THE EFFECT OF SELECTED HALOGENATED BENZOIC ACIDS ON FLOWERING

By

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A THESIS

Submitted to the School of Graduate Studies of Michigan

State University of Agriculture and Applied Science

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1956

ACKNOWLEDGEMENTS

The author wishes to express his indebtedness and sincere appreciation to Dr. C. L. Hamner for his guidance, encouragement and never failing help which made this investigation possible.

To the members of my committee, Dr. R. Bandurski, Dr. W. Haney and Dr. H. Sell goes my appreciation.

Thanks are also due to Dr. R. Leaper for synthesizing certain compounds used in this experiment and to Dr. C. E. Wildon for never failing interest and friendship.

To a man who had no active part in this research must go the greatest credit. In my undergraduate days at Colorado A&M he awakened me to the beauty of science. To George Beach goes my most heartfelt thanks.

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INTRODUCTION

The induction of flowering in plants has attracted the attention of investigators for many years. It is perhaps not surprising that different workers have chosen to emphasize different aspects (35, 38, 41) of this complex phenomenon. Thus, in their classical work on carbohydrate-nitrogen relationships, Kraus and Kraybill (19) called attention to the nutritional status of plants as a factor in flower production. Emphasis was placed upon the effect of C/N ratio on fruitfulness rather than on the initiation of flower primordia. It was stated that "The conditions for initiation of floral primordia and even blooming are probably different from those accompanying fruit setting. The greatest number of flowers are produced neither by conditions favoring highest vegetation nor by conditions markedly suppressing vegetation". Melchers (25) also investigated the C/N ratio.

Working with Maryland Mammoth tobacco, Garner and Allard (9) focussed attention on photoperiodic factors leading to flower induction. Plants in early stages of development may respond differently to a given photoperiod than similar plants at later stages of development. Cajlachjan (4) has shown conclusively that it is the leaves which are the receptors of the photoperiodic stimulus.

As has been shown by Steinburg and Garner (37) many plants respond to thermoperiodicity by flowering. The study of the interrelatedness of C/N ratio, photoperiod and thermoperiodicity has proven very productive and has shown the three factors are closely related in their affect on floral development.

Hitchcock and Zimmerman (16) induced flowering by a chemical compound having the property of a growth regulator. Tobacco plants to which indole propionic acid was applied to the soil in an aqueous solution flowered much quicker than nontreated plants. These same workers (50) reported that an aqueous solution of 2,3,5-triiodobenzoic acid (TIBA) applied as a foliar spray would induce leafy shoots of tomatoes to grow flower clusters and cause the main shoot to terminate in a flower cluster. Clark and Kerns (6) induced flowering by spraying solutions of naphthaleneacetic acid on pineapple. Many investigators, stimulated by the work of Zimmerman and Hitchcock and Clark and Kerns, have tried various chemicals for their ability to induce flowering. To date, however, no systematic study of the effect of TIBA and its derivatives has been made on commercial greenhouse crops.

The purpose of this investigation was to study the effect of foliar applications of TIBA and its derivatives on asters (*Callistephus chinensis*), petunias (*Petunia hybrida*) and zinnias (*Zinnia elegans*).

REVIEW OF LITERATURE

Photoperiodism and Flowering

The literature on photoperiodism is very extensive and numerous excellent general review articles are available (13, 15, 29 and 17). There are in addition many reviews of more restricted scope. Parker and Borthwick (31) reported on work done with economic crops and found photoperiodic responses to include vegetative extension (onions), tuber formation (sugar beets) and changes in winter hardiness (sugarcane) as well as initiation of floral primordia and development of flowers, fruits and seeds. Hamner (15) reviewed investigations of the postulated hormone-photoperiod relationship. He concluded that a hormone or hormones may be involved in the flowering process of many plants; that, whatever this hormone is, its activity is not confined to the particular plant in which it is formed but may be transferred to other species and there result in flowering; and that the environmental conditions leading to its manufacture differ with different plants. Thus, in some plants the hormone seems to be manufactured under special photoperiodic conditions and in other plants under different, but still special, photoperiodic conditions.

A comprehensive symposium by Murneek and Whyte (29) summarizes the information available on vernalization and photoperiodism as they relate to floral induction. It is generally agreed that the photoperiodic induction of flowering is hormonally controlled, that the site of induction is in the leaves; and that flower induction is a phasic development.

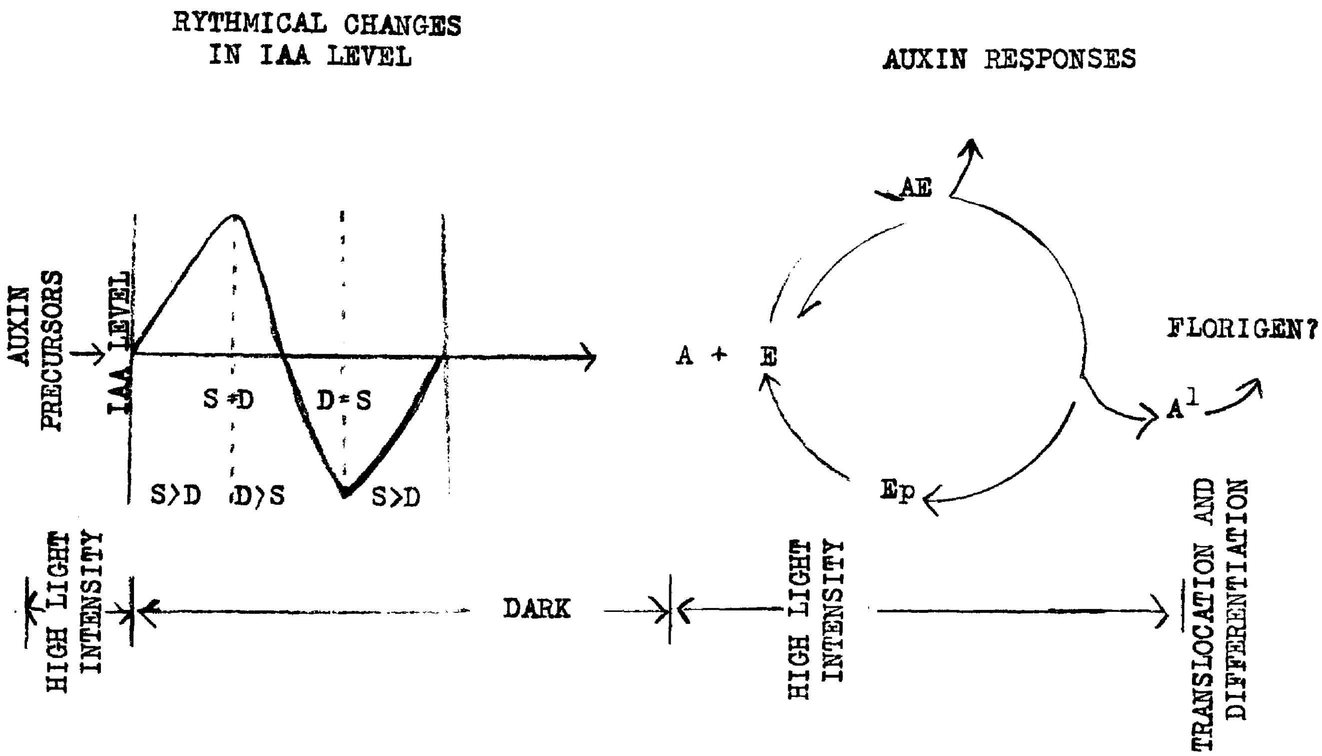
Growth Regulators and Flowering

A. Indoleacetic acid (IAA)

Two recent reviews on flowering have been prepared by Bonner and Liverman (2) and Liverman (24). Bonner and Liverman reviewed the literature on hormonal control of flower initiation. They subdivided the photoperiodic response of plants into a series of sequential partial or component processes and attempted to associate these processes with particular biochemical or physiological stages of the plant. These biochemical or physiological stages are believed to be controlled, at least in part, by auxins. With short-day plants in particular, photoperiodic induction may be associated with reduced levels of effective auxin in the plant and the interplay of light and dark in the photoperiodic process may be mediated, in part at least, through influences upon the auxin economy. Flowering in short-day plants is not a simple matter of auxin economy, however, and two or possibly more hormones of quite different chemical natures may be involved. It follows that the influence of auxin on flowering is not direct but appears rather to be exerted through control and regulation of the synthesis of a specific flowering hormone or hormones of unknown chemical nature. It is this material, the flowering hormone or hormones, and not the auxin, which has been studied by virtue of the transmission of the flowering stimulus through the plant and from plant to plant.

Investigation of flowering in long-day and day-neutral plants has shown the concept of regulation of flowering by auxin level to be of wide applicability.

Liverman (24) emphasized the importance of physiological factors on flowering. Essentially the same conclusions were reached as were reached by Bonner and Liverman (2). These conclusions are best shown graphically (Diagram I):



The high-intensity light process is characterized in part by the formation of auxin precursors. The net concentration of auxin (A) depends on the balance between its synthesis (S) and its destruction (D) by the adaptive formation and the de-adaptive disappearance of IAA oxidase. Auxin formed during the dark process combines with the auxin receptor (E) in light to form the growth-active auxin-receptor complex (AE). The complex may lead to growth responses or may be decomposed to form an auxin-nonreceptive entity (Ep) plus some compound (A^1). The auxin-nonreceptive entity (Ep) is again activated by red light to form (E). A^1 may participate in the biosynthesis of florigen.

Neither Bonner and Liverman (2) or Liverman (24) discuss or cite the work done on auxin treatment of seed which has resulted in hastening of flowering. Thimann and Lane (42) soaked tomato seed in an aqueous auxin solution and reported, under greenhouse conditions, three to seven days quicker flowering of plants from treated seed compared to plants from non-treated seeds. Stier and duBuy (39) dusted tomato seed with an auxin-talc mixture at time of sowing in quartz sand. After germination plant roots were dipped into solutions of naphthaleneacetic and indolebutyric acids and then transplanted into the field. Treated plants flowered three days earlier than nontreated plants. This hastening of flowering is not explicable by the interaction of auxin level and photoperiod hypothesized by Bonner and Liverman (2) and Liverman (24). A review of the literature on photoperiod has shown no reports of light or dark affecting seeds prior to germination. It is difficult, therefore, to see any relationship between auxin treated seed and flowering if flowering is controlled by photoperiodically induced changes in the auxin economy.

B. 2,3,5-Triiodobenzoic acid (TIBA)

A relationship between TIBA and flowering was first reported by Zimmerman and Hitchcock (50). They observed that application of a foliar spray of TIBA to tomato plants induced normally vegetative axillary buds to grow flower clusters. The terminal vegetative bud of the main axis was also induced to grow flower clusters. This discovery by Zimmerman and

Hitchcock stimulated considerable interest and they later (52) reported widespread confirmation of their initial observation of TIBA accelerating flowering in tomato plants.

On the other hand, Galston (8) reported that TIBA had no floralgeneic properties since it did not induce vegetative soybean plants to flower. However, it greatly augmented the flowering response due to photoperiodic induction.

It has been reported by Gorter (11) that the application of TIBA to the soil of Phaseolus vulgaris resulted in an increased number of flowers and fruit and an increase in the fresh weight of the fruit. In addition, early flowering was promoted. It was also found that the number of flowers on Lycopersicon esculentum was increased by soil application of TIBA.

C. Other Growth Regulators

The first large scale practical use of growth regulators in inducing flowering was made possible after Clark and Kerns (6) induced flowering of pineapples by sprays of naphthaleneacetic acid.

It has been reported that if naphthaleneacetic acid soaked tomato seeds were germinated at three degrees centigrade the numbers of days to flower was shortened when compared to nontreated plants (22).

Hamner and Bonner (14) placed vegetative cuttings of Xanthium pennsulvanicum in solutions of vitamins B_1, B_2, B_6, ascorbic acid, pantothenic acid, nicotinic acid, inositol, IAA, theelin and theelol to test their activity on floral initiation. All these substances when tested over a wide range of concentrations proved ineffective in promoting floral primordia.

Dormant Thompson seedless grape cuttings treated with a spray of the thiosemicarbazones of 2,4-dichlorobenzaldehyde, 3,4-dichlorobenzaldehyde, 4-chlorobenzaldehyde and phenylacetone were successfully induced to flower (33).

Growth Regulators and Morphology

A. Indoleacetic acid

The action of natural and synthetic growth regulators on morphological changes has been rather widely investigated (20,3,18,44). Kraus, Brown and Hammer (20) treated decapitated Red Kidney beans with IAA in lanolin. Different tissues varied in their response to IAA but induced or prolonged meristematic activity was the first reaction noted in all tissues. Epidermal cells underwent a few divisions in the radial plane. The cells of the cortical parenchyma enlarged and became meristematic. Nuclear division was greatly speeded up in epidermal cells and walls formed in all planes. The cells of the pericycle and parenchyma of primary phloem proliferated slightly while the parenchyma of the secondary phloem proliferated markedly. Numerous tumors and galls formed on the stem and leaves.

Similar results were obtained by Borthwick, Hamner and Parker (3) upon treating tomatoes in the above fashion.

B. 2,3,5-Triiodobenzoic Acid and Related Substituted Benzoic Acids

In general, both application of TIBA and related materials produce some degree of formative effects in most plants. Zimmerman and Hitchcock (50) reported that TIBA applied as a foliar spray to tomato plants induced shortening of internodes and malformed leaves. Also, TIBA applied as a foliar spray caused aberrations within soybean plants which resulted in shortening of internodes (8). Application of TIBA to bean leaves checked elongation of internodes, affected terminal and axillary bud growth and caused dwarfing and curling of leaves (21). When TIBA was applied in lanolin paste to decapitated bean plants, discoloration of tissue, necrosis of treated tissue, tumor formation and dwarfing resulted (47)

Zimmerman and Hitchcock (53) and Zeist and Koevoets (49) reported that TIBA and 2-chloro-3,5-diiodobenzoic acid when sprayed on Kalanchoe plants lead to fusing of opposite leaves, fusing of main axis and lateral shoots, increased leaf succulence and stoppage of main axis growth.

Application of TIBA to tomato leaves resulted in stoppage of longitudinal growth of root hairs whereas production of cell-wall material continued. The growing points of tomato plants treated with TIBA grew in a cone-shape. Correlated with this phenomenon was the inability of the plant to produce new leaves and the ability to produce flowers (12).

Rooting of Coleus blumei was inhibited by treatment of the cuttings with TIBA powder (36).

Flowers of tomato plants sprayed with TIBA were characterized by short to long, heavy peduncles and by fasciated, often abnormally large flowers. The flowers had circular ovaries (52).

Later, Zimmerman and Hitchcock (54) showed that a foliar application on tomato plants of 2,3,5-trichlorobenzoic acid and its aldehyde had similar but more severe formative effects than TIBA at the same concentration.

Ready, et. al. (34) reported that the application of 3-nitro-4-hydroxybenzoic acid, 3-nitro-4-methoxybenzoic acid, 3-nitro-4-ethoxybenzoic acid and 3-nitro-4-acetoxybenzoic acid to the soil caused albinism in emerging barley shoots. 3-Nitrobenzoic acid, 4-methoxybenzoic acid, 4-ethoxybenzoic acid, 4-acetoxybenzoic acid and 4-hydroxybenzoic acid were ineffective in causing albinism.

The effect of TIBA on morphogenesis of Lemna minor was studied by Wagerman and Lacey (46). They reported that fronds grown in nutrient solutions containing TIBA had a much more extensive vascular system than control fronds. A similar investigation was made by Wardlaw (46) who found that the application of a foliar spray of TIBA on tomato plants caused a cessation of growth in the most distal cells of the apex and retarded growth of the subjacent regions. As a result of this treatment apices of unusual configuration and organization were produced and some of these aspices gave rise to hollow or ring-fasciated shoots with a double vascular system. Trichlorobenzoic acid applied as a foliar spray on bean plants caused complete or partial suppression of apical growth and leaf formation, but greatly increased root formation.

Pillai and Chakrauarti (32) studied the effect of TIBA on leaf form of Brassica campastris and Lens esculenta and found that soaking mustard seed in TIBA solutions prior to germination caused coalescence of the leaves thus forming an ascidum. Treatment of Lens esculenta seed in the same manner induced shortening of the rachis and coalescence of the leaflets resulting in palmate leaves.

C. Other Growth Regulators

The morphological effects of the phenoxyacetic acids (particularly 2,4-dichlorophenoxyacetic acid) and naphthaleneacetic acids have been studied in detail. While these compounds show small differences from the response of IAA on beans and tomatoes (14), the overall response is similar. In general, those tissues that are least differentiated have the greatest potential for response, and with increasing degrees of differentiation, responsiveness declines (Leopold, 23).

Growth Regulators and Physiology

A. Indoleacetic Acid

Effects of IAA on the general physiology of the plant have been the subject of intensive investigation. Literature resulting from these investigations is voluminous and frequently contradictory. None of the literature read in preparation for this experiment offered conclusive experimental evidence linking an auxin induced physiological responses of intact plants to subsequent flowering.

B. 2,3,5-Triiodobenzoic Acid and Related Substituted Benzoic Acids

Zimmerman and Hitchcock (50) have concluded that TIBA does not cause immediate (within a few hours) cell elongation of test objects. The ultimate results of applying TIBA to plants resembled those of true hormones more than the indole and naphthalene compounds commonly referred to as auxins. Carroll (5), in studying the anti-auxin properties of TIBA in Avena tests, concluded there was a lag of four hours between the application of TIBA and its anti-auxin action. Galston (8) reported TIBA itself was without activity in Avena tests but its activity antagonized and negated the effects of IAA. Wangerman and Lacey (45) applied foliar sprays of TIBA to Lemna minor and reported no effect on length of life or rate of aging of the fronds. Lower auxin levels were found in treated fronds when compared to nontreated fronds.

Audus and Quastel (1) found that certain sulphonamides applied to the soil at 10 p.p.m. inhibited root growth of cress. p-Aminobenzoic acid solutions when applied to the soil lessened the inhibition. When the sulphonamides and p-aminobenzoic acid were added in equal concentrations root growth was normal.

C. Other Growth Regulators

Most of the work has been done with phenoxyacetic acids and naphthaleneacetic acids. Reported results are varied and do not permit pinpointing of the primary activity of these growth regulators. Secondary results are abnormal metabolism and abnormal growth patterns. There is strong evidence (Leopold, 23; Ready et. al., 34) of a relationship between these growth regulators and auxin levels.

Mode of Action

A. Indoleacetic Acid

An enzymatic mode of action for IAA in straight growth tests has been advanced by Bonner and Liverman (2). They show that IAA induced growth follows Michaelis-Menten kinetics. It is postulated that the carboxyl group and ortho ring carbon combine with a sulfhydryl group of an amino acid to form a complex. This complex dissociates to regenerate the sulfhydryl group and form products.

Little information is available on the mode of action of IAA on flowering. Bonner and Liverman (2) have attempted to correlate the observations of low auxin levels in flowering plants with photoperiod. Essentially this involved postulating the enzymatic combination of auxin with compounds photoperiodically induced in leaves, the dissociation of the complex, and formation of one or more flower inducing hormones.

B. 2,3,5-Triiodobenzoic Acid and related Substituted Benzoic Acids

Muir and Hansch (27) have postulated an anti-auxin mode of action for TIBA on flowering. They said the following structural requirements must be met for activity as growth regulators:

1. The ortho position must be substituted with an electronegative group capable of displacement by an electron-rich substrate.
2. If only meta and/or para positions are substituted the compound will be inactive.
3. If the ortho position is substituted with an electron-

positive group the compound will be inactive.

It is thought that any substituted benzoic acid possessing the necessary structural requirements will combine with the same substrate as IAA. Failure to meet these requirements gives TIBA its commonly ascribed anti-auxin action.

C. Other Growth Regulators

The mode of action of these compounds has been the subject of intensive investigation. It is thought their activity depends on a structural resemblence to IAA which permits interference with IAA catalysed reactions. The structural requirements necessary are a free or potentially free carboxyl group and a free or potentially free position having a spatial relation to the carboxyl group which permits their being linked by a sulfhydryl group. Several recent reviews on this subject are by Muir and Hansch (28) and Gordon (10).

MATERIALS AND METHODS

In order to determine the effectiveness of various substituted benzoic acids as flower inducing agents a variety of floricultural crops were grown under greenhouse conditions prevailing at East Lansing, Michigan, during Fall, Winter and Spring months of 1955-56.

Seeds of Zinnia[1] (Zinnia elegans) variety Enchantress, aster[1] (Callistephus chinesis) variety Queen of the Market Azure Blue, and petunia[2] (Petunia hybrida) variety Dream Girl, were planted in sterilized flats and later transplanted to 3-inch sterilized pots.

The chemicals used in these experiments were obtained from various sources (Table I). The solutions were prepared, first, dissolving these compounds in a minimum amount of 95 percent alcohol (usually 0.05 gram/10 ml.) and made to volume with distilled water. Concentrations of 100 and 1000 p.p.m. with a small amount of wetting agent (Dreft) were employed for all the chemicals, listed in Table 1, and foliar applications were made at a stage when the first true leaves were one-half expanded.

In another series of trials, the relative efficacy of the various chemicals was determined in relation to its pH value. Solutions of 100 p.p.m. concentrations were adjusted to 3,4,5,6,7,8 and 9 pH by 0.5 N KOH and 0.5 N HCl on a Beckman model H-2 line operated, pH meter. Later on, these solutions were used as foliar sprays.

Since modifications of soil reactions has a great effect on plant growth it was thought advisable to include the effect of soil applications of chemicals, substituted benzoic acid group as given in Table I, on plant

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Seeds obtained from (1) Vaughans and (2) Harris Seed Company

TABLE I

LIST OF THE CHEMICALS WITH SOURCE OF ORIGIN USED IN VARIOUS EXPERIMENTS FOR FLOWER INDUCTION STUDIES

o-Iodobenzoic acid	Eastman Chemical Company	2,5-Dichlorophenoxyacetic acid	Dow Chemical Company
p-Iodobenzoic acid	" " "	3,5-Dinitrobenzoic acid	" " "
2,3,5-Triiodobenzoic acid	" " "	m-Nitrobenzoic acid	" " "
2,4-Dihydroxybenzoic acid	" " "	o-Bromobenzoic acid	" " "
2,5-Dihydroxybenzoic acid	" " "	m-Bromobenzoic acid	" " "
3,5-Diiodosalicylic acid	" " "	o-Chlorobenzoic acid	" " "
p-Aminobenzoic acid	General Biochemicals, Inc	m-Chlorobenzoic acid	" " "
2,3-Diiodobenzoic acid	Dr. Leaper of Pineapple Research Institute,T.H	3,4-Dichlorobenzoic acid	" " "
		2,5-Dichlorobenzoic acid	" " "
2,5-Diiodobenzoic acid	" " "	3,5-Dinitrosalicylic acid	" " "
3,5-Diiodobenzoic acid	" " "	3,5-Diiodosalicylic acid	" " "
2-Iodo-3-nitrobenzoic acid	" " "	3-Bromosalicylic acid	" " "
2-Amino-5-iodobenzoic acid	" " "	5-Bromosalicylic acid	" " "
2-Amino-3,5-diiodobenzoic acid	" " "	3,5-Dibromosalicylic acid	" " "
2-Amino-3,5-dibromobenzoic acid	" " "	3-Chlorosalicylic acid	" " "
2,3,5-Tribromobenzoic acid	" " "	5-Chlorosalicylic acid	" " "
2,3,5-Trichlorobenzoic acid	B. F. Goodrich	3,5-Dichlorosalicylic acid	" " "

growth behavior. With a view to evaluate the relative effectiveness of some of these compounds, as a flower inducing agents, 10 ml of 50 p.p.m. solutions were applied to soil daily, for 62 days, in 3-inch pot with a seedling, at a stage, when it had fully expanded cotyledonary leaves; care was taken to avoid direct contact of solutions with the plant tissue.

All these experiments were arranged in a randomized block with 3 replications of four plants in each treatment. Greenhouse temperatures were adjusted to 50°F., during night, and about 70°F. for day time.

Plants were observed daily for any significant morphological changes. However, when the first flower on a plant reached anthesis then that plant was removed, carefully, in such a way that the roots were intact. Data were recorded for the number of flowers, buds, axillary shoots and the number of nodes to the first flower (in zinnia and aster) and for dry weight of roots and tops. The statistical treatment of the data is given in Tables II - XXII.

EXPERIMENTAL RESULTS

EFFECT OF DIFFERENT CONCENTRATIONS OF SUBSTITUTED BENZOIC ACIDS WHEN SPRAYED ON *PETUNIA HYBRIDA*

According to the results presented in Table II it is clear that chemical treatments, in petunia, did not affect the synthesis of dry matter in the aerial portion of the plants to any extent. However, results were different in the case of dry weight of roots, which indicated highly significant influence of the treatments (Table III). It is interesting to note that first order interactions, between chemicals and concentrations, were also significant. On the other hand, differences in dry weight of roots due to 100 and 1000 p.p.m. concentrations treatments, when considered, disregarding the nature of the chemicals, did not show any significance.

The data, Table III, indicated that trihalogenated acids were among the most active chemicals inhibiting root growth of petunia, followed by the substituted salicylic acids, 2,5-dichlorophenoxyacetic acid and the di and mono-substituted benzoic acids. In general, it can be stated from the observations that when the ortho position was halogenated, the compound inhibited root growth to a greater degree than did its isomers. It can be seen further (Table III)that compounds with an electro-negative group substituted in the ortho position were the most active chemicals in inhibiting root growth.

Top/root ratios of *Petunia hybrida*, as expressed on a dry weight basis, Table IV, also indicated a high signicance in favor of certain chemical treatments, concentrations and their interactions. However, the degree of biological activity, as measured by this ratio, did not establish the superiority of one chemical structure over the other.

TABLE II

EFFECT OF SUBSTITUTED BENZOIC ACIDS ON DRY WEIGHT OF *PETUNIA HYBRIDA* TOPS*

Chemical	Grams Dry Weight 100 p.p.m.	Grams Dry Weight 1000 p.p.m.
Check	1.56	1.56
2,3,5-triiodobenzoic acid	1.25	1.37
2,3,5-tribromobenzoic acid	2.56	1.41
2,3,5-trichlorobenzoic acid	1.90	0.69
p-iodobenzoic acid	1.73	2.14
o-iodobenzoic acid	2.69	1.48
m-iodobenzoic acid	2.46	2.11
2,3-diiodobenzoic acid	2.54	2.40
2,5-diiodobenzoic acid	1.52	3.08
3,5-diiodobenzoic acid	3.35	2.67
2-iodo-3-nitrobenzoic acid	3.54	1.54
p-aminobenzoic acid	2.09	2.36
2-amino-3-iodobenzoic acid	2.88	2.48
2-amino-3,5-diiodobenzoic acid	3.12	2.92
2-amino-3,5-dibromobenzoic acid	3.33	1.79
2,4-dihydroxybenzoic acid	4.13	3.31
2,5-dihydroxybenzoic acid	2.21	2.71
3,5-diiodosalicylic acid	3.43	2.52
3-bromosalicylic acid	3.13	2.54
3,5-dibromosalicylic acid	2.23	1.58
3,5-dichlorosalicylic acid	2.09	1.97
2,5-dichlorophenoxyacetic acid	1.89	1.61

L.S.D. 5% = 3.15
1% = 3.94

*Average of three replicates of four plants each

TABLE III

EFFECT OF SUBSTITUTED BENZOIC ACIDS

ON DRY WEIGHT OF PETUNIA HYBRIDA ROOTS*

Chemical	Grams Dry Weight 100 p.p.m.	1000 p.p.m.
Check	1.15	1.15
2,3,5-triiodobenzoic acid	0.53	0.48
2,3,5-tribromobenzoic acid	0.71	0.44
2,3,5-trichlorobenzoic acid	0.48	0.33
p-iodobenzoic acid	0.56	0.71
o-iodobenzoic acid	0.75	0.71
m-iodobenzoic acid	0.71	0.72
2,3-diiodobenzoic acid	0.94	0.87
2,5-diiodobenzoic acid	0.77	0.73
3,5-diiodobenzoic acid	0.98	0.73
p-aminobenzoic acid	0.96	0.71
2-amino-5-iodobenzoic acid	0.61	0.67
2-amino-3,5-diiodobenzoic acid	0.77	0.83
2-amino-3,5-dibromobenzoic acid	0.71	0.77
2,4-dihydroxybenzoic acid	0.90	0.67
2,5-dihydroxybenzoic acid	0.92	1.04
3,5-diiodosalicylic acid	1.88	0.63
3-bromosalicylic acid	0.54	1.15
3,5-dibromosalicylic acid	1.38	0.65
3,5-dichlorosalicylic acid	0.46	0.57
2,5-dichlorophenoxyacetic acid	0.52	0.71
2-iodo-3-nitrobenzoic acid	0.81	0.71

L.S.D. 5% = 0.006
1% = 0.008

*Average of three replicates of four plants each

TABLE IV

EFFECT OF SUBSTITUTED BENZOIC ACIDS ON RATIO

OF DRY WEIGHT OF PETUNIA HYBRIDA

TOP/ROOT*

Chemical	Dry Weight 100 p.p.m.	1000 p.p.m.
Check	1.23	1.57
2,3,5-triiodobenzoic acid	4.67	2.92
2,3,5-tribromobenzoic acid	4.57	3.46
2,3,5-trichlorobenzoic acid	4.21	2.13
p-iodobenzoic acid	3.44	3.58
o-iodobenzoic acid	4.21	2.90
m-iodobenzoic acid	3.04	3.99
2,3-diiodobenzoic acid	3.65	3.89
2,5-diiodobenzoic acid	4.05	4.09
3,5-diiodobenzoic acid	3.51	5.45
2-iodo-3-nitrobenzoic acid	8.20	3.24
p-aminobenzoic acid	2.91	3.30
2-amino-5-iodobenzoic acid	6.54	3.30
2-amino-3,5-diiodobenzoic acid	6.04	6.24
2-amino-3,5-dibromobenzoic acid	3.78	2.65
2,4-dihydroxybenzoic acid	5.14	6.73
2,5-dihydroxybenzoic acid	3.45	3.64
3,5-diiodosalicylic acid	3.88	7.23
3-bromosalicylic acid	7.76	6.48
3,5-dibromosalicylic acid	5.53	3.30
3,5-dichlorosalicylic acid	4.59	4.28
2,5-dichlorophenoxyacetic acid	3.59	3.09

L.S.D. 5% = 0.60
1% = 0.79

*Average of three replicates of four plants each

The chemical treatments influenced the number of flowers and buds on petunia plants (Table V). Differences due to concentrations and its interaction with chemicals were, also, highly significant. All compounds, with the exception of 2,3,5-trichlorobenzoic acid and 2,5-dichlorophenoxyacetic acid, significantly increased floral initiation. In general conclusion, it can be pointed out that concentrations of the chemicals at 100 p.p.m. were much more effective in promoting floral initiation than 1000 p.p.m. Results indicated that the more electro-positive the ortho substitution of the chemical the more effective the chemical was in promoting floral initiation. The action of the chemical was enhanced when an electro-negative group was ortho to the electro-positive group.

Observations for the number of axillary shoots produced indicated that magnitude of this phase of biological activity was influenced by the rate and nature of the chemical used (Table VI). Accordingly, plants treated with 100 p.p.m developed more axillary shoots than plants treated with 1000 p.p.m. Differences in plant growth behavior, when analyzed in terms of chemical activity, followed the same general trend as expressed previously for the initiation of increased number of flowers and buds. That is, the more electro-positive ortho substituted compounds produced more axillary shoots than the electro-negative ortho substituted compounds; the effect was enhanced by an electro-negative group ortho to the electro-positive group. All plants except those sprayed with 2,3,5-trichlorobenzoic acid and 2,5-dichlorophenoxyacetic acid had more axillary shoots than plants receiving no treatment.

The chemical treatments and the concentrations of the applications affected the petunia plants in such a manner that the flowering phenomena

TABLE V

EFFECT OF SUBSTITUTED BENZOIC ACIDS ON FLORAL INITIATION OF *PETUNIA HYBRIDA**

Chemical	Number of Flowers 100 p.p.m.	1000 p.p.m.
Check	4.33	4.33
2,3,5-triiodobenzoic acid	4.83	4.08
2,3,5-tribromobenzoic acid	8.00	4.50
2,3,5-trichlorobenzoic acid	4.67	1.50
p-iodobenzoic acid	6.58	5.33
o-iodobenzoic acid	6.50	3.92
m-iodobenzoic acid	5.92	4.69
2,3-diiodobenzoic acid	7.00	5.83
2,5-diiodobenzoic acid	6.17	7.17
3,5-diiodobenzoic acid	5.83	7.08
2-iodo-3-nitrobenzoic acid	9.83	4.17
p-aminobenzoic acid	6.65	7.58
2-amino-5-iodobenzoic acid	8.33	6.33
2-amino-3,5-diiodobenzoic acid	9.17	8.42
2-amino-3,5-dibromobenzoic acid	9.35	6.92
2,4-dihydroxybenzoic acid	12.00	10.75
2,5-dihydroxybenzoic acid	5.17	5.25
3,5-diiodosalicylic acid	6.83	7.08
3-bromosalicylic acid	8.17	6.17
3,5-dibromosalicylic acid	5.00	3.83
3,5-dichlorosalicylic acid	5.25	3.58
2,5-dichlorophenoxyacetic acid	4.92	2.83

L.S.D. 5% = 0.88
       1% = 1.16

*Average of three replicates of four plants each

TABLE VI

EFFECT OF SUBSTITUTED BENZOIC ACIDS

ON NUMBER OF AXILLARY SHOOTS OF *PETUNIA HYBRIDA**

Chemicals	Number of axillary shoots 100 p.p.m.	1000 p.p.m.
Check	4.60	4.50
2,3,5-triiodobenzoic acid	4.08	4.50
2,3,5-tribromobenzoic acid	6.50	4.66
2,3,5-trichlorobenzoic acid	5.92	3.75
p-iodobenzoic acid	6.25	6.00
o-iodobenzoic acid	6.92	4.50
m-iodobenzoic acid	6.25	5.08
2,3-diiodobenzoic acid	6.50	5.58
2,5-diiodobenzoic acid	6.92	6.83
3,5-diiodobenzoic acid	6.00	6.42
2-iodo-3-nitrobenzoic acid	7.25	5.58
p-aminobenzoic acid	5.42	6.08
2-amino-5-iodobenzoic acid	7.58	6.25
2-amino-3,5-diiodobenzoic acid	7.66	7.08
2-amino-3,5-dibromobenzoic acid	7.78	6.42
2,4-dihydroxybenzoic acid	9.25	1.50
2,5-dihydroxybenzoic acid	6.17	6.33
3,5-diiodosalicylic acid	5.83	5.66
3-bromosalicylic acid	7.75	6.66
3,5-dibromosalicylic acid	5.66	5.00
3.5-dichlorosalicylic acid	5.83	5.08
2,5-dichlorophenoxyacetic acid	4.50	4.42

L.S.D. 5% = 0.60
1% = 0.80

*Average of three replicates of four plants each

was influenced. There were highly significant variation in the number of days to flower (Table VII). However, there appeared to be no correlation between structure of the chemical compound and their ability to either hasten or delay flowering. Observations seemed to indicate that ortho substitution tended to cause more delay in flowering at 1000 p.p.m. than meta or para substitutions at the same concentration.

No teratological changes in Petunia hybrida were observed with any of the chemical treatments. Morphological changes were limited to the formation of succulent leaves and more leaf hairs on plants sprayed with ortho electro-positive substituted benzoic acids. When Petunia hybrida was treated with 100 p.p.m. of either 2,3,5-trichlorobenzoic acid or 2,5-dichlorophenoxyacetic acid, a slight to severe epinasty occurred within twelve hours and persisted for two to six days after treatment. Extreme epinasty and dwarfing resulted when plants were treated with the above chemicals at concentrations of 1000 p.p.m.

TABLE VII

EFFECT OF SUBSTITUTED BENZOIC ACIDS

ON DAYS TO FLOWER IN PETUNIA HYBRIDA*

Chemical	Days to Flower 100 p.p.m.	1000 p.p.m.
Check	89.33	89.25
2,3,5-triiodobenzoic acid	88.08	88.33
2,3,5-tribromobenzoic acid	91.00	89.58
2,3,5-trichlorobenzoic acid	89.58	92.66
p-iodobenzoic acid	93.58	92.83
o-iodobenzoic acid	89.41	89.75
m-iodobenzoic acid	80.00	78.58
2,3-diiodobenzoic acid	87.66	89.25
2,5-diiodobenzoic acid	89.08	89.16
3,5-diiodobenzoic acid	88.33	89.00
2-iodo-3-nitrobenzoic acid	91.41	91.50
p-aminobenzoic acid	88.58	86.83
2-amino-5-iodobenzoic acid	89.33	84.41
2-amino-3,5-diiodobenzoic acid	91.66	91.75
2-amino-3,5-dibromobenzoic acid	90.75	84.08
2,4-dihydroxybenzoic acid	91.16	92.25
2,5-dihydroxybenzoic acid	87.25	86.08
3,5-diiodosalicylic acid	92.91	90.58
3-bromosalicylic acid	85.83	80.41
3,5-dibromosalicylic acid	82.75	83.58
3,5-dichlorosalicylic acid	87.00	85.66
2,5-dichlorophenoxyacetic acid	79.75	84.41

L.S.D. 5% = 1.15
1% = 1.53

*Average of three replicates of four plants each

EFFECT OF DIFFERENT ACIDITY LEVELS OF SUBSTITUTED BENZOIC ACIDS WHEN SPRAYED ON PETUNIA HYBRIDA

The synthesis of dry matter in aerial portions of Petunia hybrida was highly significantly affected by the chemical treatments (Table VIII). There were no significant affects of pH nor pH-chemical interactions. With the exception of 2,3,-diiodobenzoic and 3,5-diiodosalicylic acid there was no significant difference between the chemicals in their effect on top growth. There was a significant difference between the chemical treated plants and controls. All chemical treatments resulted in reduced synthesis of dry matter as compared to control plants.

There were highly significant correlations between dry weight in grams of Petunia hybrida roots and chemical treatments but no correlations between dry root weight and pH or chemical-pH interactions (Table IX). No significant differences were noted between chemicals in their action on root growth and all chemicals reduced root growth as compared to control plants.

Highly significant correlations were noted between the dry weight of top/root ratio of Petunia hybrida and chemical treatments, pH and pH-chemical interactions (Table X). All chemicals substituted in the para position affected plants more than plants treated with non-para substituted chemicals. All chemicals applied to plants in solutions at pH 6 affected subsequent growth and produced the lowest value for top/root ratio (Figure 1). The highest ratio was obtained at pH 4 with a slightly smaller ratio at pH 8.

Highly significant correlations were observed between chemical treatments and floral initiation and significant correlations between pH and

Figure 1

Effect of pH of substituted Benzoic Acids on Top/Root Ratio Dry Weight of *Petunia Hybrida*

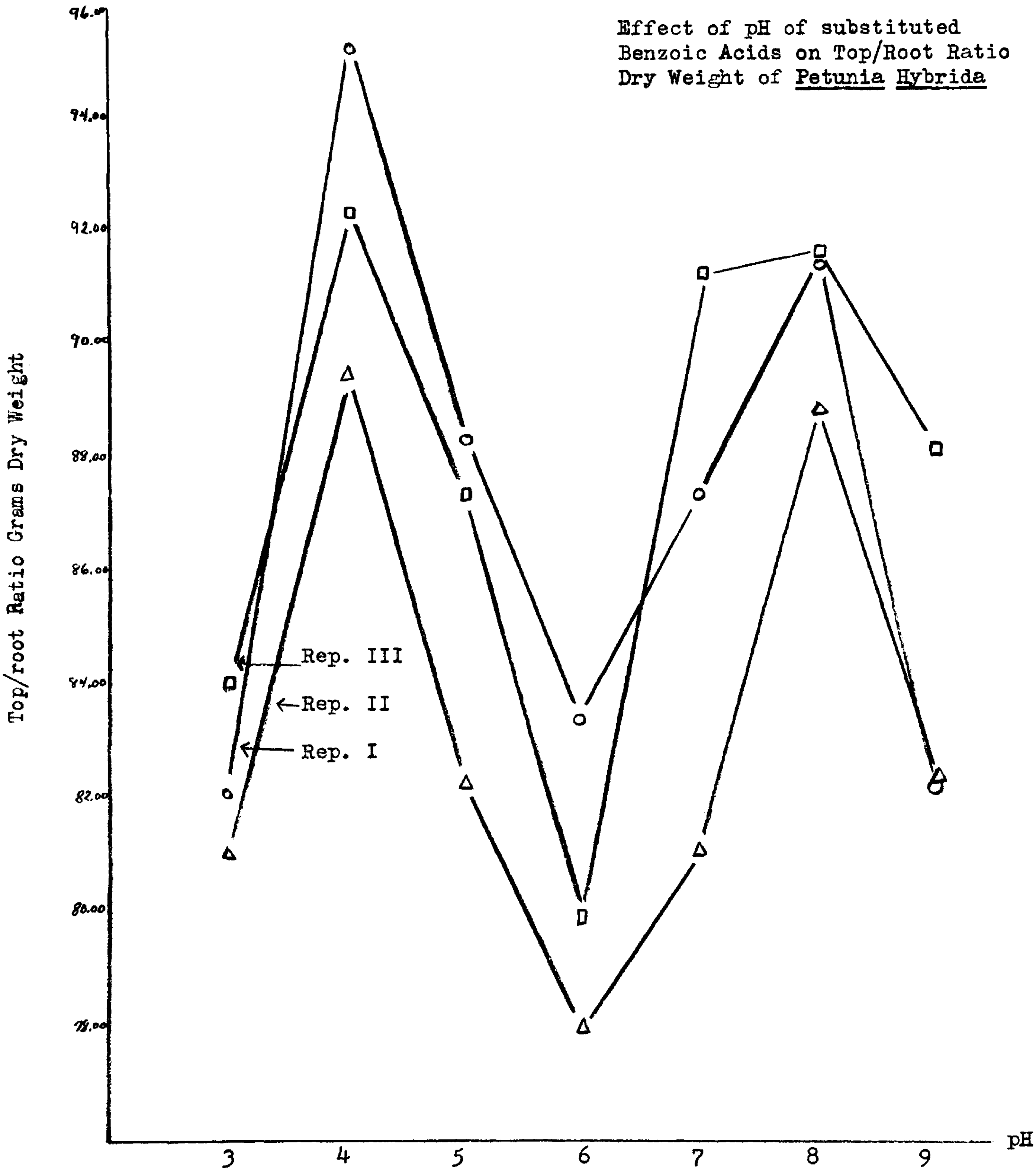


TABLE VIII

EFFECT OF SUBSTITUTED BENZOIC ACIDS AT 100 P.P.M. CONCENTRATION ON DRY WEIGHT OF *PETUNIA HYBRIDA* TOPS*

Chemical	Grams Dry Weight
Check	19.01
2,3,5-triiodobenzoic acid	12.53
2,3,5-tribromobenzoic acid	13.60
2,3,5-trichlorobenzoic acid	14.07
o-iodobenzoic acid	15.37
m-iodobenzoic acid	16.48
p-iodobenzoic acid	15.78
2,3,-diiodobenzoic acid	19.50
2,5-diiodobenzoic acid	16.23
3,5-diiodobenzoic acid	16.03
2-iodo-3-nitrobenzoic acid	13.99
2-amino-5-iodobenzoic acid	16.26
2-amino-3,5-diiodobenzoic acid	16.16
3,5-diiodosalicylic acid	17.83

L.S.D. 5% = 3.94
1% = 5.20

*Average of three replicates of twenty-eight plants each

TABLE IX

EFFECT OF SUBSTITUTED BENZOIC ACIDS

AT 100 P.P.M. CONCENTRATION ON DRY WEIGHT OF

PETUNIA HYBRIDA ROOTS*

Chemical	Grams Dry Weight
Check	8.32
2,3,5-triiodobenzoic acid	2.80
2,3,5-tribromobenzoic acid	3.38
2,3,5-trichlorobenzoic acid	2.61
o-iodobenzoic acid	3.08
m-iodobenzoic acid	3.26
p-iodobenzoic acid	2.53
2,3-diiodobenzoic acid	3.07
2,5-diiodobenzoic acid	3.05
3,5-diiodobenzoic acid	2.68
2-iodo-3-nitrobenzoic acid	3.01
2-amino-5-iodobenzoic acid	3.31
2-amino-3,5-diiodobenzoic acid	2.86
3,5-diiodosalicylic acid	2.93

L.S.D. 5% = 2.84
1% = 3.74

*Average of three replicates of twenty-eight plants each

TABLE X

EFFECT OF SUBSTITUTED BENZOIC ACIDS

AT 100 P.P.M. CONCENTRATION ON TOP/ROOT DRY WEIGHT RATIO

OF <u>PETUNIA HYBRIDA</u>*

Chemical	Ratio Grams Dry Weight Top/Root
Check	33.86
2,3,5-triiodobenzoic acid	38.16
2,3,5-tribromobenzoic acid	34.26
2,3,5-trichlorobenzoic acid	45.36
o-iodobenzoic acid	41.53
m-iodobenzoic acid	40.51
p-iodobenzoic acid	52.07
2,3-diodobenzoic acid	48.85
2,5-diiodobenzoic acid	42.84
3,5-diiodobenzoic acid	49.43
2-iodo-3-nitrobenzoic acid	39.55
2-amino-5-iodobenzoic acid	43.99
2-amino-3,5-diiodobenzoic acid	44.68
3,5-diiodosalicylic acid	45.00

L.S.D. 5% = 0.59
1% = 0.78

*Average of three replicates of twenty-eight plants each

floral initiation (Table XI). There were no correlations between pH-chemical interactions and floral initiation. Any relationships between chemical structure and activity (as judged by the number of floral initials) were not evident. Plants sprayed with chemicals in solution at pH7 resulted in the lowest increase of floral initials. Treatment with solutions at pH 9 resulted in the greatest production of floral initials in Petunia hybrida. Plants treated with chemicals at pH 4 or 5 produced an intermediate increase of floral initials in Petunia hybrida. Plants treated with chemicals at pH 4 or 5 produced an intermediate increase of floral initials (Figure 2).

The number of axillary buds on Petunia hybrida were highly significantly correlated with the various chemical treatments and pH but no significant differences with pH-chemical interactions were recorded (Table XII). There appeared to be no correlation between chemical structure and ability to either inhibit or enhance the axillary shoot production of Petunia hybrida. The least number of axillary shoots was obtained on plants sprayed with solutions at pH 6 (Figure 3), and the greatest number of axillary shoots was noted on plants treated with solutions at pH 9.

There were highly significant correlations between days to flower and chemical treatments in Petunia hybrida, (Table XIII), as well as significant correlations between days to flower and pH (Table XIII). No correlations between days to flower and pH-chemical interactions were observed. In general, compounds with a para substitution (either alone or in conjunction with other position substitutions) were the least effective in hastening flowering. Ortho-meta substitutions had the greatest affect in accelerating flowering. As far as acidity levels were concerned, greatest

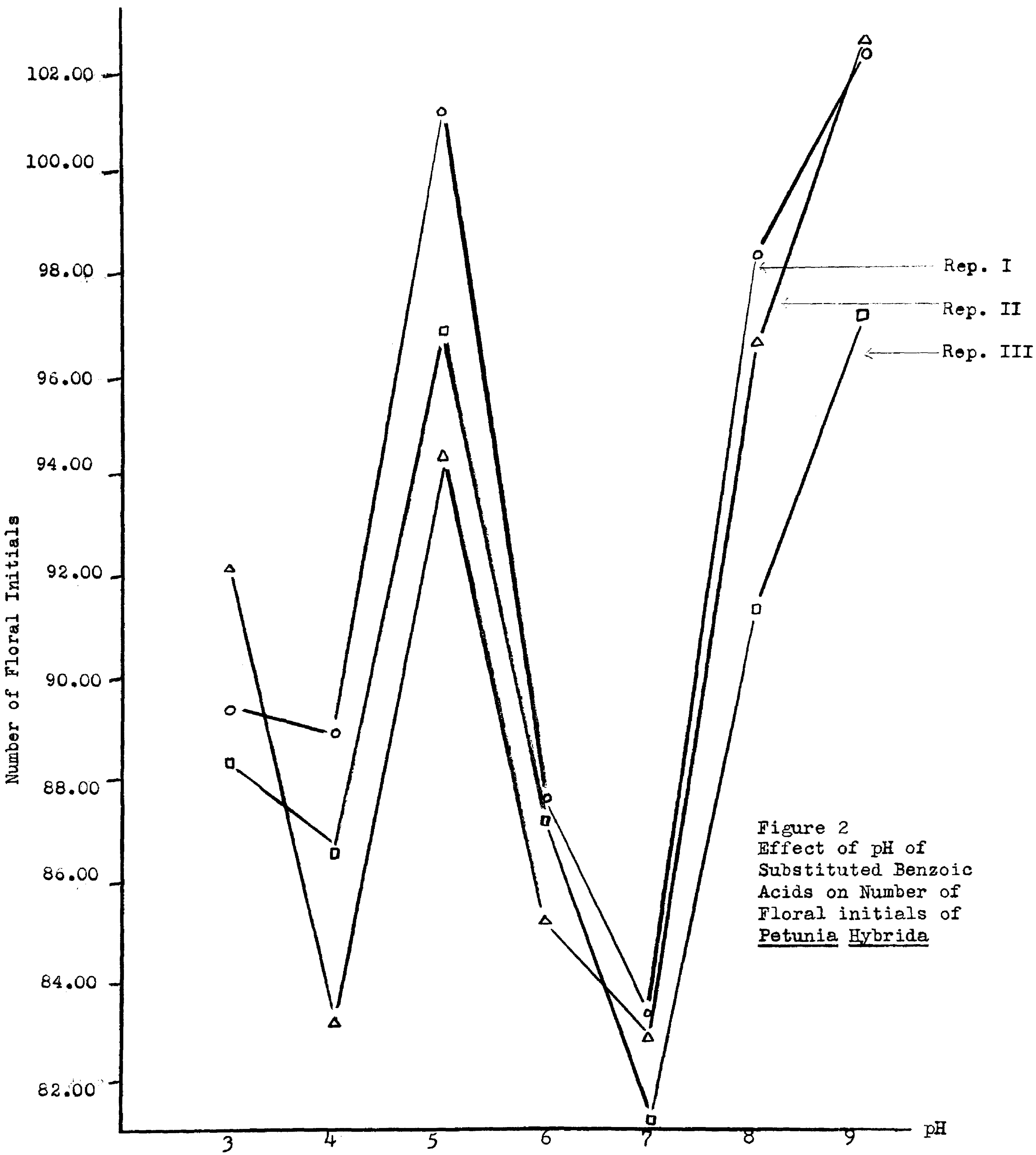


Figure 2
Effect of pH of Substituted Benzoic Acids on Number of Floral initials of <u>Petunia Hybrida</u>

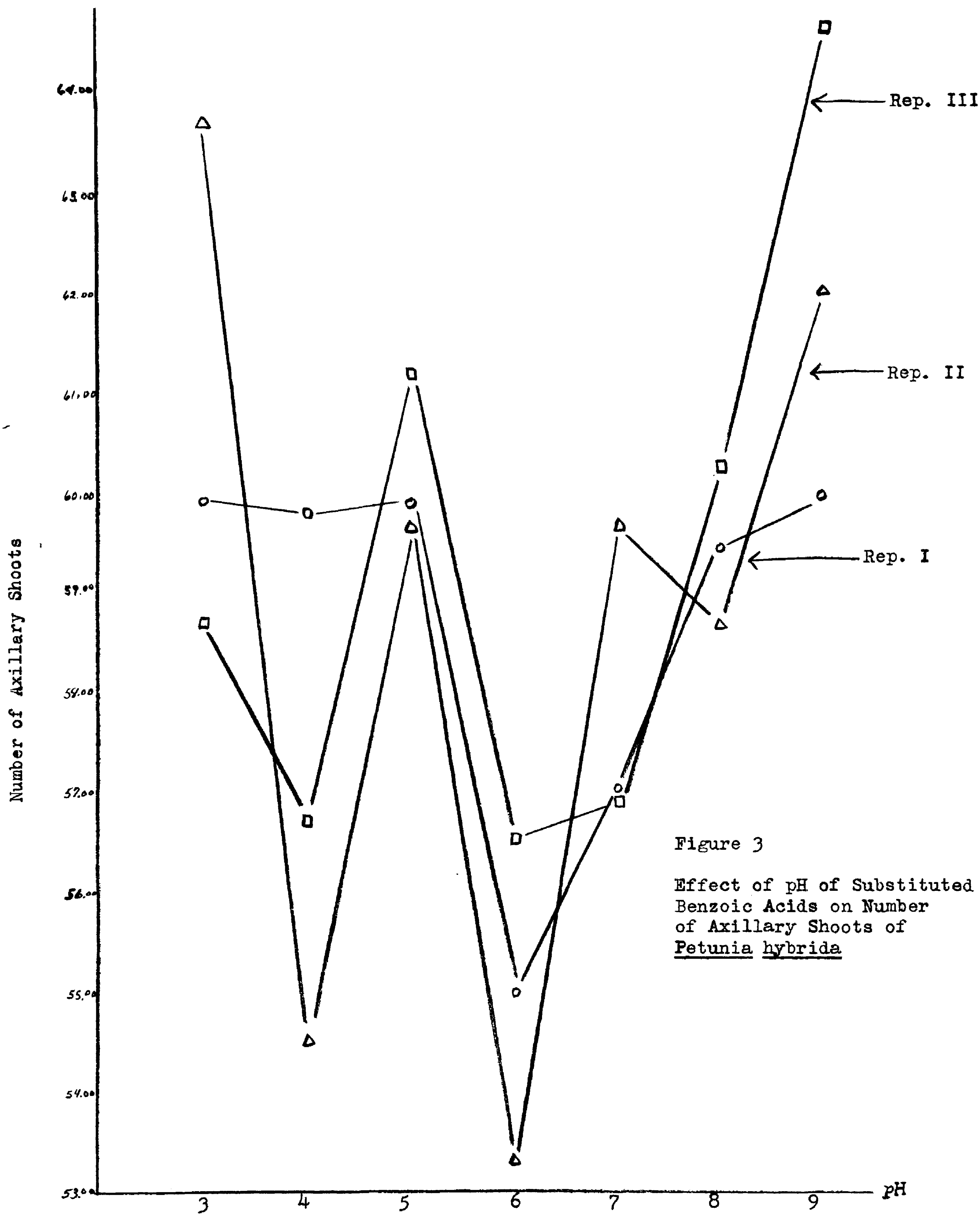


Figure 3

Effect of pH of Substituted Benzoic Acids on Number of Axillary Shoots of Petunia hybrida

TABLE XI

EFFECT OF SUBSTITUTED BENZOIC ACIDS AT 100 P.P.M. CONCENTRATION ON THE NUMBER OF FLORAL INITIALS OF *PETUNIA HYBRIDA**

Chemical	Number of Floral Initials
Check	31.92
2,3,5-triiodobenzoic acid	40.65
2,3,5-tribromobenzoic acid	43.83
2,3,5-trichlorobenzoic acid	49.50
o-iodobenzoic acid	40.33
m-iodobenzoic acid	51.00
p-iodobenzoic acid	52.25
2,3-diiodobenzoic acid	52.83
2,5-diiodobenzoic acid	43.33
3,5-diiodobenzoic acid	43.25
2-iodo-3-nitrobenzoic acid	44.83
2-amino-5-iodobenzoic acid	49.50
2-amino-3,5-diiodobenzoic acid	47.92
3,5-diiodosalicylic acid	47.17

L.S.D. 5% = 0.95
1% = 1.25

*Average of three replicates of twenty-eight plants each

TABLE XII

EFFECT OF SUBSTITUTED BENZOIC ACIDS AT 100 P.P.M. CONCENTRATION ON THE NUMBER OF AXILLARY SHOOTS OF *PETUNIA HYBRIDA**

Chemical	Number of Axillary Shoots
Check	31.77
2,3,5-triiodobenzoic acid	23.98
2,3,5-tribromobenzoic acid	26.15
2,3,5-trichlorobenzoic acid	27.50
o-iodobenzoic acid	28.67
m-iodobenzoic acid	31.58
p-iodobenzoic acid	29.08
2,3-diiodobenzoic acid	36.33
2,5-diiodobenzoic acid	27.75
3,5-diiodobenzoic acid	30.33
2-iodo-3-nitrobenzoic acid	24.50
2-amino-5-iodobenzoic acid	31.50
2-amino-3,5-diiodobenzoic acid	29.92
3,5-diiodosalicylic acid	32.42

L.S.D. 5% = 0.59
1% = 0.78

*Average of three replicates of twenty-eight plants each

TABLE XIII

EFFECT OF SUBSTITUTED BENZOIC ACIDS AT 100 P.P.M. CONCENTRATION ON DAYS TO FLOWER OF *PETUNIA HYBRIDA**

Chemical	Days to Flower
Check	122.30
2,3,5-triiodobenzoic acid	82.42
2,3,5-tribromobenzoic acid	86.70
2,3,5-trichlorobenzoic acid	83.50
o-iodobenzoic acid	89.51
m-iodobenzoic acid	81.45
p-iodobenzoic acid	93.94
2,3-diiodobenzoic acid	83.29
2,5-diiodobenzoic acid	89.03
3,5-diiodobenzoic acid	93.00
2-iodo-3-nitrobenzoic acid	87.10
2-amino-5-iodobenzoic acid	88.85
2-amino-3,5-diiodobenzoic acid	92.26
3,5-diiodosalicylic acid	89.62

L.S.D. 5% = 2.92
1% = 3.84

*Average of three replicates of twenty-eight plants each

acceleration of flowering occurred when plants were treated with solutions on pH 7 and least acceleration with solutions of pH 4 (Figure 4).

Acidity levels apparently did not affect the chemical in inducing morphological changes in Petunia hybrida. All morphological changes differing from control plants were previously noted and recorded in the section under concentration affects. These changes were increased leaf succulence and increased leaf hairs on all plants treated with electro-positive ortho substituted benzoic acids. There was, in addition, slight to severe epinasty and slight to severe dwarfing of plants sprayed with 2,3,5-trichlorobenzoic acid and 2,3,5-triiodobenzoic acid.

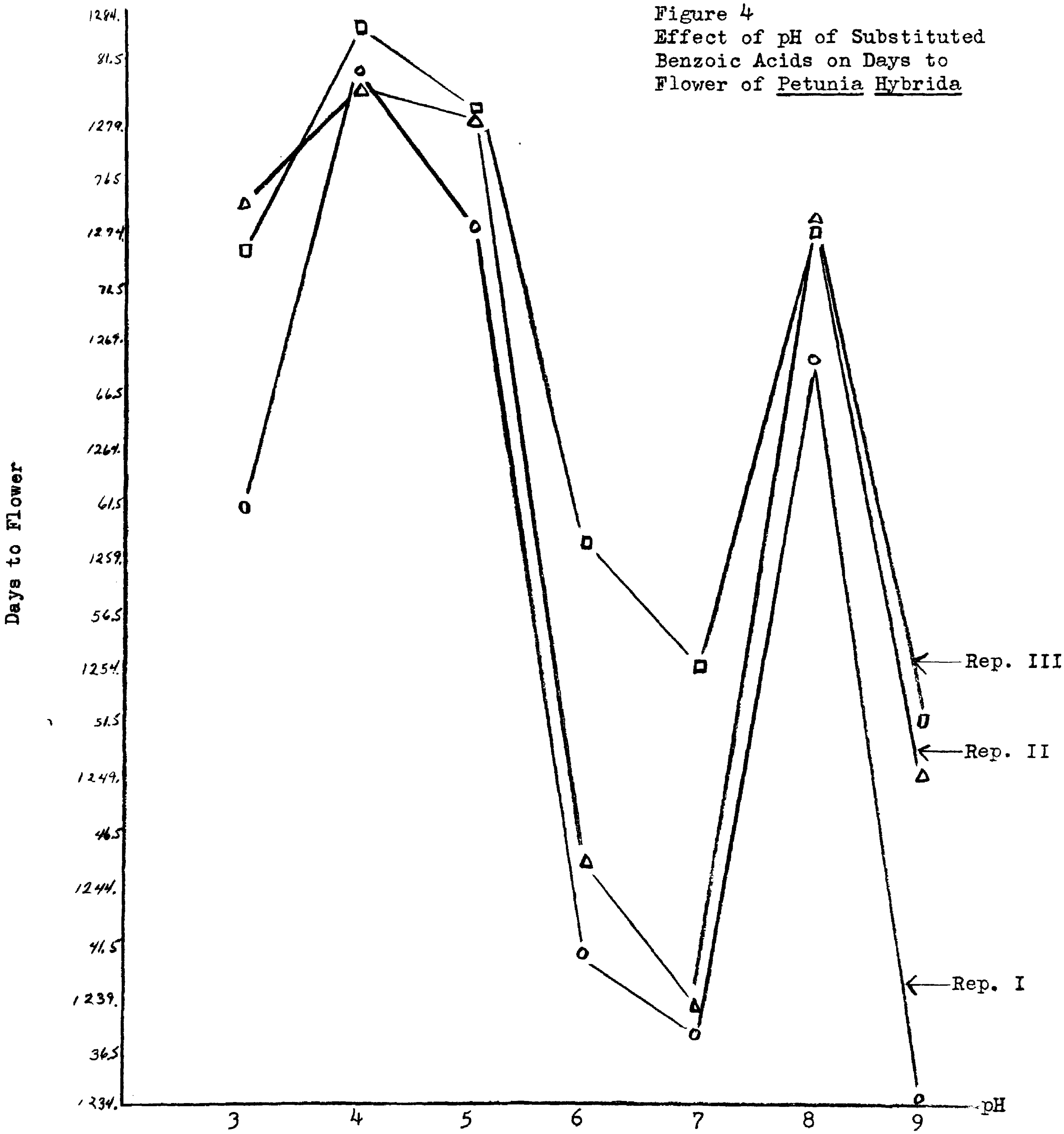


Figure 4
Effect of pH of Substituted Benzoic Acids on Days to Flower of <u>Petunia Hybrida</u>

EFFECT OF DIFFERENT CONCENTRATIONS OF SUBSTITUTED BENZOIC ACIDS ON *ZINNIA ELEGANS*

Dry weights in grams of *Zinnia elegans* tops was highly significantly correlated with chemical treatments, concentrations and chemical-concentration interactions (Table XIV). There were no apparent trends relating chemical structure to activity. In general, the para and meta substituted compounds showed greater activity than ortho and combinations of 2,5 substitutions as judged by increase in dry matter over control plants.

No treatment resulted in any difference of number of nodes to flower. Almost uniformly plants flowered at the fifth node. Occasionally a plant would flower at the sixth node and rarely at the fourth node.

The first plant flowered in forty-seven days and the last plant flowered in fifty-eight days. There were no significant differences between days to flower and chemical treatments, concentrations or chemical-concentration interactions.

Profound morphological and teratological changes were induced in *Zinnia elegans* by several of the treatments with substituted benzoic acids. Within twenty-four hours following spraying, 2,3,5-trichlorobenzoic acid at 1000 p.p.m. induced epinasty. The growing point turned down and in several cases touched the soil. At the end of 120 hours after spraying the plants had straightened up but the leaves still showed epinasty. The subsequent growth of these plants is shown in Figure 5. The first true leaves were normal and the remaining leaves appeared in a cluster due to much shortened internodes. Leaves were abnormal in size (smaller, strap-like or round, Figure 8) and had much vascular tissue proliferation. Plants were a pale to

TABLE XIV

EFFECT OF 100 AND 1000 P.P.M. CONCENTRATIONS OF

SUBSTITUTED BENZOIC ACIDS ON DRY WEIGHT OF ZINNIA ELEGANS TOPS*

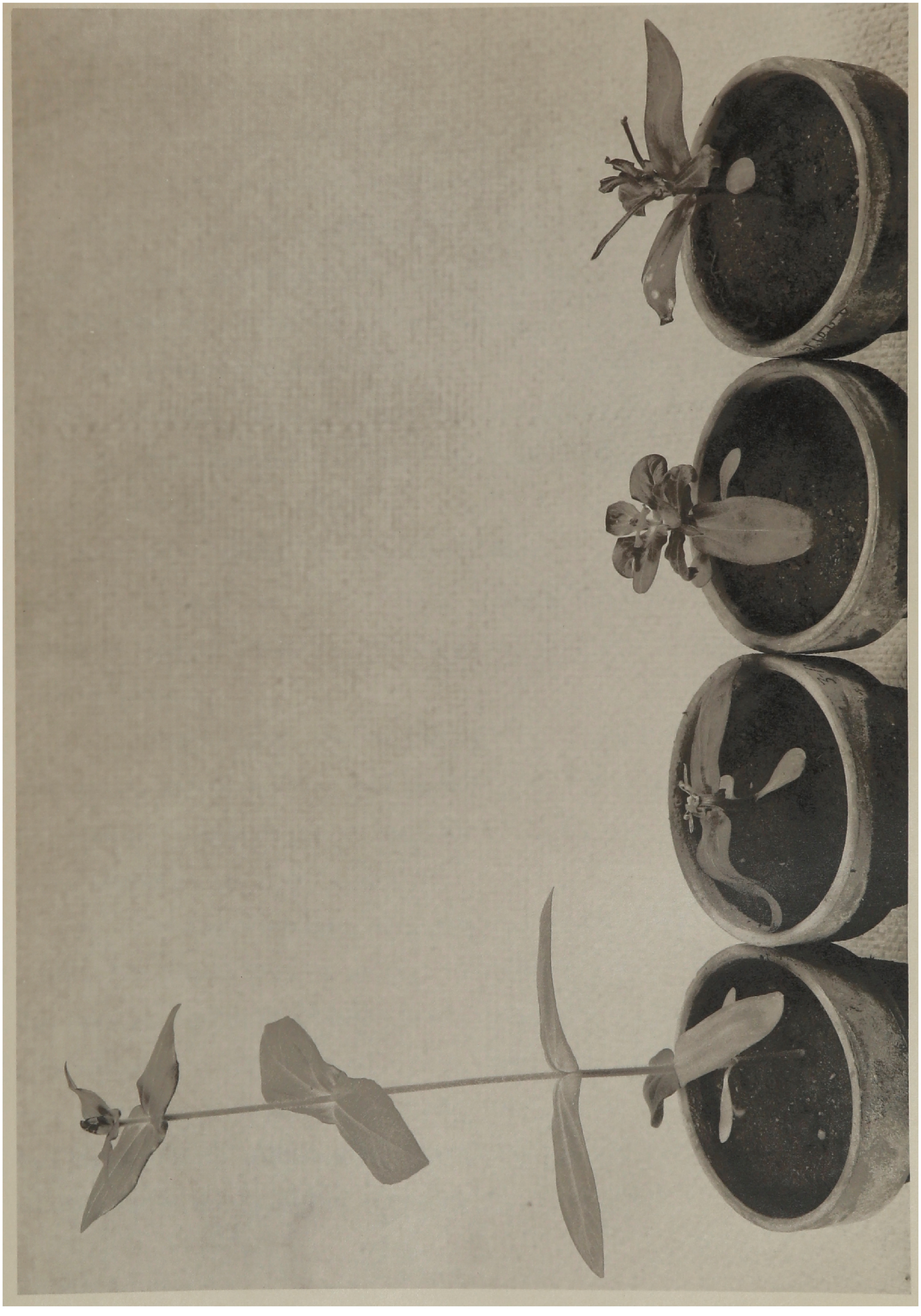
Chemical	Grams Dry Weight 100 p.p.m.	1000 p.p.m.
Check	27.2	23.6
2,3,5-triiodobenzoic acid	24.9	35.8
2,3,5-tribromobenzoic acid	37.2	33.3
2,3,5-trichlorobenzoic acid	27.5	8.3
o-iodobenzoic acid	34.9	26.1
p-iodobenzoic acid	34.2	45.0
2,3-diiodobenzoic acid	36.0	33.1
2,5-diiodobenzoic acid	29.8	34.2
3,5-diiodobenzoic acid	28.1	38.9
2-iodo-3-nitrobenzoic acid	41.7	24.7
p-aminobenzoic acid	29.8	41.5
2-amino-5-iodobenzoic acid	30.9	28.9
2-amino-3,5-diiodobenzoic acid	45.8	25.2
2-amino-3,5-dibromobenzoic acid	29.1	36.2
2,5-dihydroxybenzoic acid	27.3	28.3
3,5-diiodosalicylic acid	33.2	67.5

L.S.D 5% = 0.90
1% = 1.20

*Average of three replicates of four plants each

Figure 5

Zinnia elegans sprayed with solutions of 1000 p.p.m. of 2,3,5-trichlorobenzoic acid. Note shortened internodes leading to a terminal leaf cluster; plant on left is control.



bright yellow color and soon died. A 100 p.p.m. spray of 2,3,5-Trichlorobenzoic acid induced similar but less severe symptoms than the 1000 p.p.m. spray. Growth of these plants is shown in Figure 6. The first nodal leaves were normal. The basal portions of the second nodal leaves were much reduced or strap-like (Figure 8). Proliferation of vascular tissue occurred and parenchymous tissue was almost lacking in the strap-like leaves. Third nodal leaves were either strap-like or else lacking in distal parenchymous tissue. Vascular tissue was much proliferated and confined to the middle of the leaf. The fourth node leaves were fused into a cone-shape and varied from a tight tube to a slight cup (Figure 4). Internodal distances were much shortened compared to control plants.

A spray 1000 p.p.m. spray of 2,3,5-Triiodobenzoic acid induced slight to medium epinasty. Seventy-two hours after spraying all plants appeared normal.

Leaves on plants treated with 2,3,5-tribromobenzoic acid at 1000 p.p.m. showed much swollen parenchymous tissue with sunken veins on all leaves. Leaves at the lower nodes were more severely affected than those leaves at the upper nodes.

Profound changes, as compared with check plants, were induced by 2-Iodo-3-nitrobebzoic acid (Figure 7). Plants treated with 100 p.p.m. of the chemical caused the second pair of leaves to be strap-like with some proliferation of the vascular tissue (Figure 8). The usual three midribs were coalesced into one and the third nodal leaves were lobed at the base. The three midribs were loosely interlaced. One side of the basal portion of the leaves was joined at node four and in addition, the three midribs

Figure 6

Zinnia elegans sprayed with 100 p.p.m. 2,3,5-trichlorobenzoic acid. Note tight to loose terminal cone, proliferated vascular tissue (light center portion of leaf), strap-like leaves and leaves deficient in parenchymous tissue; plant on left is control.

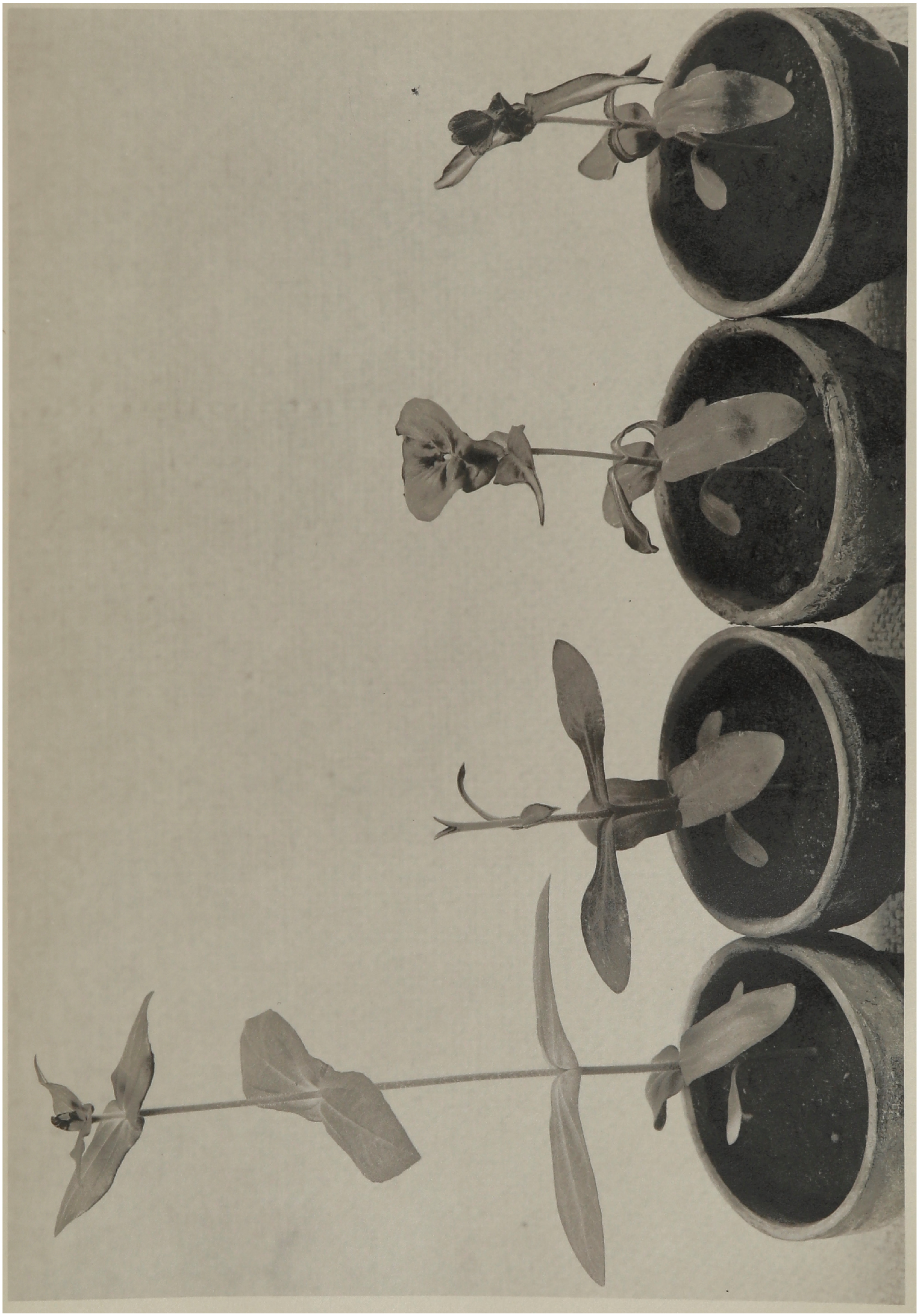


Figure 7

Zinnia elegans sprayed with (left to right) water, 2-iodo-3-nitrobenzoic acid (100 p.p.m.) and remaining plants with 2-iodo-3-nitrobenzoic acid (1000 p.p.m.). On plant B (100 p.p.m.) note strap-like leaves at second node, loose coalescense of three midveins at third node and complete coalescense of three midveins at fourth node; on plants C and D note strap-like second nodal leaves with vascular tissue proliferation, shortened third-fourth internode, coalescense and proliferation of vascular tissue at third node, lost tip at fourth node.

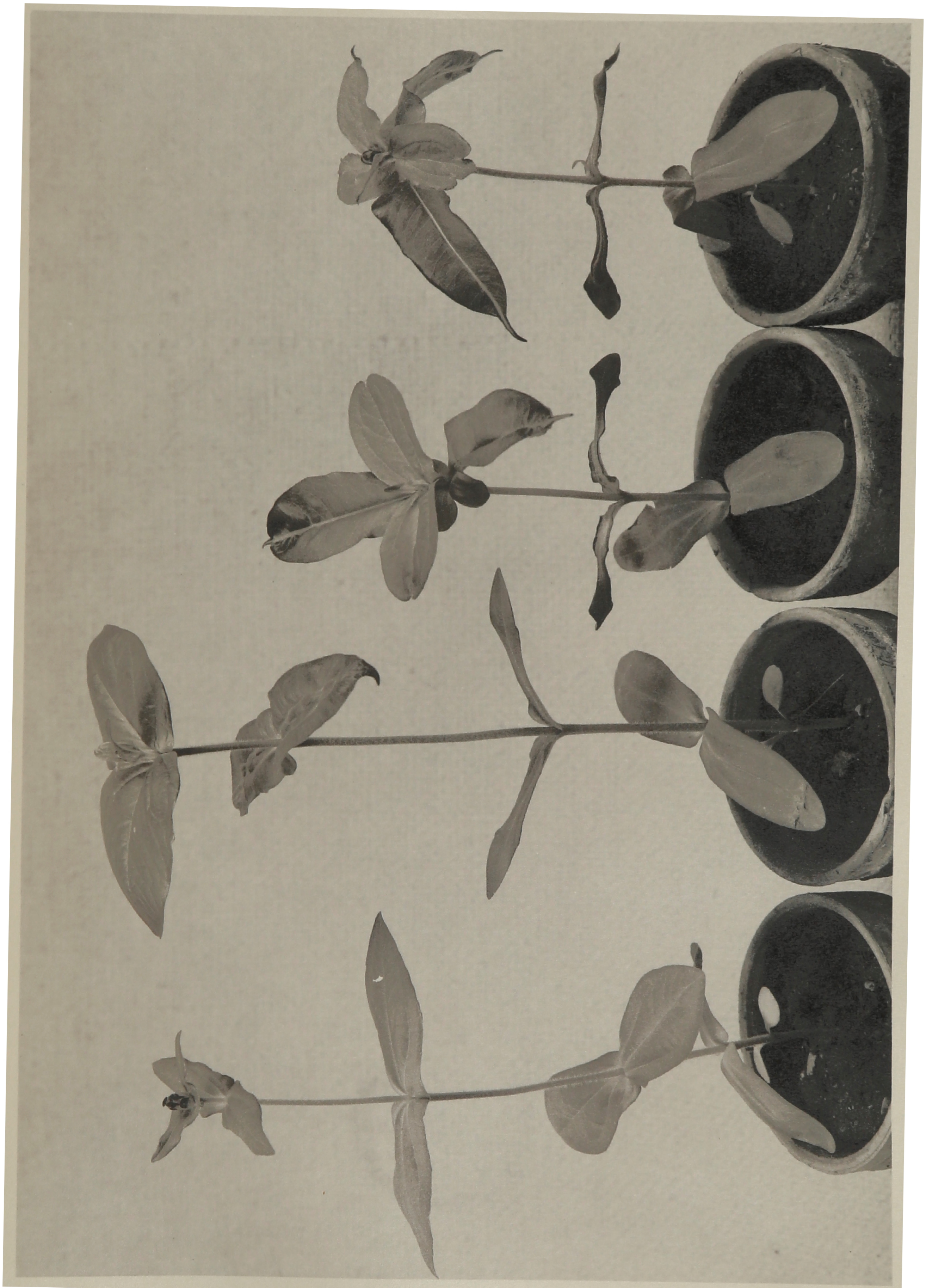


Figure 8

Zinnia elegans leaves sprayed with substituted benzoic acids. Row 1 is control leaf; row 2 (left to right) leaves A, B and C have loose to tight cone of terminal growth of 100 p.p.m. 2,3,5-trichloro-benzoic acid sprayed plants; leaf E is strap-like with proliferated vascular tissue from 1000 p.p.m. 2,3,5-trichlorobenzoic acid sprayed plants; leaf 5 shows proliferation and coalescense of vascular tissue, lack of distal parenchymous tissue in 1000 p.p.m. 2-iodo-3-nitrobenzoic acid sprayed plants; Row 3 leaf A shows loose coalescense of three main veins in 100 p.p.m. 2-iodo-3-nitrobenzoic acid sprayed plants; leaf B shows fusion of opposite leaves of 100 p.p.m. 2-iodo-3-nitrobenzoic acid sprayed plants; leaf C shows vascular proliferation and coalescense in 1000 p.p.m. 2-iodo-3-nitrobenzoic acid sprayed plants.

were coalesced. Plants treated with 1000 p.p.m. of the chemical were also affected and the second pair of leaves were similar to those receiving 100 p.p.m. although the abnormalities were somewhat more pronounced (Figures 7 and 8). Third nodal leaves had much vascular tissue proliferation which coalesced to form midribs. Internodal distances between the third and fourth, and fourth and fifth nodes were much shortened. A much reduced distal portion of the fourth pair of leaves was common. The fifth leaf-pair was normal. Normal flowering occurred at both concentrations.

To further test the effect of concentration of other substituted benzoic acids more seed of Zinnia elegans was sown on December twenty-seventh, 1955.

Chemicals used in this test were m-iodobenzoic acid, o-chlorobenzoic acid, m-chlorobenzoic acid, o-bromobenzoic acid, m-bromobenzoic acid, m-nitrobenzoic acid, 2,5-dichlorobenzoic acid, 3,5-dichlorobenzoic acid, 3,5-dinitrobenzoic acid, 3-chlorosalicylic acid, 5-chlorosalicylic acid, 3-bromosalicylic acid, 5-bromosalicylic acid, 3,5-diiodosalicylic acid, 3,5-dichlorosalicylic acid, 3,5-dibromosalicylic acid and 3,5-dinitrosalicylic acid.

There were no correlations between days to flower and chemical treatments, concentrations, or chemical-concentration interactions. The first plant flowered in twenty-nine days and the last plant flowered in forty-nine days.

Statistical analysis revealed no correlation between dry weight of aerial portions and chemical treatments, concentrations or chemical-concentration interactions. Non-treated plants were uniformly lower in weight than test plants but the difference was not significant at the five percent level. Nodes to first flower in both non treated plants and treated plants were usually four and in a few cases there were five nodes to first flower. Each plant under treatment had one or two axillary shoots per plant while no control plant produced any axillary shoots.

As in theprevious treatment of Zinnia elegans with substituted benzoic acids, this treatment also produced profound morphological and teratological changes within the plant. A concentration of 1000 p.p.m. of 2,5-dichlorobenzoic acid produced adventitious buds or internodal dichotomous branching (Figure 9), on three out of twelve plants. This is believed to be the first time a growth regulator has brought has brought about this response. Further effects of 2,5-dichlorobenzoic acid at 1000 p.p.m. are shown on Figures 10 and 11. Leaves at the second node are either non-expanded at the base or else strap-like with proliferation of vascular tissue. At the second node,(Figure 10), either a strap-like leaf arose directly from the node or a cluster of strap-like leaves on a stem arose from the node. The terminal growing point either produced a yellow anther-like fringe (plant A) or a mass of malformed (mostly bilobed) leaves with proliferated vascular tissue (light bands on leaf). Two plants at a later stage of growth are shown in Figure 11. Plant A has the yellow anther-like fringe on top and in addition, at the third node, an axillary shoot has branched after producing one leaf. Plant B has fused leaves at the second node, a missing leaf at the third node, two axillary shoots on the same side of the node at the third node and a missing leaf at the fifth node.

The effect of 2,5-dichlorobenzoic acid at 100 p.p.m. is shown in

Figure 9

Zinnia elegans treated with 2,5-dichlorobenzoic acid at 1000 p.p.m. showing adventitious bud; bud on left is usual terminal bud.

Figure 10

Zinnia elegans treated with 2,5-dichlorobenzoic acid at 1000 p.p.m. Note strap-like leaves with proliferated vascular tissue at second node on plants A and B with basal unexpanded leaves at second node of plant C. Plant A has much shortened axillary shoot at second node and plants B and C have upright growing strap-like leaf at second node. On plants B and C note terminal leaf cluster of bilobed, heavily vascular leaves.

Figure 11

Zinnia elegans sprayed with 1000 p.p.m. 2,5-dichlorobenzoic acid pictured at the flowering stage. On plant A note basal unexpanded leaves at second node, strap-like leaves at third node and fused leaves at fourth node. Axillary shoot at third node has much reduced internodes. On plant B note fused second and third node leaves and missing leaf at fifth node. Note much reduced axillary shoot at third node.

figure 12. First nodal leaves are normal and leaves at the second node are non-expanded at the base and have proliferated vascular tissue. Distal parenchymous tissue is lacking on leaves at the third node, vascular tissue is greatly proliferated and forms a more or less massive midrib. On plant D the internodal distance between the fourth and fifth node is lacking and the four leaves are fused as a unit.

The effects of 5-chlorosalicylic acid at 1000 p.p.m. are shown in Figure 13. In addition to abnormal leaves (strap-like, proliferation of vascular tissue, fusing) plant A has three adjoining blossom heads while plant B has two adjoining blossom heads.

Morphological and teratological changes induced by compounds in addition to these described above are shown in Table XV. A para position substitution appears to confer more formative activity on a compound than any other substitution.

Figure 12

Zinnia elegans sprayed with 100 p.p.m. 2,5-dichlorobenzoic acid. Plant on left is a control; plants B and C show basal unexpanded second node leaves, third node leaves have tight to loosely coalesced proliferation of vascular tissue. Plant on right has fourth and fifth internode completely lacking with the four leaves fused.

Figure 13

Zinnia elegans sprayed with 1000 p.p.m. of 5-chlorosalicylic acid. Plant A has three adjoining blooms and plant B has two adjoining blooms.

TABLE XV

EFFECT OF 100 AND 1000 P.P.M. CONCENTRATION OF SUBSTITUTED BENZOIC ACIDS ON MORPHOLOGY AND TEROTOLOGY OF ZINNIA ELEGANS

Chemical	Conc. p.p.m.	Epinasty	Malformed Leaves	Proliferated Vascular Tissue	Fused Leaves	Axillary Shoots	Multiple Bud
Check	0	0	0	0	0	0	0
o-chlorobenzoic acid	100	0	0	0	1	4	0
	1000	0	0	0	2	4	0
m-chlorobenzoic acid	100	0	0	0	0	4	0
	1000	0	0	0	0	4	0
o-bromobenzoic acid	100	0	0	0	0	4	0
	1000	0	0	0	0	4	0
m-bromobenzoic acid	100	0	0	0	0	4	0
	1000	0	0	0	0	4	0
m-nitrobenzoic acid	100	0	0	0	0	4	0
	1000	0	0	0	0	4	0
m-iodobenzoic acid	100	0	0	0	0	4	0
	1000	0	0	0	0	4	0
2,5-dichlorobenzoic acid	100	4	3	3	2	4	0
	1000	4	4	4	3	4	1
3,4-dichlorobenzoic acid	100	0	0	0	0	4	0
	1000	0	0	0	1	4	0
3,5-dinitrobenzoic acid	100	0	0	0	0	4	0
	1000	4	0	0	0	4	0
3-chlorosalicylic acid	100	4	0	0	0	4	0
	1000	4	0	0	1	4	0
5-chlorosalicylic acid	100	4	0	0	0	4	0
	1000	4	2	3	3	4	1
3-bromosalicylic acid	100	0	0	0	0	4	0
	1000	0	0	0	0	4	0
5-bromosalicylic acid	100	4	0	1	1	4	0
	1000	4	0	2	2	4	0
3,5-diiodosalicylic acid	100	0	0	0	0	4	0
	1000	0	0	0	0	4	0

TABLE XV (Continued)

Chemical	Conc. p.p.m.	Epinasty	Malformed Leaves	Proliferated Vascular Tissue	Fused Leaves	Axillary Shoots	Multiple Bud
3,5-dichlorosalicylic acid	100	4	0	0	0	4	0
	1000	4	0	2	2	4	0
3,5-dibromosalicylic acid	100	4	0	0	0	4	0
	1000	4	0	3	3	4	0
3,5-dinitrosalicylic acid	100	0	0	0	0	4	0
	1000	0	0	0	0	4	0

1 = 0% to 25% of plants
2 =26% to 50% of plants
3 =51% to 75% of plants
4 =76% to 100% of plants

EFFECT OF DIFFERENT ACIDITY LEVELS OF SUBSTITUTED BENZOIC ACIDS SPRAYED ON *ZINNIA ELEGANS*

The following compounds were used in this experiment: 2,3,5-triiodobenzoic acid, 2,3,5-tribromobenzoic acid, 2,3,5-trichlorobenzoic acid o-iodobenzoic acid, m-iodobenzoic acid, p-iodobenzoic acid, 2,3-diiodobenzoic acid, 2-amino-5-iodobenzoic acid, 2-amino-3,5-diiodobenzoic acid and 3,5-diiodosalicylic acid, 2,5-diiodobenzoic acid, 3,5-diiodobenzoic acid and 2-iodo-3-nitrobenzoic acid.

Due to cultural difficulties many plants died and it became necessary to regroup the replications into two rather than three sets. Just before flowering occurred, it became necessary to remove the plants from thebench and place them in four groups on the greenhouse floor.

No statistical analysis was possible but several trends were observed. Non-treated plants were noticably taller than the treated plants. Plants treated with 3,5-diiodosalicylic acid appeared to be slightly taller than the other treated plants but not as tall as control plants. Plants sprayed with ortho and orth-para substituted benzoic acids appeared to flower a few days earlier than the control plants or plants treated with benzoic acids substituted in other positions.

Morphological and teratological changes were evident in treated plants. The changes seemed to be related more closely to chemical structure than to acidity or chemical-pH interactions. Changes induced in plants sprayed with 2,3,5-trichlorobenzoic acid at 1000 p.p.m. concentration are shown in figures 14 and 15. Terminal growth formed a tight to loose cone with vascular tissue proliferation and absence of distal parenchymous tissue.

Figure 14

Zinnia elegans sprayed with 1000 p.p.m. 2,3,5-trichlorobenzoic acid. Note cone-shaped terminal leaves, proliferation and coalesing of vascular tissue. Plant A shows terminal tip growing down and terminating in a leaf cluster.

Figure 15

Zinnia elegans sprayed with 1000 p.p.m. 2,3,5-trichlorobenzoic acid. This plant at an earlier stage of growth is shown in Figure 10, plant A. Note alternate leaves.

Leaves at lower nodes were characteristically strap-like with much vascular tissue proliferation. Additional leaves appeared at the node (plant B, Figure 14) and grew straight up. If apical growth continued the polarity was reversed causing the new growth to grow down and terminate in a leaf cluster. Usually a lateral shoot (Figure 14) assumed dominance and produced a flower head. Characteristically leaves were alternate and not opposite on the lateral shoot. At all pH levels compounds with para or ortho-para substitutions tended to produce plants with alternate and not opposite leaves from the third node up.

To test the morphological effects of adding substituted benzoic acids to the soil, Zinnia elegans seed was sown on February twenty-fifth, 1956, directly into three-inch pots. Starting on February twenty-eighth, 1956, 10 ml. of 50 p.p.m. of substituted benzoic acids (Table XVI) were added to the soil daily. Daily observations were made and are recorded in Table XVI. In general, morphological and teratological changes previously recoreded for Zinnia elegans were evident: fused leaves, proliferation of vascular tissue and strap-like leaves. One new observation was that plants treated with solutions of 3,5-diiodobenzoic acid and 2-amino-3,5-diiodobenzoic acid lost their polarity and grew prostrate. All such plants died as soon as, or immediately after, the third nodal leaves expanded.

TABLE XVI

EFFECT OF DAILY SOIL ADDITION OF 50 P.P.M. OF SUBSTITUTED BENZOIC ACIDS ON MORPHOLOGY OF ZINNIA ELEGANS

Chemical	Effect
2,3,5-triiodobenzoic acid	Stunted
o-iodobenzoic acid	Stunted and leaves more narrow than check
m-iodobenzoic acid	Stunted
p-iodobenzoic acid	As second leaves were opening, polarity was lost and plant grew prostrate. As third nodal leaves opened the plant died.
2,3-diiodobenzoic acid	Second and third nodal leaves fused together
2,5-diiodobenzoic acid	Stunted
3,5-diiodobenzoic acid	As first leaves were opening, polarity was lost and plant grew prostrate. After third nodal leaves opened the plant died.
2,3,5-trichlorobenzoic acid	First nodal leaves strap-like, much vascular proliferation, second nodal leaves tight tube
o-chlorobenzoic acid	First nodal leaves fused
m-chlorobenzoic acid	Proximinal parenchymus tissue lacking on second nodal leaves, third and fourth nodal leaves have swollen parenchyma and imbedded vascular tissue
2,5-dichlorobenzoic acid	Similar to, but not as severe as, 2,3,5-trichlorobenzoic acid
2-amino-5-iodobenzoic acid	Stunted
2-amino-3,5-diiodobenzoic acid	As first leaves were opening polarity was lost and plant grew prostrate. After third nodal leaves opened, all plants died.
2-iodo-3-nitrobenzoic acid	First node has fused leaves; remaining leaves have swollen parenchymous tissue with iibedded vascular tissue
3-chlorosalicylic acid	Stunted; all plants breaking at first node
5-chlorosalicylic acid	Stunted; all plants breaking at first node
3,5-dichlorosalicylic acid	Stunted; leaves alternate instead of opposite
3,5-diiodosalicylic acid	Second nodal leaves fused
2,5-dichlorophenoxyacetic acid	All plants died before first leaf expanded; a large (three times stem diameter) swelling occurred at soil line before death.

EFFECT OF DIFFERENT CONCENTRATIONS OFSUBSTITUTED BENZOIC ACIDS ON *CALLISTEPHUS CHINENSIS*

There were highly significant correlations between dry weight of aster tops and chemical treatments but no correlations between concentrations and chemical-concentration interactions (Table XVII).

There were highly significant correlations between dry weight in grams of aster roots and chemical treatments, concentrations and chemical-concentration interactions (Table XVIII). In general, chemical sprays at 1000 p.p.m. concentration tended to inhibit root growth more than did sprays at 100 p.p.m. concentration. There appeared to be little relationship between chemical structure and dry weight of roots.

There were highly significant correlations between top/root ratio of aster and chemical treatment but no correlations between concentrations or chemical-concentration interactions (Table XIX). All treated plants had a lower value than did control plants. No generalization could be drawn relating chemical structure to top/root ratio in asters.

There were highly significant correlations between number of floral initials, chemical treatments and concentrations but no correlations between number of floral initials and chemical-concentration interactions (Table XX). Plants sprayed with compounds substituted in the ortho position with an electro-negative group had the most floral initials and were followed by plants sprayed with compounds substituted in the para position with an electro-negative group. The trend was for 100 p.p.m. sprayed to have more floral initials than plants sprayed with 1000 p.p.m.

The node number of first flower was correlated highly significantly

TABLE XVII

EFFECT OF SUBSTITUTED BENZOIC ACIDS AT 100 AND 1000 P.P.M.

CONCENTRATIONS ON DRY WEIGHT OF *CALLISTEPHUS CHINENSIS**

Chemical	Grams Dry Weight	
	100 p.p.m.	1000 p.p.m.
Check	7.42	7.52
2,3,5-triiodobenzoic acid	8.15	7.69
2,3,5-tribromobenzoic acid	8.90	8.38
2,3,5-trichlorobenzoic acid	2.15	dead
o-iodobenzoic acid	7.50	8.09
p-iodobenzoic acid	7.56	8.54
2,3-diiodobenzoic acid	7.23	7.39
2,5-diiodobenzoic acid	8.77	8.02
3,5-diiodobenzoic acid	8.69	8.23
2-iodo-3-nitrobenzoic acid	7.77	5.83
p-aminobenzoic acid	10.84	9.73
2-amino-5-iodobenzoic acid	9.52	9.59
2-amino-3,5-diiodobenzoic acid	7.33	8.19
2-amino-3,5-dibromobenzoic acid	10.63	11.10
2,5-dihydroxybenzoic acid	10.42	9.84
3,5-diiodosalicylic acid	9.58	8.67

L.S.D. 5% = 2.80
1% = 3.72

*Average of three replicates of four plants each

TABLE XVIII

EFFECT OF SUBSTITUTED BENZOIC ACIDS

AT 100 P.P.M. AND 1000 P.P.M. CONCENTRATIONS

ON DRY MATTER OF CALLISTEPHUS CHINENSIS ROOTS*

Chemical	Grams Dry Weight 100 p.p.m.	1000 p.p.m.
Check	2.46	2.48
2,3,5-triiodobenzoic acid	2.42	2.02
2,3,5-tribromobenzoic acid	2.38	2.25
2,3,5-trichlorobenzoic acid	0.73	dead
o-iodobenzoic acid	2.53	1.33
p-iodobenzoic acid	2.65	2.46
2,3-diiodobenzoic acid	2.36	2.58
2,5-diiodobenzoic acid	3.04	2.81
3,5-diiodobenzoic acid	2.91	2.81
2-iodo-3-nitrobenzoic acid	3.09	1.58
p-aminobenzoic acid	3.42	2.89
2-amino-5-iodobenzoic acid	2.39	2.02
2-amino-3,5-diiodobenzoic acid	3.54	3.81
2-amino-3,5-dibromobenzoic acid	4.09	4.08
2,5-dihydroxybenzoic acid	3.48	3.66
3,5-diiodosalicylic acid	2.86	2.15

L.S.D. 5% = 0.28
1% = 0.37

*Average of three replicates of four plants each

TABLE XIX

EFFECT OF SUBSTITUTED BENZOIC ACID

AT 100 AND 1000 P.P.M. CONCENTRATIONS ON DRY WEIGHT

OF <u>CALLISTEPHUS</u> <u>CHINENSIS</u> TOP/ROOT RATIO*

Chemical	Grams Dry Weight 100 p.p.m.	1000 p.p.m.
Check	3.30	3.36
2,3,5-triiodobenzoic acid	3.51	4.27
2,3,5-tribromobenzoic acid	4.50	3.74
2,3,5-trichlorobenzoic acid	2.61	dead
o-iodobenzoic acid	3.45	3.04
p-iodobenzoic acid	3.01	3.75
2,3-diiodobenzoic acid	3.39	3.15
2,5-diiodobenzoic acid	3.04	3.29
3,5-diiodobenzoic acid	3.31	2.84
2-iodo-3-nitrobenzoic acid	2.99	3.08
p-aminobenzoic acid	3.55	3.55
2-amino-5-iodobenzoic acid	4.07	4.61
2-amino-3,5-diiodobenzoic acid	2.52	2.66
2-amino-3,5-dibromobenzoic acid	2.81	3.77
2,5-dihydroxybenzoic acid	3.71	3.14
3,5-diiodosalicylic acid	3.74	3.93

L.S.D. 5% = 1.30
1% = 1.72

*Average of three replicates of four plants each

TABLE XX

EFFECT OF SUBSTITUTED BENZOIC ACIDS AT 100 AND 1000 P.P.M.

CONCENTRATIONS ON NUMBER OF FLORAL INITIALS

OF CALLISTEPHUS CHINENSIS*

Chemicals	Number of Floral Initials 100 p.p.m.	1000 p.p.m.
Check	10.08	10.13
2,3,5-triiodobenzoic acid	12.33	12.00
2,3,5-tribromobenzoic acid	14.58	11.92
2,3,5-trichlorobenzoic acid	4.92	dead
o-iodobenzoic acid	10.75	13.33
p-iodobenzoic acid	15.67	14.25
2,3-diiodobenzoic acid	12.17	12.38
2,5-diiodobenzoic acid	15.17	15.08
3,5-diiodobenzoic acid	16.00	14.75
2-iodo-3-nitrobenzoic acid	12.42	10.63
p-aminobenzoic acid	17.92	15.33
2-amino-5-iodobenzoic acid	14.50	14.54
2-amino-3,5-diiodobenzoic acid	16.25	16.29
2-amino-3,5-dibromobenzoic acid	20.25	20.04
2,5-dihydroxybenzoic acid	16.08	15.21
3,5-diiodosalicylic acid	15.67	14.38

L.S.D. 5% = 1.72
1% = 2.38

*Average of three replicates of four plants each

with chemicals and concentrations but not correlated with chemical-concentration interactions (Table XXI). No trend was discernable as to whether 100 p.p.m. or 1000 p.p.m. concentration caused the lowest nodes to flower. Plants sprayed with compounds having ortho-meta substitutions had the lowest number of nodes to flower. The type of substitution did not seem to be a factor in flowering. Para substituted compounds when sprayed on plants seemed to give the highest number of nodes to flower.

Number of days to flower in the aster is highly significantly correlated with chemical treatments and chemical-concentration interactions but is not correlated with concentrations (Table XXII). In general, plants sprayed with compounds ortho-substituted with an electro-negative group flowered in the shortest time; electro-negative 5-position substitutions were almost as effective in hastening flowering.

Morphological and teratological changes induced in the aster by substituted benzoic acid were few. The effects of 2,3,5-trichlorobenzoic acid (100 p.p.m.) and 2-iodo-3-nitrobenzoic acid (1000 p.p.m.) are shown in Figure 16. Terminal growth has stopped and as a result of shortening of internodal distance, a leaf cluster is formed. Leaves in the cluster are small, entire margined and are mostly vascular tissue. Parenchymous tissue is limited to a margin around the vascular tissue.

Leaves at the second, third and fourth nodes are usually strap-shaped, have proliferated vascular tissue and little parenchymous tissue.

The effect of 2,3,5-trichlorobenzoic acid at 1000 p.p.m. on plant growth is shown in Figure 17. Terminal growth has stopped and a leaf cluster, or more commonly, a round knob with small, stipule-like leaves arise from the knob. These leaves turn a bright yellow and soon die. Nodes below the knob

Figure 16

Callistephus chinesis showing effects of substituted benzoic acids applied as foliar spray. Control plant is on left; top right sprayed with 2,3,5-trichlorobenzoic acid; bottom right sprayed with 2-iodo-3-nitrobenzoic acid. Note short internodes with terminal leaf cluster; leaf margins entire, proliferated vascular tissue, parenchymous tissue reduced in amount.

Figure 17

Callistephus chinensis sprayed with 1000 p.p.m. 2,3,5-trichlorobenzoic acid. Control plant is right row center; note dwarfing, entire margins, cup-shaped leaves and fused leaves on treated plants.

have large, malformed, cup-shaped leaves. Mature leaf margins are often entire, a characteristic of the young leaf. All plants turned bright yellow and died.

TABLE XXI

EFFECT OF SUBSTITUTED BENZOIC ACIDS AT 100 AND 1000 P.P.M. CONCENTRATIONS ON NUMBER OF NODES TO FIRST FLOWER IN <u>CALLISTEPHUS CHINENSIS</u>*

Chemical	Nodes to First Flower 100 p.p.m	1000 p.p.m.
Check	37.42	37.17
2,3,5-triiodobenzoic acid	31.92	31.83
2,3,5-tribromobenzoic acid	31.67	31.00
2,3,5-trichlorobenzoic acid	17.66	dead
o-iodobenzoic acid	30.58	33.00
p-iodobenzoic acid	30.92	31.33
2,3-diiodobenzoic acid	28.33	29.58
2,5-diiodobenzoic acid	36.25	31.17
3,5-diiodobenzoic acid	28.50	30.25
2-iodo-3-nitrobenzoic acid	25.25	27.00
p-aminobenzoic acid	35.17	34.67
2-amino-5-iodobenzoic acid	34.33	34.33
2-amino-3,5-diiodobenzoic acid	25.50	25.75
2-amino-3,5-dibromobenzoic acid	32.01	30.00
2,5-dihydroxybenzoic acid	32.01	30.00
3,5-diiodosalicylic acid	34.83	30.50

L.S.D. 5% = 3.72
1% = 4.95

*Average of three replicates of four plants each

TABLE XXII

EFFECT OF SUBSTITUTED BENZOIC ACIDS AT 100 AND 1000 P.P.M. CONCENTRATIONS ON DAYS TO FLOWER IN

CALLISTEPHUS CHINENSIS*

Chemical	Days to Flower 100 p.p.m.	1000 p.p.m.
Check	138.13	148.25
2,3,5-triiodobenzoic acid	130.42	136.33
2,3,5-tribromobenzoic acid	140.00	139.00
2,3,5-trichlorobenzoic acid	128.67	dead
o-iodobenzoic acid	137.00	141.50
p-iodobenzoic acid	145.67	142.92
2,3-diiodobenzoic acid	143.83	137.92
2,5-diiodobenzoic acid	143.58	138.92
3,5-diiodobenzoic acid	145.83	142.67
2-iodo-3-nitrobenzoic acid	140.92	139.67
p-aminobenzoic acid	144.00	141.92
2-amino-5-iodobenzoic acid	143.42	143.17
2-amino-3,5-diiodobenzoic acid	144.83	143.92
2-amino-3,5-dibromobenzoic acid	148.67	155.83
2,5-dihydroxybenzoic acid	141.92	142.75
3,5-diiodosalicylic acid	143.83	140.08

L.S.D. 5% = 11.66
1% = 15.48

*Average of three replicates of four plants each

DISCUSSION

It is obviously difficult to discuss in detail the many effects of the thirty-four chemicals at three concentrations, seven different acidity levels and two modes of application on three such different plants as *Petunia hybrida*, *Zinnia elegans*, and *Callistephus chinensis*. Even with the aid of statistical analysis it is difficult to pinpoint differences among the 5000 plants involved in this experiment. Certain trends are evident, however, and it is possible to correlate these trends so as to form a coherent hypothesis for results reported in this experiment.

Plant growth was divided, in this experiment, into two broad phases -- the vegetative phase and the reproductive phase. By measuring the various phasic components it was possible to determine the effects of substituted benzoic acids on each phase. The vegetative phase growth was determined by measuring the dry root weight, dry aerial weight, dry root/dry aerial ratio, number of axillary shoots and number of floral initials. Floral initials, while considered by some investigators as being part of the reproductive phase, anatomically and phytogenetically belong to the vegetative phase. The reproductive phase of plant growth is, strictly speaking, only that portion of the plant's life cycle during which fertilization can take place. It is unusual for fertilization to take place within floral initials and only rarely does fertilization take place before anthesis. Despite repeated attempts no self or cross fertilization could be achieved before anthesis in the asters, petunias and zinnias used in this experiment. After anthesis both self and cross fertilization was achieved. Therefore, all growth components occurring durin the period from spray application to anthesis were used as a measure of the vegetative phase; the number of days from spray application to anthesis was used to determine the change from the vegetative phase to the reproductive phase.

Two observations led to the conclusion that the substituted benzoic acids had two actions in plants. These observations were (1) ortho electro-negative substituted benzoic acids affected reproductive growth while ortho electro-positive substituted benzoic acids affected vegetative growth, and (2) different and reciprocal acidity optimums were observed for vegetative and reproductive growth.

The Muir-Hansch hypothesis of ortho electro-negative substitutions activating substituted benzoic acids more than other types of substitutions is supported by all data reported here on reproductive growth. As far as vegetative growth is concerned, benzoic acids substituted with an ortho electro-positive group gave better growth (though still not as good as control plants) than ortho electro-negative substitutions. This lends support to the idea of substitute benzoic acids having two reactions in plants. One reaction, when the benzoic acid is ortho electro-negative substituted, affects reproductive growth; another reaction, when the benzoic acid is ortho electro-positive substituted, affects vegetative growth.

Acidity optimums were also different for the vegetative and reproductive phases of growth. Vegetative growth was most enhanced at pH 9 and most inhibited at pH 6 or 7; reproductive growth was most enhanced at pH 7 and most inhibited at pH 8. At pH 4 (the pH region where halogenated benzoic acids are at their iso-electric point) the action of benzoic acids could be expected to be anomalous. This was experimentally confirmed when slight inhibition of both vegetative and reproductive growth occurred at pH 4. There is overwhelming evidence that the degree of ionization of a compound profoundly affects its penitration into a leaf. A non-ionized molecule will enter a leaf much faster than an ionized molecule of the same size. In addition, a non-ionized compound will reach a higher concentration in a leaf than an ionized compound if their other characteristics are similar. Compounds applied as a foliar spray at pH 6 or 7 lead to the lowest top/root ratio, lowest number of flowers and lowest number of axillary shoots but to the greatest acceleration of flowering (as measured by days from spray application to anthesis). Conversely, compounds applied as a foliar spray at pH 4 accelerated flowering the least while, at the same time, giving the highest top/root ratio. The affect of compounds at pH 4 was anomalous as far as number of flowers and number of axillary shoots was concerned. Compounds sprayed on plants at pH 9 caused the highest number of flowers, the highest number of axillary shoots while accelerating flowering to some degree.

The hypothesis put foreward by Thimann and Bonner (43) is the basic assumption upon which is based both the acidity reactions and the ortho substituted reactions. They assume that an anti-auxin may react with IAA substrate molecules to a large extent but still leave enough substrate molecules open to react with IAA to cause growth with the expenditure of a smaller number of auxin molecules. The rapid reaction in the leaves with IAA substrate molecules of both ortho electro-negative substituted benzoic acids and substituted benzoic acids at pH 7 goes almost to completion and seriously lowers the auxin content in the leaves. This leads to florigen production. The slow reaction in the entire plant with IAA substrate molecules of both ortho electro-positive substituted benzoic acids at pH 9 doesn't limit auxin concentration in any one orgin very drastically. There are enough substrate molecules free to combine with IAA to produce normal vegetative growth.

The recent observation of Niedergang-Kamien and Skoog (30) that TIBA destroys polarity and thus limits movement of IAA within excised plant tissue is difficult to interpret in terms of reaction mechanisms. The observation of substituted benzoic acids affecting polarity in intact plants has been reported under experimental results. This loss of polarity was of transient nature, however, and the plants soon grew normally. Two compounds, 3,5-diiodobenzoic acid and 2-amino-3,5-diiodobenzoic acid, were notable in causing a prolonged loss of apical dominance which ultimately resulted in the death of all treated plants. Street (40), who also worked with excised tissue, found evidence of polarity reversal.

This experiment affords strong support for two way movement of substituted benzoic acids in plants. Statistical analysis shows the compounds affect roots even when sprayed on the first node shows the compounds move up. The first nodal leaves of zinnias, asters and petunias are apparently formed in the seed since no effect was noticed on them. Leaves at the second node are apparently well developed initials since only the most formatively active compounds (Trihalogenated and 2-iodo-3-nitrobenzoic acid) caused profound changes in their morphology. These changes, which resulted in a strap-like leaf, were chiefly the lack of parenchymous tissue and proliferation of vascular tissue. When a 100 p.p.m. spray of the less formatively active compounds were used proliferation of vascular tissue still occurred but parenchymous tissue was characterized by persistent marginal meristems which lead to basilarly fused leaves. Plants sprayed with concentrations of 1000 p.p.m. of the less formatively active compounds were characterized by clustering of leaves due to shortening of internodes, proliferation of vascular tissue, and lack of parenchymous tissue.

These observations are consistent with leaf histogenesis. Esau (7) (page 444, Figure 16.13) shows a leaf primordium with the veinal net work well developed while parenchyma tissue is still only partially differentiated.

When high concentrations of formatively active benzoic acids are sprayed on further growth of metistematic tissue is stopped and only elongation of tissue already present takes place. This results in strap-like leaves with proliferated vascular tissue. Whether the vascular tissue is actually proliferated or merely more prominant due to lack of parenchyma tissue is undetermined. Since Wardlaw (46) who first reported the phenomena used the term proliferation the term is used here. At lower concentrations the distal region of theprimordium remains meristematic and results in a loose cone or fused basal leaves with proliferated vascular tissue. Any study, therefore, of the affects of substituted benzoic acids on leaf histogenesis must include the morphological age of the plant as well as the structure of the chemical.

SUMMARY

1. Substituted benzoic acids have two effects on plants. At pH 9 and with ortho electro-positive substituted compounds vegetative growth is affected; at pH 7 and with ortho electro-negative substituted compounds reproductive growth is affected. Low pH affects both vegetative and reproductive growth regardless of the type of substitution. These affects were linked to the ionic structure of the compounds at various acidity levels.

2. The Muir-Hansch hypothesis of substituted benzoic acid mechanism is apparently valid for reproductive growth but may not be valid for vegetative growth.

3. Substituted benzoic acids have the following effects on plants:

   1) inhibit root growth
   2) hasten days to flowering
   3) increase number of axillary shoots

4. Substituted benzoic acids have no effect on weight of aerial portions of plants.

5. Morphological changes of leaves of plants sprayed with substituted benzoic acids can be explained in terms of leaf histogenesis.

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