replaced by auxin, then it would be expected that the freed TIBA would bring about other effects. Since no formative effects were noted with Coleus it is doubtful if such occurred. Although there is no evidence to indicate that destruction of TIBA occurs either within the plant or when mixed in solution or agar with IAA or IBA, such an explanation should be considered. Still another possibility of why the inhibitory effect of TIBA does not persist is that molecules of TIBA may unite with the substrate and thus render the substrate ineffective in its role in the growth of the plant. In this way, TIBA may be permanently tied up, and new positions formed on the substrate may therefore be utilized by other growth substances.

Effect of TIBA and IBA on development of axillary buds

MATERIALS AND METHODS.—Red kidney bean (*Phaseolus vulgaris* L.) and California privet (*Ligustrum ovalifolium* Hassk.) were used as the test plants.

Red kidney bean seeds were planted in ordinary potting soil in 4-inch The seeds were germinated under greenhouse conditions and seven pots. days later seedlings were removed from each pot so that only two uniform plants remained. The beans were allowed to grow until the first trifoliate leaves were expanding, at which time selection was made for uniform plants to be treated. In the first experiment, the treatments (listed in table IV) were so arranged that a comparison could be made of the activity of the axillary buds of the primary leaves. In one series of treatments applications of the lanolin mixtures were made to intact plants, whereas, in a second series the plants were decapitated two to three cm. above the primary leaves. Application of two per cent. TIBA or two per cent. IBA in lanolin was made: 1) as complete rings around the internodes one and one-half to 2 cm. above the primary leaves; 2) as half-rings directly above one of the axillary buds; and 3) to various portions of the cut surface of the decapitated plants. In a second experiment with intact bean plants, lanolin containing two, one, or one-half per cent. of TIBA or IBA was applied as a ring two cm. above or below the primary leaves.

The California privet were single stem plants and had been maintained under greenhouse conditions over winter. The terminal meristem was active, leaves were present, but all axillary buds were dormant. In all instances treatments were made one centimeter above or below one of the lowest pairs of opposite axillary buds. Leaves were present at these pairs of buds. A small section of the bark, approximately five mm. square, was removed so that the chlorenchyma tissue of the stem was exposed. Lanolin mixtures containing two, one, and one-half per cent. TIBA or IBA were applied to these exposed surfaces.

RESULTS AND DISCUSSION.—With intact bean plants, a complete ring application of two per cent. TIBA in lanolin stimulated the development

TABLE IV

EFFECT OF 2% TIBA OR 2% 1BA IN LANOLIN ON DEVELOPMENT OF AXILLARY BUDS OF PRIMARY LEAVES OF RED KIDNEY BEAN. SIXTEEN PLANTS IN EACH TREATMENT. DATA TAKEN 10 DAYS AFTER TREATMENT.

	Num Showi	IBER OF NG ELOI	BUDS NGATION	TOTAL ELONGATION OF AXILLARY BUDS IN MM.		
TREATMENT	TREA	ATED	Noт	TRE	ATED	Noт
	TIBA	IBA	TREATED	TIBA	IBA	TREATED
TERMINAL BUD PRESENT						
No treatment			0-0			0-0
Lanolin rings above			0.0			0.0
TIBA ring above			0-0			0-0
primary leaves	16-14			286-231		
TIBA 4-ring above	10 11					
primary leaves	15		11	347		103
IBA ring above						
primary leaves		0-0			0-0	
IBA <u>1</u> -ring above						
primary leaves		0	0		0	0
TIBA ring above IBA						
loove primary		0 0			0_0	
IBA ring over TIBA		0-0			0-0	
ring above primary						
leaves	8-0			64 - 0		
DECAPITATED 2 CM. ABOVE						
PRIMARY LEAVES						
No Treatment			11-9			424–395
Lanolin on decapitated			10 11			FFF 504
TTRA on deconitated			19-11			555-594
surface	12-11			321-364		
TIBA on 4-decapitated	10 11			021 001		
surface	16		12	734		119
IBA on decapitated						
surface		3-6			17 - 38	
IBA on $\frac{1}{2}$ -decapitated						
		4	15		12	216
IBA on 1 surface	16	0		650	104	
TIBA ring above	10	9		059	104	
primary leaves	16 - 16				707-308	
TIBA 3-ring above						
primary leaves	15		13	535		234
IBA ring above						
primary leaves		1-0			5 - 0	
IBA z-ring above		0	•		0	^
TIBA ½-ring—IBA ½-		U	U		U	0
leaves	13	e		940	55	
104108	10	U		417	00	

of axillary buds of the primary leaves. When the ring was incomplete a greater stimulation of the bud below the application resulted. IBA applied as rings to intact plants did not result in the stimulation of bud development.

With decapitated plants, a marked stimulation of the axillary bud below the treatment was obtained with applications of TIBA to half of the decapitated surface or as a half-ring. A similar response in the case of a complete ring of TIBA may have resulted from application of a greater quantity of the material to one side. Applications of IBA to decapitated plants, either to the cut surface or as rings, inhibited bud development in all cases.

Application of both TIBA and IBA to the decapitated surface or as a ring resulted in a stimulation of only those buds below the application of TIBA. Buds below the IBA application showed significantly less activity

·	·					
TREATMENTS No Treatment		NO. OF PRI- MARY AXIL-	TOTAL ELONGA	No. of trifoli-		
		LARY BUDS SHOWING PRIMARY AXIL- ELONGATION LARY SHOOTS		TERMINAL SHOOT	ATE LEAVES ON PRIMARY AXIS	
		8	10	612	80	
. . .	Lanolin	8	10	594	73	
r s	2% TIBA	19	228	106	93	
av	1% TIBA	20	280	108	93	
le So	1% TIBA	19	193	168	91	
r al	2% IBA	2	2	291	46	
n ng	1% IBA	0	0	404	59	
코	1% IBA	2	2	415	57	
	Lanolin	8	10	599	72	
i s	2% TIBA	6	7	473	69	
d D	1% TIBA	8	11	517	72	
below y lea	1% TIBA	7	8	482	64	
lg lar	2% IBA	2	2	374	48	
	1% IBA	5	5	520	55	
-	4% IBA	3	3	544	56	

TABLE V

Responses of red kidney bean to ringing with TIBA or IBA in lanolin applied above and below the primary leaves. Each treatment consisted of ten plants. Data taken 20 days after treatments

than the control groups. Data taken 20 days after treatment showed that there was no significant change in the number of active axillary buds than had been noted ten days after treatment.

The results of the second experiment (table V) not only confirm the stimulating effect of TIBA on growth of axillary buds, but also show that only applications made above the buds were effective. Applications of TIBA and IBA resulted in less growth of the terminal shoot. TIBA was more inhibitory than was IBA in this respect and application made above the primary leaves was more inhibitory than that made below. Plants treated with TIBA above the primary leaves produced more trifoliate leaves than did the controls, whereas IBA applied either above or below

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the primary leaves resulted in the formation of fewer leaves on the primary axis.

The results with California privet show that development of axillary buds occurred only when application of TIBA was made above the bud. Two per cent. TIBA resulted in significantly more bud growth than either of the other concentrations (table VI). Data taken 67 days after treatment showed no significant difference in the number of stimulated buds.

	32 DAYS AFTER TREATMENT							
		No. of ELONG	F BUDS ATING	TOTAL ELONGA- TION IN CM.				
	TREATMENT	TREATED	Not treated	TREATED	NOT TREATED			
No Treatment		0	0	0	0			
Application below bud	Lanolin 2% TIBA 1% TIBA ½% TIBA 2% IBA 1% IBA ½% IBA	0 0 0 0 1 0	0 1 0 0 0 0 0	0 0 0 0 1.9 0	0 5.5 0 0 0 0 0 0			
Application above bud	Lanolin 2% TIBA 1% TIBA ½% TIBA 2% IBA 1% IBA ½% IBA	0 6 2 2 1 0 0	0 1 0 0 0 0 0	0 50.8 22.4 4.2 9.2 0 0	0 1.2 0 0 0 0 0			

TABLE VI EFFECT OF VARIOUS APPLICATIONS OF TIBA AND IBA IN LANOLIN ON AXILLARY BUDS

OF CALIFORNIA PRIVET. ALL BUDS DORMANT AT TIME OF TREATMENT. APPLICA-TIONS MADE 1 CM. ABOVE OR BELOW ONE BUD OF THE LOWEST PAIR OF LEAVES OF EACH PLANT. SEVEN PLANTS IN EACH TREATMENT. DATA TAKEN

Terminal as well as the stimulated axillary shoots were in an active state of growth.

The data presented showing the stimulating effect of TIBA on axillary bud development does not clarify the specific mechanism of bud inhibition. The observed anti-auxenic effects of TIBA, including the stimulatory effect on bud development, lend support to the explanations of bud inhibition based on auxin relationships. It is suggested that use of this substance, or similar materials, in specifically designed experiments might add to our understanding of bud inhibition.

Formative and injurious effects of TIBA

COLEUS.—High concentrations of TIBA (100 and 250 p.p.m.) used as a single soak for terminal cuttings, or repeated treatment with lower concentrations (25 and 50 p.p.m.) resulted in severe injury to the treated

portion of the stem. Those cuttings which were soaked in solutions containing 100 and 250 p.p.m. TIBA had a brown-to-black discoloration at the completion of the soaking treatment. After 12 days in the rooting medium, the base of the cuttings were either almost completely rotted or the lower portion of the stem had partially disintegrated. Repeated soakings each fourth day resulted in an earlier appearance of these conditions.

Representative rooted cuttings of the various treatments were potted and at no time were significant differences noted between the controls and those whose rooting was inhibited by the TIBA treatments.

BEAN.—Formative effects of TIBA reported above included shortening



FIG. 1. Red kidney bean plants ten days after lanolin treatment of internode as indicated: A. pure lanolin; B. 2% IBA as one-half ring above right primary axillary bud; C. 2% TIBA as one-half ring above primary axillary bud on the left, showing abnormal leaves and stimulation of axillary shoot below TIBA application; D. 2% IBA as one-half ring on right and 2% TIBA as one-half ring on left, showing extreme curvature of stem and abnormally developing leaves.

of the internodes, production of more trifoliate leaves on the main stem axis, and stimulation of axillary bud activity.

Application of lanolin mixtures of TIBA to the cut stem surface and along the internode did not stimulate significant callousing. In those treatments where both TIBA and IBA were applied, callousing and formation of adventitious roots occurred only where the IBA was applied. Curvature of the stem occurred toward the side to which TIBA was applied (fig. 1D). The degree of curvature was greater in those treatments which received application of IBA.

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Most obvious of the formative effects was the appearance of the leaf tissue of certain treatments (fig. 1). Leaves of treated plants were darker green than the controls and the veins were pronounced. The leaves were dwarfed, brittle, and curved inward toward the undersurface. Epidermal hairs were very prominent. There was a marked tendency for premature death and abscission of the terminal meristem as well as of many of the axillary buds of the trifoliate leaves. These conditions were more pronounced after 20 days and were developing in some of the stimulated axillary shoots. The abnormalities noted in the developing axillary shoots were less intense with decreased concentration of the TIBA application as well as when application was made above the primary leaves. It is quite possible that lower concentrations than were used might stimulate axillary bud development without the accompanying abnormalities.

The plants which received TIBA application below the primary leaves possessed less abnormal terminal growth than the plants treated above the leaves; however, in the former group many axillary buds of the primary leaves showed symptoms of death or were abscissing. It is quite obvious that with this herbaceous plant, the chemical application was in toxic concentrations.

CALIFORNIA PRIVET.—No formative effects of the chemical have been noted on either the terminal or stimulated shoots. New growth was of normal color and shape and neither leaf nor bud drop have been noted.

Summary

Inhibition of rooting of terminal stem cuttings of *Coleus Blumei* have been obtained following a 24-hour soaking in solutions containing five to 250 p.p.m. TIBA. The degree of inhibition increased rapidly with increased concentration of TIBA. The inhibition of rooting gradually disappeared unless reapplication of the chemical was made. Cuttings treated with solutions containing TIBA followed by soaking in solutions containing IBA produced fewer roots than did cuttings treated with these chemicals in the reverse sequence.

Stimulation of axillary bud development was obtained with red kidney bean and California privet, providing the applications of TIBA in lanolin were made above the bud. Treatments containing IBA in lanolin did not stimulate bud activity in privet and inhibited bud development of decapitated bean plants.

Formative and injurious effects of TIBA noted included:

- a) COLEUS.—Injury to stem tissue subjected to solutions of TIBA containing 25 or more p.p.m. if the treatments were repeated and to a single treatment of 100 or more p.p.m. No formative effects noted.
- b) BEAN.—Shortening of internodes and production of more trifoliate leaves on the main shoot when TIBA was applied above the primary leaves, lack of callous formation and adventitious root production by TIBA treated tissues, negative curvature of the stem at the point of

application, abnormal leaf development, and premature death and abscission of the apical and, in some cases, the axillary buds.

c) CALIFORNIA PRIVET.—No formative effects resulted from treatment with TIBA.

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