ACTION OF 2,3,5-TRIIODOBENZOIC ACID ON GERMINATION
AND GROWTH OF ORNITHOGALUM CAUDATUM AIT. POLLEN TUBES

by

ABDUS SADIQ

B.Sc., University of Peshawar, West Pakistan, 1959
M.Sc., University of Peshawar, West Pakistan, 1962

———————————————

A MASTER'S THESIS

submitted in partial fulfillment of the
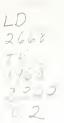

requirements for the degree

MASTER OF SCIENCE

Division of Biology
Botany

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1968

Approved by:

James A. Don
Major Professor

TABLE OF CONTENTS

INTRODUCTION

The growth regulator, 2,3,5-triiodobenzoic acid, abbreviated TIBA, has been recommended to increase the number of pods on soybean plants (Burton and Curley, 1966; Anderson, 1966). Some investigators consider it to be an auxin (Thimann and Bonner, 1948; Muir and Hansch, 1951; Zimmerman et al., 1952; Aberg, 1953) and others consider it to be an antiauxin (Galston, 1947; Grunshaw and Murey, 1955; Greer and Anderson, 1965). When used to stimulate pod production in soybeans, the results have been variable, with some investigators reporting stimulation (Zimmerman and Hitchcock, 1942; Waard and Roodenburg, 1948; Gorter, 1949), and others reporting no significant increase or even a decrease in pod production (Whiting and Murray, 1948; Hartwig, 1966; Smith, 1967). Perhaps the inconsistency of the results could be due to the effects of the TIBA on pollen development, since growth regulators have been shown to reduce germination and tube growth of pollen at concentrations used to promote pod formation on soybeans. To test this hypothesis, this study was undertaken. It would have been desirable to use soybean as the source of pollen and attempts were made to do so. However, due to the small number of flowers produced per plant, the small amount of pollen produced per flower, and the difficulty of extracting the pollen, this species was discarded and Ornithogalum caudatum Ait. and Petunia hybrida Vilm. used as a source of pollen for this study.

The results indicate that TIBA is inhibitory to pollen germination at the concentrations usually applied to stimulate soybeans. Also, some interactions among TIBA and other growth regulators were found and are described.

MATERIALS AND METHODS

Freshly harvested pollen from <u>Ornithogalum caudatum</u> Ait. and <u>Petunia hybrida</u> Vilm. were used for this study. <u>Ornithogalum caudatum</u> plants were raised in soil in 9"-12" pots in the greenhouse and <u>Petunia</u> seeds were germinated in Vermiculite in growth chambers. The seedlings of <u>Petunia</u> were transplanted into 9 inch pots containing soil and a moderate amount of fertilizer. These pots were also kept in greenhouse. Although the main emphasis was on <u>Ornithogalum</u> pollen, <u>Petunia</u> pollen were used for comparison in some of the experiments.

<u>Ornithogalum caudatum</u> Ait. is a member of the Liliaceae, and occasionally grown as an ornamental. It is native to South Africa (Bailey, 1916). Its pollen is binucleate, with the generative cell often in a state of an "arrested prophase in the mature pollen grain" (Brewbaker, 1959b), but it divides to form two sperm cells during growth of the pollen tube. Brewbaker (1959a) observed that binucleate pollen have a long storage life and germinate readily in culture.

<u>Petunia</u> belongs to the family Solanaceae. These are small herbs grown for their showy bloom as garden annuals. <u>Petunia hybrida</u> Vilm., the common garden petunia, probably is a hybrid derivative of <u>P. axillaris</u> BSP. and <u>P. violacea</u> Lindl. (Bailey, 1916).

Both species when greenhouse grown provide pollen throughout the year and are suitable for physiological studies. Pollen were collected from freshly opened flowers for each experiment.

Germination studies were carried out using a modified hanging drop method in petri dishes (Goss, 1962). Six circles (corresponding

to the number of treatments for one replication) of about one cm diameter were drawn with a red glassmarking pencil on lower surface of the cover. This confined the germination medium to a given area. Each petri dish represented one replication. In the circle marked, a drop (approximately 0.01 ml) of germination medium was placed and then the fresh pollen grains were sprinkled on the surface of the drop with the help of a dissecting needle. Fresh solutions and clean glassware were always used. Pollen from a single flower, preferably from one anther, were used for each replication. A small quantity of water was added at the base of the petri dish and one drop of Kodak Photo-flo solution was mixed with the water to keep the water surface uniform at the bottom of the petri dish. The water in the bottom of the petri dish increased the vapor pressure of the atmosphere during pollen germination. The cover was slowly inverted over the bottom and these petri dishes were incubated at a favorable temperature (24-29° C) for 30-60 minutes. After this the petri dishes were taken out and photomicrographs were taken of each treatment with a 50X magnification for Ornithogalum and 100X magnification for Petunia. The films were developed and prints made. The printed pictures were analyzed to determine the percentage of pollen grains germinated and average tube length. The values reported in the results are percentages of the maximum germination (control) recorded for each study. LSD$_{.05}$ values were determined for each study.

The germination media were prepared using deionized distilled water and analytical-reagent grade chemicals. The basal medium of Goss (1962) was used for supplying the inorganic nutrients needed, viz. boric acid (1/2 ml, of 630 mg H_3BO_3/250 ml per 10 ml) plus 1 g/10 ml

(10%) sucrose were used for Ornithogalum and 1/2 ml of boric acid

(1.428 g H_3BO_3/250 ml) per 10 ml plus 1.5 g/10 ml (15%) sucrose for

Petunia. For Ornithogalum 5 ml of macro and 1 ml of micronutrients per

10 ml were used. The macronutrient stock solution consisted of:

$$Ca(NO_3)_2 \cdot 4H_2O \text{ ----------- } 2.86 \text{ g}$$
$$KNO_3 \text{ -------------------- } 8.00 \text{ g}$$
$$KH_2PO_4 \text{ ------------------ } 1.00 \text{ g}$$
$$MgSO_4 \cdot 7H_2O \text{ -------------- } 6.00 \text{ g}$$

These compounds were dissolved in water and diluated to one liter.

The micronutrient stock solution consisted of:

$$FeSO_4 \cdot 7H_2O \text{ -------------- } 50 \text{ mg}$$
$$MnCl_2 \cdot 4H_2O \text{ -------------- } 5 \text{ mg}$$
$$ZnCl_2 \text{ ------------------- } 2 \text{ mg}$$
$$CuCl_2 \cdot 2H_2O \text{ -------------- } 1 \text{ mg}$$

These were dissolved in water and diluted to one liter.

The macro- and micronutrient stock solutions were kept in the re-
frigerator. To this basic medium were added the pure growth regulators.
The pH values of the media were maintained at pH 4.2-5.4 for
Ornithogalum and 5.9-6.7 for Petunia studies and adjusted with 0.01N
NaOH.

TIBA was obtained from Eastman Kodak Company, Rochester, New York,
on 30 September, 1966. It is insoluble in water. Weeraratne (1965)
dissolved TIBA in 0.1N NaOH and then neutralized the base with 0.1N HCl
and used the neutralized solution for the experimental studies. In
the latter part of his investigations he obtained a water soluble sodium

salt of TIBA from commercial sources and used this for the tests.
Greer and Anderson (1965) prepared the stock solution by dissolving 10 g
of TIBA in 95% ethanol to a volume of one liter. The author prepared
the stock solution of TIBA by dissolving 10 mg in 4 ml of 95% ethanol
and making the volume to 10 ml by deionized distilled water. 10 mg of
TIBA could be dissolved in 3 ml of 95% ethanol and made the volume to
10 ml by adding water but on standing for an hour or so it precipitates
out. A fresh stock solution was prepared for each experiment.

Indole-3-acetic acid (IAA) was obtained from Nutritional
Biochemical Corporation, Cleveland, Ohio, in April, 1967. This auxin
is insoluble in water. 10 mg of IAA was dissolved in 1 ml of 95%
ethanol and the volume was made to 10 ml to prepare the stock solution.
This stock solution was also prepared fresh for each experiment, as it
decomposes on standing overnight.

Trans-cinnamic acid, abbreviated t-CA, was obtained from Eastman
Kodak Company, Rochester, New York in October 1967. Nagao and Ohwaki
(1955) recrystallized t-CA from alcohol (M.P. of product 133° C, re-
ported 133° C). The author, for these studies, prepared the stock
solution by dissolving 10 mg of t-CA in 2 ml of 95% ethanol and the
volume was made to 10 ml by adding water.

Puromycin dihydrochloride was obtained from Nutritional Biochemical
Corporation, Cleveland, Ohio, in September, 1967. This salt is water
soluble, so 10 mg was dissolved in water and diluted to 10 ml and was
used for the experimental studies. This stock solution was kept in
refrigerator for use, when required.

All the measurements for calculating the average length of pollen tubes were done in millimeters. Very long tubes were discarded and the photomicrographs were taken from the most representative field views under the microscope. Population effect was eliminated by uniform pollen distribution. All the experiments were repeated at least twice before reporting the results.

EXPERIMENTAL RESULTS

A. Effects of Growth Regulators

Experiments using two important growth regulators viz. TIBA and
Puromycin were performed to determine the effects of these growth
regulators on germination and growth of pollen tubes of Ornithogalum
caudatum at different concentrations. Pollen of Petunia hybrida were
also used to study the effect of TIBA on germination and tube growth.
The growth regulators were added to the culture medium and pollen
germinated and studied using the hanging drop method. Observations
were made 30 to 60 minutes after inoculation (Tables 1, 2, and 3).

Table 1 shows the effect of various concentrations of TIBA on
germination and tube growth of Ornithogalum caudatum pollen. The test
consisted of six treatments and eight replications. It was found that
TIBA at more than 20 ppm inhibited germination significantly. More
than 50% inhibition over control was found at 100 ppm concentration of
TIBA. The inhibition of pollen tube growth was more significant than
inhibition of germination. At 20 ppm concentration of TIBA more than
70% inhibition of the pollen tube growth over control was found. A
subsequent increase in TIBA concentration did not produce a proportion-
ate inhibition in pollen growth as was also the case for germination.

Table 2 shows the inhibition effect of TIBA on germination and
pollen tube growth of Petunia hybrida. In this experiment six treat-
ments and six replications were used. A 50% inhibition of germination
over the control was found at a concentration of 40 ppm TIBA. At higher

Table 1. Effect of TIBA concentration on Germination and Tube
Growth of <u>Ornithogalum</u> pollen (after 30 mins. at 24° C)

TIBA conc.(ppm)	Germination (% of control)	Tube length (% of control)
0	100	100
20	82	27
40	66	21
60	67	22
80	62	18
100	41	17
LSD_{o5}	19	6

Table 2. Effect of TIBA concentration on Germination and Tube
Growth of <u>Petunia</u> pollen (after 1 hr. at 28° C).

TIBA conc.(ppm)	Germination (% of control)	Tube length (% of control)
0	100	100
20	67	42
40	48	37
60	29	37
80	16	37
100	4	13
LSD_{o5}	10	6

concentrations (80 and 100 ppm) the inhibition effect was greater than in Ornithogalum caudatum. The differences between the treatments are statistically significant. Pollen tube growth was found to be more sensitive than germination. A 58% inhibition of pollen tube growth over the control was found at a concentration of 20 ppm TIBA. Subsequent increase in TIBA concentration did not effect pollen tube growth until a concentration of 100 ppm was used.

Data are presented in Table 3 to show the effect of Puromycin on germination and tube growth of Ornithogalum caudatum pollen. During this study it was found that this growth regulator proved neither stimulatory nor inhibitory for both the germination and tube growth, over the concentration range used. The test contained six treatments and four replications. Similar results were found in preliminary investigation on pollen of Antirrhinum majus at this laboratory.

B. Interaction Among Growth Regulators

Table 4 shows the effect of TIBA at a constant level and IAA at various levels on germination and tube growth of Ornithogalum caudatum pollen. From the studies of other workers (Smith, 1939; Goss, 1963; Panchall, 1964, etc.) it is known that IAA alone at a very low concentration has a slight stimulatory effect on pollen germination and tube growth and at higher concentrations it is inhibitory for both. Tables 1 and 2 show that TIBA is also an inhibitor of germination and tube growth of Ornithogalum and petunia pollen. With a LSD$_{.05}$ of 40 for germination and 30 for tube growth Table 4 shows that there is an

Table 3. Effect of Puromycin on Germination and Tube Growth
of <u>Ornithoqalum</u> pollen (after 50 mins. at 26° C)

Puromycin conc. (ppm)	Germination (% of control)	Tube Length (% of control)
0	100	100
20	119	120
40	121	101
60	105	98
80	115	101
100	78	120
LSD_{o5}	32	25

Table 4. Effect of the Combination of TIBA at Constant Level
and IAA at Various Levels on Germination and Tube
Growth of <u>Ornithogalum</u> pollen (after 40 mins. at 27° C)

Conc. (ppm) TIBA + IAA	Germination (% of control)	Tube Growth (% of control)
40 + 0	100	100
40 + 10	140	111
40 + 20	133	129
40 + 30	117	108
40 + 40	116	117
40 + 50	123	122
LSD_{o5}	40	30

interaction between these two growth regulators. For this test there were six treatments and six replications. At higher concentrations of IAA bursting of pollen grains and deformation of tubes was observed.

The effect of the same growth regulators were studied but in reverse order (Table 5). In the first treatment, i.e., IAA + TIBA with 50 + 10 ppm concentration respectively, it was found that there is an interaction between these two growth regulators, but with the increased concentrations of TIBA and IAA at a constant level (50 ppm) no interaction between these regulators was found. In this experiment it appears that TIBA does not alleviate the inhibitory effects of IAA at the concentration range used. It is rather more convincing that both of these two growth regulators act independent of each other. Differences between the treatments were not very significant but an inhibitory trend is more pronounced in tube growth than germination. These results were not as expected and this discrepancy will be discussed in the Discussion and Literature Review. In this experiment the same number of treatments but eight replications were used.

The effect of t-CA at a constant level and TIBA at various levels on germination and tube growth of Ornithogalum pollen were studied and results are given in Table 6. This experiment was based on six treatments and six replications. This study shows that there is no interaction between these two growth regulators at the concentrations used. At a concentration of 30 + 100 ppm of t-CA and TIBA respectively, an 81% inhibition over the control in germination and 83% inhibition over the control in tube growth was observed.

Table 5. Effect of the Combination of IAA at Constant Level
and TIBA at Various Levels on Germination and Tube
Growth of Ornithogalum pollen (40 mins. at 26° C)

Conc. (ppm) IAA + TIBA	Germination (% of control)	Tube Length (% of control)
50 + 0	100	100
50 + 10	126	99
50 + 20	78	46
50 + 30	70	32
50 + 40	63	26
50 + 50	59	21
LSD_{o5}	28	24

Table 6. Effect of the Combination of Trans-cinnamic Acid
(t-CA) at Constant Level and TIBA at Various Levels
on Germination and Tube Growth of Ornithogalum
pollen (1 hr. at 26° C)

Conc. (ppm) t-CA + TIBA	Germination (% of control)	Tube Length (% of control)
30 + 0	100	100
30 + 20	64	43
30 + 40	44	31
30 + 60	25	23
30 + 80	25	22
30 + 100	19	17
LSD_{o5}	17	12

Table 7 is presented to show the effect of TIBA and Puromycin on germination and tube growth of <u>Ornithogalum</u> pollen. No interaction between these regulators was found at the concentration range used, again showing an independent activity of both the regulators. The results are based on six treatments and six replications.

From these experiments one may conclude that pollen tube growth responds more to these growth regulators than does germination.

Table 7. Effect of the Combination of TIBA at Constant Level
and Puromycin at Various Levels on Germination and
Tube Growth of Ornithogalum pollen (45 mins. at 26° C)

Conc. (ppm) TIBA + Puromycin	Germination (% of control)	Tube Length (% of control)
20 + 0	100	100
20 + 20	98	89
20 + 40	95	98
20 + 60	74	78
20 + 80	84	90
20 + 100	89	94
LSD_{o5}	20	21

DISCUSSION AND LITERATURE REVIEW

Since Von Mohl (1839) observed that pollen of some species would form tubes readily in moist air, numerous attempts have been made to cultivate pollen artificially. It has been found that different species vary considerably in the ease with which the pollen may be germinated in vitro (Brink, 1924a). In some species, the pollen may germinate under a wide variety of conditions, in other species the requirements for germination are quite specific and with not a few species the methods thus far devised have given only negative results. Pollen tube growth is likewise sensitive to temperature, moisture, sugar concentration, pH, growth substances, inorganic elements, etc. If the right medium is available, tubes will be produced within a few hours (Brink, 1924a,b,c). Brink (1924a) found a semi-solid agar medium to be suitable for cultivating pollen artificially. Since Schleiden (1849) and Van Tieghem (1869) made the first serious attempts to cultivate pollen artificially, cane sugar (sucrose) solutions have been frequently employed as media and with some success. Of numerous studies that have been reported, only a few contribute evidence of critical value in determining the part played by sugar as a nutrient. The reserve nutrients in the pollen grains are probably sufficient to support an appreciable amount of growth, and it must be considered that the supplementary sugar by a purely osmotic action, might alter the physiological conditions of the medium so that growth could proceed until this stored material is exhausted. Brink (1924d) interpreted the bursting of tubes so frequently encountered in the artificial culture of pollen as an osmotic phenomenon. Vasil (1963) reported that a study of metabolic activity of pollen in

relation to utilization of exogenous sugar has proved that the pollen tubes cannot grow merely on the endogenous food reserves.

Although pollen grains from some species can germinate and grow readily in water or in sugar media without an external supply of nutrients, their rate of growth can be accelerated by the crushed tissues of the stigma or their extracts (Knowlton, 1922; Sasaki, 1928; Noguchi, 1931), by vitamins (Dandliker, Cooper, and Taub, 1938; Cooper 1939), and by growth promoting substances such as 3-IAA, gibberellic acid and kinin (Smith, 1938; Addicott, 1943; Loo, et al., 1944; Kato, 1955; Chandler, 1957; Konar, 1958; Bose, 1959; Goss, 1963, etc.).

Among the inorganic substances, manganese sulphate and boric acid have been found to be highly growth-promoting. Loo and Hwang (1944) have reported the stimulation of pollen-tube growth with the addition of manganese sulphate in pollen from nine different species. Molisch (1893) observed that the stigmatic secretions of many plants are acids and with studies on Azolla indica, Rhododendron ponticum and R. arboreum found beautiful germination of pollen in distilled water containing 0.01% malic acid or a little calcium malate. Jost (1907) secured pollen-tubes of Rhododendron species 12 mm long on a medium containing 5% cane sugar and 0.01% citric acid. Brink (1924b) reported that strong acids and basis beyond very low concentrations totally inhibited germination. The same author (1924d) demonstrated that a small amount of sterile yeast added to sugar media stimulated growth.

The suggestion that boron may be an essential element required for normal growth was made by Agulhon in 1910. A few years later this was confirmed beyond any doubt by the work of Maze (1915) on Zea mays and of

Warington (1923) on Vicia faba. A great deal of work has been done in the past 50 years on the effect of boron deficiency as well as toxicity and the role of boron in plant growth. Boron has also been said to play an essential role in fertilization in higher plants. Anthers appear to be especially sensitive to boron deficiency. Pollen of most species seem to be naturally deficient in boron.

During the years 1932, 1933 and 1935 Schmucker published a series of papers from Göttingen on the stimulating effect of boron on pollen germination and pollen tube growth. He observed that pollen of tropical Nymphaea hardly germinated in 1% glucose but satisfactory germination could be obtained by supplementing the glucose medium with stigmatic extracts. On analysis, the stigmatic extract was shown to contain appreciable quantities of boric acid. Later, he successfully germinated the pollen of Nymphaea in 1% glucose supplemented with boric acid. In fact, on quantitative analysis it was found that the pollen grain required almost the same concentration of boric acid as was present in the stigmatic secretion. Johri and Vasil (1961) reported that the effect of boron on pollen germination and on elongation of tubes was much more marked than the effect of any known hormone, vitamin or chemical. It promoted absorption of sugar and other metabolism by forming sugar-borate complexes, increased oxygen uptake and was involved in the synthesis of pectic materials required for the wall of the actively elongating pollen tube. Vasil (1963) studied five concentrations of boric acid along with sucrose on three varieties of Arachis hypogaea pollen and concluded that low concentrations of boric acid (0.01 to 0.015%) gave the optimum percentage of germination and maximum lengths

of the tubes. He also reported that the stimulatory effect of boron
was direct and not secondary in nature and that effect of boron was
much more pronounced on the growth of pollen tubes than the germination
of pollen grains. Goss (1962) demonstrated a need of boron for Ornithogalum
pollen germination.

Since pollen of various species have specific nutrient requirements
for germination and subsequent tube growth, it was necessary to test
various media to determine the optimum composition. This was done by
Goss (1962) and used in this study.

Opinions are so conflicting that it is difficult to decide whether
pH, over a reasonable range, influence the germination of pollen.
Gotch (1924) pointed out that the dissolved alkali of the cover glass
greatly affects the pollen germination. Brink (1925) assumed that pH
may modify the growth of the pollen tubes through a direct effect on
the chemical reactions attending the digestion of the reserve food
materials. He also demonstrated that the range of the hydrogen ion
concentration that will permit the strong growth is significantly
narrower than that allowing a high percentage of germination. Berg
(1930) and Sisa (1930), Vasil and Bose (1959) are of the opinion that
pH is not an important factor for pollen germination. Panchal (1964)
reported a range of pH 6.0 to 6.5 the optimum pH both for germination
and tube growth of Petunia pollen. The pH of the nutrient medium is a
very important factor for proper tube growth as stated earlier by
Brink (1925). Panchal (1964) also reported that the effectiveness of
growth regulators depends upon their concentration and the pH of the
medium in which they exist. Goss (1962) reported 4.5-4.7 optimum pH

for germination of <u>Ornithogalum</u> pollen. During this study it was found

that the germination and tube growth of <u>Petunia</u> pollen was more sensi-

tive to pH and temperature than <u>Ornithogalum</u> pollen.

Growth regulators were defined by Van Overbeek <u>et</u> <u>al</u>. (1954) as

regulators which affect flowering. Leopold (1964) has defined them as

chemicals which may participate in the control of growth. Plant

physiologists have been able to recognize four types: auxins, gibber-

ellin, kinin and inhibitors. Van Overbeek <u>et</u> <u>al</u>. (1954) reported that

auxin is a generic term for compounds characterized by their capacity

to induce elongation in shoot cells. They resemble indole-3-acetic

acid in physiological action. Auxins may, and generally do, affect

other processes besides elongation, but elongation is considered criti-

cal. Auxins are generally acids with an unsaturated cyclic nucleus or

their derivatives. These authors also defined antiauxins as compounds

which inhibit competitively with action of auxin. The inhibitors include

a wide array of chemical entities which may inhibit growth or development

functions or may inhibit some component reactions relating to the growth

regulators. Inhibitors are at present a poorly defined group of growth

regulators (Leopold, 1964). From the survey of literature there seems

to be an agreement that IAA is an auxin in nature and Puromycin is an

antibiotic. But there is a difference of opinion among the various in-

vestigators whether TIBA and t-CA are antiauxins or uncoupling agents.

Effect of Growth Regulators

2,3,5-triiodobenzoic acid (TIBA) is a well-known auxin antagonist

the precise mode of action of which is still unknown; however, in

general it is thought that its action is effected through an interfer-
ence with the normal auxin system of the plant (Grunshaw and Morey,
1965). Burton and Curley (1966) found a reduced plant height by 16%
during the early blossom period of soybeans by spray application of
TIBA. They also found an increased branching, shortened leaf petioles
and a conical-shaped row profile a 4-15% increase in pod number. They
believed that these TIBA effects are brought by interference with
auxin transport in the plant and other possible interactions. Greer
and Anderson (1965) came out with the same type of result except that
they found a vertical orientation of the upper leaflets and an indirect
effect of TIBA: a decrease in lodging of soybeans. Weeraratne (1965),
by using tobacco mosaic virus (TMV) obtained a reduced infectivity by
TIBA and suggested that inhibitory effect of TIBA on TMV infection is
due to the influence of TIBA on the host metabolism. Galston (1947)
noted an epinasty of young leaves, shortening of internodes near the
apex, loss of apical dominance, and abscission of the apical bud of
Peking and Biloxi soybeans. He negated the idea of florigenic activity
of TIBA and suggested that TIBA may augment the action of florigen, by
affecting other metabolic conditions at the meristem.

TIBA has been classified as a plant growth regulating substance
which is active in inducing modification of organs but is inactive in
causing cell elongation or root formation. Whiting and Murray (1948)
separated the TIBA from IAA, Naphthaleneacetic acid (NAA), and substi-
tuted phenoxy compounds due to the lack of cell elongation and absence
of adventitious roots and tumor formation of bean plants (Phaseolus vulga-
ris var. Red Kidney). These authors also attributed an antiauxin activity

to TIBA. Zimmerman and Hitchcock (1942) did not get cell elongation
with TIBA on Lycopersicum esculentum Mill. and excluded the TIBA from
the definition of auxins. But these authors reported a florigenic
activity of this growth regulator to which later studies by various
workers did not agree. Gorter (1949) said that there is only one
synthetic substance to influence the formation of flowers:
2,3,5-triidobenzoic acid. Snyder (1949) reported an inhibition of
rooting of cuttings (of Coleus Blumei) as well as the average number of
roots formed. He found that the degree of inhibition increased rapidly
with increased concentration of TIBA. Tables 1 and 2 show an inhibi-
tion in germination and tube growth of Ornithogalum and Petunia pollen.
My results agree with most of the investigators who have been studying
the effect of TIBA on various parts of the plant body but these results
are quite comparable with Snyder (1949) and Nagao and Ohwaki (1955) that
an increased inhibition was found by increased concentration of TIBA
and more so that tube growth was greatly influenced by TIBA than germi-
nation. Kiermayer (1961) also found that TIBA inhibited growth in length
of tomato leaves and separation of their primordia from the apical
meristem.

Very recently Smith (1967) found that TIBA causes a suppression of
branching, decrease in flowering responses, as expressed by the number
of inflorescences produced. He also found that the number of flowers
per inflorescence increased by Kinetin, decreased by TIBA and unaffected
by the auxins. Guha, et al., (1966) obtained an enhanced auxin-effect
instead of reversing it by antiauxin like substance, TIBA. Anderson
(1966), working with northern soybeans obtained a decrease in the

growth at the stem apex which speeds up the reproduction and 20% more pods were found on the plants. Seed yield increased by 10%, but he reported a tricky timing for application and concentration of TIBA also plays an important role in getting the desired effects. Hartwig (1966) came out with the results that Southern type of soybeans did not show the same effects as did the northern types. He attributed this difference to the determinate growth type of southern type of soybeans. Hartwig says that the results of his experiments offer no encouragement for using TIBA to increase the seed yield of adapted varieties in Mississippi. Tumanove and Lizandr (1946) mentioned that different plants showed very different sensitivity towards TIBA. Plants of Perilla nankinensis showed a retarded growth while spraying with weaker solutions of TIBA produced an increased yield of alfalfa seeds in a long day variety. Although the mechanism of TIBA inhibition on germination and tube growth of pollen was not investigated in this study but the opinions differ about the inhibition mechanism of TIBA. Some investigators (Aberg, 1953; McRae and Bonner, 1953; etc.) are of the opinion that inhibition mechanism of TIBA is of a competitive type while others (Nagao and Ohwaki, 1955) have reported that TIBA acts as an uncoupling agent like 2,4-dinitrophenol (DNP). Kennedy and Farrar (1965) supported the idea that TIBA blocks the IAA transport and inhibition effects are in response to auxin deficiency. TIBA in low concentration has also been said to possess an auxinic activity (Thimann and Bonner, 1948; Muir and Hansch, 1951; Zimmerman et al., 1952; Aberg, 1953, etc.).

The concentrations of TIBA used (20 through 100 ppm) to study the effect on germination and pollen tube growth of Ornithogalum and petunia

pollen were similar to and often less than used by others, as field sprays to alter flowering. This study indicates the possibility that some of the variations observed in the field may be due to the effects of TIBA in inhibiting pollen tube development, and therefore the normal functioning of pollen in fertilization.

Another growth regulator which was tried to see the effect on germination and tube growth of Ornithogalum pollen was Puromycin (generic name for Achromycin). Puromycin is an antibiotic produced by actinomycetes, Streptomyces alboniger. This was formerly known as Achromycin, the trade name of Lederle Laboratories Division, American Cyanamide Company (Yarmolinsky and De La Haba, 1959). Stylomycin is the current trade name of Lederle Laboratories for Puromycin. Puromycin has a broad range of biological activity, being effective against bacteria, protozoa, parasitic worms (Halliday, 1955), an alga (Tomisek, 1957), and cells of mammalian origin (Halliday, 1955; Lieberman, 1959). Antibiotic action of Puromycin is due to an inhibition of protein synthesis; specifically at a site involving transfer RNA. Yarmolinsky and De La Haba (1959) contributed that the wide variety of plant and animal material showing growth inhibition by Puromycin suggests that the antibiotic interferes with some major biochemical process not differing in detail from one organism to another. Richmond and Biale (1966) studied the inhibition of the incorporation of valine and leucine into protein of climacteric tissues but Puromycin, in this study, did not effect the oxygen uptake of the discs. Tokeda et al. (1960) and Sells (1964) considered Puromycin to be a potent inhibitor of protein synthesis in microorganisms and Muller et al. (1962) found the same effect in mammalian cells. In E. Coli 15T Puromycin inhibited protein formation

while allowing ribosomal and transfer RNA (Sells, 1964) to accumulate.
Sells (1965) indicated that synthesis of individual protein by the
same organism may be effected quite differently by Puromycin. In in-
tact plant tissues, higher concentrations of Puromycin are required.
Mann (1965) concluded that experimental treatment of plants with hor-
mones or with environmental changes frequently lead both to physiological
responses and to alterations in the rate of protein or RNA synthesis.
He questioned that are the changes in protein and RNA the causes of
subsequent physiological responses or do the physiological responses,
in turn, affect protein and RNA synthesis?

In the present studies the author did not find any inhibitory or
other effect of Puromycin on germination or tube growth of Ornithogalum
pollen (Table 3). Similar results were found in preliminary investi-
gation in this laboratory on Antirrhinum pollen. It is possible that
pollen germination and tube growth may not be controlled by protein
synthesis or the concentration of Puromycin used in this study was not
sufficient to bring about any inhibition. Also, the membrane may not
be sufficiently permeable to Puromycin. Walton (1966) while studying
the germination of Phaseolus vulgaris concluded that initiation of axis
elongation is apparently dependent on synthesis of RNA and protein and
Puromycin inhibited the incorporation of C^{14}-leucine into axis protein.
Puromycin inhibited the increased rate of oxygen uptake that occurred
when the axes were incubated in buffer without affecting the initial
rate of uptake. Marcus and Feeley (1964) suggested that activation or
synthesis of mRNA is a pre-requisite for the germination of peanuts and
wheat while Dure (1964) proved that mRNA synthesis is not required for

initial growth of cotton embryos. Key (1964) agrees with Marcus and

Feeley that Puromycin inhibited the continuing elongation of excised

soybean hypocotyl tissue and that RNA and protein synthesis were

essential for the process of cell elongation to proceed at the normal

rate. Inhibition of incorporation of amino acids into protein by dia-

phragm in the presence of Puromycin was studied by Wool, Castles, and

Moyer (1965), and the inhibition by Puromycin of incorporation into

muscle protein of radioactivity from several amino acids has been

demonstrated (Eboue-Bonis et al., 1963; Fritz and Knobile, 1963; Carlin

and Hechter, 1964; Castles and Wool, 1964), and seems, therefore, to be

a general phenomenon applied to all amino acids. It has been suggested

(Castles and Wool, 1964; Elsas et al., 1967) that the membrane carrier

for amino acids may be a protein with a rapid rate of turnover, because

the extensive incubation with Puromycin lowers the capacity of muscle

to take up amino acids, although the effect is not seen in shorter

incubations. Manchester (1967) has also confirmed the inhibitory

effect of Puromycin and cycloheximide on accumulation of aminoisobuty-

rate.

Interaction Among Growth Regulators

The discovery of auxins was the outcome of experiments designated

to explain a correlation effect: phototropism. The term auxin in-

cludes two types of the materials: the growth hormones, which are

natural plant constituents and which regulate cell enlargement in the

manner of indoleacetic acid (IAA), and the natural or synthetic

materials, which can also stimulate cell enlargement in the manner of

IAA but which are not natural components of the growth-regulating
system in the plant. Since chemists use different schemes for naming
components, indoleacetic acid is also called indolylacetic acid,
3-indole-acetic acid, beta-indolyleacetic acid and indole-3-acetic
acid. Further, since indoloeacetic acid induces cell elongation it
belongs to the class of compounds which are designated "auxins" and
since IAA is found occurring naturally in plants, it may also be called
a hormone (Tukey, 1954). Recently Galston (1967) has also defined the
hormones as organic substances, synthesized in minute quantities in
one part of the organism and transported to another locale where they
are active and in order for a hormone to act as a regulator it must
constantly be present in short supply. Kögl et al. (1934) described
IAA as auxin and this chemical was purified from plant materials by
Kögl and Kostermans (1934) and by Thimann (1935). The subsequent study
of auxins has been dominated by interest in its control over growth.
Inhibitory responses to auxin are very general. Roots are particularly
sensitive to auxins. It is known that plant contains many chemically
and physiologically distinct types of hormones, including auxin,
gibberellin, cytokinin, abscisin, and ethylene (Leopold, 1964).

Not only the developing seeds but the pollen of many plants has
been shown to be a rich source of natural auxin. As early as 1902
Massart observed a slight enlargement of the ovary in certain orchids
as a result of placing dead pollen on the stigma, and later Fitting
(1909) obtained similar results using water extracts of both dead and
living pollen. Yasuda (1934) succeeded in stimulating the growth of
unpollinated ovaries of the aubergine by injecting them with a water

extract of Petunia pollen; and Gustafson (1936) obtained similar re-
sults using chloroform extracts applied in lanolin to the cut bases of
styles. In general, it seems that the amount of auxin contained in
the pollen which reaches the stigma in normal pollination is quite inade-
quate to initiate fruit development. This auxin may nevertheless play
an important role in the growth of the pollen tubes themselves, and
in some plants it may also serve to prevent premature abscission of the
style. The auxin of the pollen grains may also play a vital part in
bringing the ovules into condition for fertilization. It is now known
that IAA is widespread in plants. It is present particularly in growing
regions, such as shoot tips and young leaves. Pollen is a rich source.
The presence of IAA has been reported in pollen of apple (Larsen and
Tung, 1950), orchid (Muller, 1953), tobacco (Lund, 1956), date palm
(Lunden, 1954) and corn (Fukui et al., 1958).

Smith (1939) while working on the influence of 3-IAA on pollen of
Tradescantia, Polygonatum, Lathyrus and Pinus species, found the
following effects at 1 ppm: i) germination was stimulated in such a
way that the pollen tube appeared in considerably less time than in
control; ii) rate of elongation of the tube was increased; iii) a
much higher percentage of germination was achieved; iv) greater tube
lengths were found at the end of 4 hours; v) the pollen of Pinus
austriacea was stimulated to germinate, whereas no germination occurred
in control.

Goss (1963) reported that an exogenous supply of IAA was not
necessary for in vitro germination of Ornithogalum caudatum pollen.
Panchal (1964) reported that low concentration (10 ppm) of IAA promoted

germination and tube growth of Petunia pollen. Addicott (1943) reported
that IAA at 1 ppm was more stimulatory than any other concentration on
the germination and the tube growth of Milla biflora and Tropoeolum majus
pollen. He stated that germination of the pollen grains and subsequent
growth of the pollen tubes can be stimulated independently. Recently
Burg and Burg (1966) reported that in the tissues of Avena coleoptile
and etiolated pea epicotyl show auxin maxima at low concentration
$(10^{-6}M)$ of IAA because at IAA concentration higher than this, ethylene
formation is initiated, and ethylene inhibits growth. Galston (1967)
demonstrated that action of auxin involves the synthesis of new pro-
teins, through mechanisms involving the nucleic acids which regulate
that synthesis.

In the present studies an interaction between TIBA and IAA was
studied by the author. This experiment was done in two ways; firstly
keeping the TIBA at a constant level and using IAA at various concen-
trations and in the second experiment in reverse order. In the first
case (Table 4) no effect on germination or tube growth over control
was found, which suggests that there is an interaction between the two
substances. The results can be interpreted that TIBA acts as an IAA
antagonist through direct competition with auxin. Waard and Florschutz
(1948) showed that the influence of TIBA on the growth of tomato plants
is rather complicated, bringing about stimulation and inhibition of
growth at the same time in different parts of the plants, and they sug-
gested an interaction between TIBA and the growth substance of the

plant. Galston (1947) carried out experiments with TIBA in the stand-ard _Avena_ test and concluded that, this substance, itself without auxin activity, antagonized and might completely negate the effect of IAA. He supposed that this effect to be due to a destruction of the later sub-stance by the former.

As mentioned earlier TIBA is a well-known auxin antagonist, the precise mode of action of which is still unknown, however, in general it is thought that its action is effected through an interference with the normal auxin system of the plant. Grunshaw and Morey (1965) while working on the induction of tension wood of TIBA reported that the tension wood is formed under condition of IAA deficiency and tension wood is formed below the rings of TIBA and in petioles of sweet potato they have shown that TIBA effectively blocks the auxin transport. This idea is also supported by Kennedy and Farrar (1965). Burton and Curley (1966) showed that TIBA inhibited apical dominance, increased branching, enhanced flower formation and modified leaf structure. They believed that these TIBA effects were brought by interference with auxin trans-port in the plant and other possible interactions.

Christie and Leopold (1965) studied the manner of TIBA inhibition of auxin transport using corn coleoptile as experimental materials. They reported that TIBA only slightly inhibited IAA entry but severely suppressed IAA exit. Ability of increasing concentration of IAA to overcome TIBA inhibition of entry but not of exit implies differences in the TIBA actions on the two processes. Entry and exit phases of trans-port are different, that at least at higher auxin concentrations exit is the rate-limiting feature of the transport, and that TIBA selectively

suppresses the exit phase. Kuse (1953, 1954) reported that TIBA blocked, at the site of its application the basipetal translocation of natural auxin and IAA. The same author (1958) suggested two alternative explanations for the effect of TIBA blocking the downward propagation of the gibberellin effect namely: 1) the translocation of gibberellin is inhibited by TIBA, just as natural auxin and IAA, and 2) the translocation of gibberellin is not blocked, but its growth promoting effect is inhibited at and below the site of TIBA application. He noticed a synergism between gibberellin and auxin.

Thimann and Bonner (1948) while studying the effect of a mixture of TIBA and auxin obtained a much greater curvature than the auxin alone. They suggested that if auxin brings about its growth-promoting action by combining with a special substrate, then TIBA is to be considered as sufficiently alike in structure to IAA to be able to combine with the same substrate. This combination did not bring about growth but it still leaves open a small number of spaces or active groups on the substrate with which auxin can combine. Hay (1956) reported that pre-treatment with 2,4-D or TIBA inhibited subsequent translocation of IAA. Gorter (1949) suggested an interaction of TIBA with auxin. The blockade of transport of numerous substances was described by Libbert (1959). Sebanek (1966) reported an antagonism between IAA and chlorocholine chloride (CCC) as between IAA and TIBA.

In another experiment IAA was used at a constant level and TIBA at various concentrations to study the effect of this combination on germination and tube growth of Ornithogalum pollen. The results obtained were rather unexpected as compared to the previous test. Table 5

presents these results showing that at low concentration of TIBA (10
ppm) a synergistic activity was observed in germination as well as in
tube growth while subsequent increase in TIBA concentration proved to
be individually effective. A well-marked inhibition was found in ger-
mination and tube growth. This discrepancy cannot totally be resolved
at present. However, these results can be studied in the light of Waard
and Florschutz (1948) work. These authors studied the effect of a
combination of TIBA and IAA in succession and came out with the results
that TIBA proved to antagonize heteroauxin activity; 5-50 mg/1 of the
former substance inhibited the action of IAA. Their experimental results
proved that TIBA, when applied during one hour in a concentration of
1 mg/1 inhibited the subsequent action of IAA. When IAA was applied
first, the resulting curvature was not influenced by a subsequent treat-
ment with TIBA. Galston (1947) reported that TIBA causes auxin abbera-
tion in the plant and inhibits the action of IAA, and when present in
greater molar excess, completely negates the effect of the latter. He
could not decide whether the effect was due to destruction of IAA, or
to a competitive inhibition effect. Skoog, Schneider and Malan (1942)
reported that phenylbutyric acid might possess slight auxin activity,
but when it is administered in combination with IAA, its main effect is
to inhibit the activity of the latter. The extent of this inhibition
is a function of the relative concentrations of the two substances and
is therefore, ascribed to competitive action between them. They sug-
gested that the phenylbutyric acid blocks the action of IAA in accord-
ance with the relative proportion in which the two substances are
supplied. Thimann and Bonner (1948) suggested that higher TIBA con-

centration takes up all the spaces or active groups, so that auxin is excluded and growth is inhibited.

In Table 6 the effect of t-CA and TIBA is shown. Cinnamic acid is found in two forms, cis form is considered to be an auxin (COOH group placed above the ring) and trans form as antiauxin (COOH group pointing away from the ring). Haagen-Smith and Went (1935) reported cis-cinnamic acid to be an auxin and t-CA to be inactive. Van Overbeek et al. (1951) considered t-CA as antiauxin. t-CA is also considered to be a naturally occurring precursor to indole (Mitchel, 1949). Consequently, via trypto-phan, indolepyruvic acid and indole-acetaldehyde it may be considered a precursor of IAA. The acid inhibited growth of young guayule seedlings more than of tomato seedlings (Bonner, 1950). Table 6 indicates that the inhibition in pollen germination and tube growth is well marked as compared to their individual effects. This suggests that there is no interaction between the two-growth-regulators at the concentration range used here. These results are supported by the studies of Nagao and Ohwaki (1955). While studying the effect of t-CA and TIBA on the growth Nagao and Ohwaki reported that both t-CA and TIBA inhibit the growth in any concentration of IAA and that inhibition increases with increasing concentration of these inhibitors. That t-CA is able to inhibit the growth of pea stem sections was verified by Van Overbeek et al. (1951). Nagao and Ohwaki (1955), Tanaka (1964), and Goss (1968) did not consider t-CA as an antiauxin.

The effect of TIBA and Puromycin was investigated and results are shown in Table 7. These results suggest that there is no synergistic activity between the two substances. The results were as expected because

in Table 3 it is shown that Puromycin has no effect on germination and tube growth of Ornithogalum pollen and that the pre-treatment or presence of TIBA in the mixture at a constant level did not alter the results. TIBA at a constant level has produced its inhibitory effect and Puromycin was considered to be not controlling germination of Ornithogalum pollen, hence no synergistic activity between the growth regulators was observed.

SUMMARY

In vitro studies of the effect of two growth-regulators viz TIBA
and Puromycin on germination and tube growth of Ornithogalum caudatum
Ait. pollen have been made. Petunia hybrida Vilm. pollen were also used
to determine the effect of TIBA on germination and tube growth. Fresh
pollen were germinated by the modified hanging drop method, using a
suitable nutrient medium for each. The tests were made at 24-29° C and
optimum pH (4.2-5.4 pH for Ornithogalum and 5.9-6.7 for Petunia). Ob-
servations on percentage germination and tube growth were made 30-60
minutes after inoculation.

It was found that TIBA at the concentration range used inhibited
the germination and tube growth in both species. The response of pollen
tube growth to various concentrations of this growth-regulator in vitro
experiments was found to be more significant than germination. It was
also found that petunia pollen are more sensitive to TIBA concentrations
than Ornithogalum pollen. Puromycin, an antibiotic, is considered to be
an inhibitor for RNA and protein synthesis showed no inhibition in
Ornithogalum pollen germination and tube growth.

The effect of combination of TIBA with IAA, t-CA and Puromycin on
germination and tube growth of Ornithogalum pollen was also studied.
An interesting phenomenon was observed with the TIBA and IAA combination.
Keeping TIBA concentration constant and using IAA at different concen-
trations, it was found that the inhibition effect of one was alleviated
by the other, which might be due to an interaction between these two
growth regulators. On the other hand no such interaction was observed

when IAA concentration was kept constant and using TIBA at various con-
centrations.

A constant inhibition on germination and tube growth was observed
with the combination of t-CA and TIBA. Hence the effect is supplementary.
Puromycin did not alleviate the inhibition effect of TIBA on germination
and tube growth of Ornithogalum pollen.

ACKNOWLEDGMENTS

LITERATURE CITED

Aberg, B. 1953. On the interaction of 2,3,5-triiodobenzoic acid and malate hydrazide with auxin. Physiol. Plant. 6:277-291.

Addicott, F. T. 1943. Pollen germination and pollen tube growth, as influenced by pure growth substances. Plant Physiol. 18:270-279.

Agulhon, H. 1910. Empoi du bare comme engrais catalytique. Compt. Rend. Acad. Sci. (Paris). 150:288-291 (cited from Warington, 1923).

Anderson, I. C. 1966. TIBA: What it can and can't do. Crops and Soils. 18(8):8-9.

Bailey, L. H. 1916a. Standard Cyclopedia of Horticulture. Vol. IV. The Macmillan Company, New York, p. 2408-2409.

_____. 1916b. Standard Cyclopedia of Horticulture. Vol. V The Macmillan Company, New York, p. 2563-2565.

Berg, H. V. 1930. Beitrage Zur Kenntnis der Pollenphysiologie. Planta 9:105-144.

Bonner, J. 1950. The role of toxic substances in the interactions of higher plants. Bot. Rev. 16:51-65.

Bose, N. 1959. Effect of gibberellin on the growth of pollen tubes. Nature (Lond.) 184:1577.

Brewbaker, J. L. 1959a. Incompatibility and the pollen grain. Proc. IX Intern. Botan. Congress. 2:46.

_____. 1959b. Biology of angiosperm pollen grain. Indian J. Genetics and Plant Breeding 19:121-133.

Brink, R. A. 1924a. The Physiology of pollen I. The requirements for growth. Am. J. Bot. 11:218-223.

_____. 1924b. The Physiology of pollen II. Further considerations regarding the requirements for growth. Am. J. Bot. 11:283-294.

_____. 1924c. The Physiology of pollen III. Growth in vitro and in vivo. Am. J. Bot. 11:351-364.

_____. 1924d. The Physiology of pollen IV. Chemotropism; Effects on growth of grouping grains; formation and function of callose plugs; summary and conclusions. Am. J. Bot. 11:417-436.

_____. 1925. The influence of hydrogen-ion concentration on the development of the pollen tube of sweet pea (L. odoratus). Am. J. Bot. 12:149-182.

Burg, S. P. and E. A. Burg. 1966. Proc. Natl. Acad. Sci. (U.S.) 55:262 (cited from Galston, 1967).

Burton, J. C. and R. L. Curley. 1966. Influence of triiodobenzoic acid on growth, nodulation and yields of inoculated soybeans. Agr. J. 58:406-408.

Carlin, H. and O. Hechter. 1964. Insulin Action and protein synthesis in diaphragm muscle. Proc. Soc. Exptl. Biol. Med. 115:127.

Castles, J. J. and I. G. Wool. 1964. Effect of Puromycin and insulin on amino acid accumulation by isolated intact rat diaphragm. Biochem. J. 91:11C.

Chandler, Clyde. 1957. The effect of gibberellic acid on germination and pollen tube growth. Contr. Boyce Thompson Institute 19:215-23.

Christie, A. E. and A. C. Leopold. 1965. On the manner of TIBA inhibition of auxin transport. Plant and Cell Physiol. 6(2):337-345.

Cooper, W. C. 1939. Vitamins and germination of pollen grains and fungus spores. Bot. Gas. 100:844-53.

Dandliker, W. B., W. B. Cooper, and H. P. Taub. 1938. Vitamin B and the germination of pollen. Science 88:622.

Dure, L. and L. Waters. 1965. Long-lived messenger RNA. Evidence from cotton seed germination. Science 147:410-12.

Eboue-Bonis, D., A. M. Chambant, P. Volfin, and H. Clauser. 1963. Action of insulin on the isolated rat diaphragm in the presence of actinomycin D and Puromycin. Nature (Lond.) 199:1183-84.

Elsas, L. J., I. Albrecht, W. Koehue, and L. E. Rosenberg. 1967. Effect of Puromycin on insulin-stimulated amino-acid transport in muscle. Nature (Lond.) 214:916-16.

Fritz, G. R., and E. Knobil. 1963. In vitro stimulation by insulin of α-amino-isobutyric acid transport in the absence of protein synthesis. Nature (Lond.) 200:682.

Fitting, H. 1909. Die Beeinflussung der Orchideenbluten durch die Bestaubung und durch andere Umstande. Zeitsch. Bot. 1:1-86.

Fukui, H. N., F. G. Teubner, S. H. Wittwer, and H. M. Sell. 1958. Growth substances in corn pollen. Plant Physiol. 33:144-146.

Galston, A. W. 1947. The effect of 2,3,5-triiodobenzoic acid on the growth and flowering of soybean. Am. J. Bot. 34:356-60.

_____. 1967. Regulatory systems in higher plants. Am. Scient. 55:141-160.

Gorter, Chr. J. 1949. The influence of 2,3,5-triiodobenzoic acid on the growing points of tomatoes. Proc. Kon. Ned. Akad. Wetensch. Amsterdam. 52:1185-1193.

Goss, J. A. 1962. In vitro germination of Ornithagalum caudatum pollen. Kansas Acad. Sci. Trans. 65:310-317.

_____. 1963. Indole acetic acid and the germination of Ornithogalum caudatum pollen. Kansas Acad. Sci. Trans. 66:727-731.

_____. 1968. Trans-cinnamic acid and some related compound effects on Ornithogalum caudatum pollen. Am. J. Bot. 55 (in press).

Gotch, K. 1924. On the influence of dissolved alkali out of cover glass on pollen germination. Bot. Mag. Tokyo. 38:65-75.

Greer, H. A. L., and I. C. Anderson. 1965. Response of soybeans to TIBA under field conditions. Crop Sci. 5(3):229-232.

Grunshaw, J. and P. R. Morey. 1965. Induction of tension wood by 2,3,5-triiodobenzoic acid. Nature (Lond.) 205(4973):816-818.

Guha, S., S. C. Maheshwari, and B. M. Johri. 1966. Alteration in the geotropic response of Allium seedlings by growth regulators. Physiol. Plant. 19(4):861-868.

Gustafson, F. G. 1936. Inducement of fruit development by growth-promoting chemicals. Proc. Natl. Acad. Sci., 22:628-636.

Haagen-Smit, A. J., and F. W. Went. 1935. A physiological analysis of a growth substance. Proc. Kon. Ned. Akad. Wetensch. Amsterdam 38:852-857.

Halliday, S. L., P. L. Bennett, and J. J. Oleson. 1955. Cancer Res. 15:693 (cited from Yarmolinsky & De La Haba, 1959).

Hartwig, E. E. 1966. TIBA lowers southern soybeans yields. Crops and Soils 18(9):17-18.

Hay, J. R. 1956. The effect of 2,4-dichlorophenoxy-acetic acid and 2,3,5-triiodobenzoic acid on the transport of IAA. Plant Physiol. 31:118-120.

Johri, B. M. and I. K. Vasil. 1961. Physiology of pollen. Bot. Rev. 27:325-381.

Jost, L. 1907. Über die Selbststerilität einiger Blüten. Bot. Zeit. 65:77-117.

Kato, Y. 1955. Response of plant cells of gibberellin. Bot. Gaz. 117:16-24.

Kennedy, R. W., and J. L. Farrar. 1965. Induction of tension wood with the antianxin 2,3,5-triiodobenzoic acid. Nature (Lond.) 208:406-407.

Key, J. L. 1964. RNA and protein synthesis as essential processes for cell elongation. Plant Physiol. 39:365-370.

Kiermayer, O. 1961. Investigation on changes in time course of flower development in Lycopersicum esculentum with 2,3,5-triiodobenzoic acid. Ber deutsch. Bot. Ges. 74(1):15-22 (Biol. Abst. 37(5):1982. 1962).

Knowlton, H. F. 1922. Studies in pollen with reference to longevity. Cornell Univ. Agri. Expt. Sta. Man. 52:751-779.

Kögl, F. A., A. J. Haagen-Smit, and H. Erxleben. 1934. Über ein neues auxin ("Heteroauxin") aus Harn. XI. Z. Physiol. Chem. 228:90-103.

_____, and D. G. F. R. Kostermans. 1934. Heteroauxin als stoffwechselprodukt niederer pflanzlicher organismen. XIII. Z. Physiol. Chem., 228:113-121.

Konar, R. N. 1958. Effect of IAA and kinetin on pollen tube growth of Pinus roxburgii Sar. Curr. Sci. 27:216-217.

Kuse, G. 1953. Mem. Coll. Sci. Univ. Kyoto. Ser B. 20:207 (cited from Kuse, 1958).

_____. 1954. Mem. Coll. Sci. Univ. Kyoto. Ser. B. 21:107 (cited from Kuse, 1958).

_____. 1958. Necessity of Auxin for the growth effect of gibberellin. Bot. Mag. (Tokyo) 71:151-159.

Larsen, P., and S. Tung. 1950. Growth promoting and growth retarding substances in pollen from diploid and triploid apple varieties. Bot. Gaz. 111:436-447.

Leopold, A. C. 1964. Plant Growth and Development. The McGraw-Hill Co., p. 79-80.

Libbert, E. 1959. Trijodbenzoesäure und die stoffleitung bie höheren Pflanzen. Planta 53:612 bis 627.

Liebermann, I., and P. Ove. 1959. Proc. Natl. Acad. Sci. 45:867 (cited from Yarmolinsky and De La Haba, 1959).

Loo, Tsung-Le., and Hwang, Tsung-Chen. 1944. Growth stimulation by manganese sulphate, indole-3-acetic acid and colchicin in pollen germination and pollen tube growth. Am. J. Bot. 31:356-67.

Lund, H. A. 1956. The biosynthesis of IAA in the setting of fruit. Plant Physiol. 31:334-339.

Lunden, R. 1954. A short introduction to the literature on pollen chemistry. Svenski. Kem. Tidskr. 66:201-13.

Manchester, K. L. 1967. Effect of antibiotics on protein synthesis in muscle and implications with regard to the action of insulin. Nature (Lond.) 216:394-395.

Mann, J. D. 1965. Use and misuse of antibiotics in botanical research. Bioscience 15(7):464-465.

Marcus, A., and J. Feeley. 1964. Activation of protein synthesis in the inhibition phase of seed germination. Proc. Natl. Acad. Sci. 51:1075-1079.

Massart. 1902. (cited from Tukey, 1954).

Maze, P. 1915. Determination of elements necessary to the development of maize. Compt. Rend. Acad. Sci. (Paris). 160:211-14.

McRae, D. H. and J. Bonner. 1953. Chemical structure and antiauxin activity. Physiol. Plant. 6:485-510.

Molisch, H. 1893. Zur Physiologie des pollens mit bensonderer Rücksicht auf die chemotropische Bewegungen der pollenschläuche. Sitzungsber. Wien. Akad. Wiss. Mathnaturw. Kl. 102:423-448 (cited from Brink, 1924d).

Muir, R. M., and C. Hansch. 1951. The relationship of structure and plant-growth activity of substituted benzoic and phenoxyacetic acids. Plant Physiol. 26:369-374.

Muller, R. 1953. Zur quantiativen Bestimmung von Indolylessingsaure mittles papierchromatographie und papiereclectrophorese. Beitr. Biol. Pflanz. 30:1-32 (cited from Panchal, 1964).

Muller, G. C., K. Kajiwara, E. Stubbleefield, and R. Rueckct. 1962. Cancer Res. 22:1084. (cited from Sells, 1965).

Nagao, M., and Y. Ohwaki. 1955. The action of trans-cinnamic and 2,3,5-triiodobenzoic acids in the rice seedlings. Sci. Rep. Tohuku Univ. Ser. IV (Biol.) 21:96-108

Noghuchi, Y. 1931. Studie inter Reispflanzen II. Pollenkuing und pollenschlauch wachstum. Jap. J. Bot. 5:351-368.

Panchal, Y. C. 1964. Some physiological studies of <u>Petunia hybrida</u> Vilm. pollen. Ph.D. thesis. Kansas State University, Manhattan, Kansas.

Richmond, A., and J. B. Biale. 1966. Protein synthesis in avocado fruit tissue. Arch Biochem. Biophys. 115(1):211-214.

Sasaki, T. 1928. On the pollen tube growth in some species of Brassica. Res. Bull. No. 11. Imp. Hort. Exptl. Station. Okita, Japan.

Schleiden, M. J. 1849. Principles of Botany (trans. E. R. Lonkester) London.

Schmucker, T. 1932a. Bor als Physiologisch entscheidendes Element Naturwiss. 20:839.

_____. 1932b. Physiologische und Okölogische untersuchungen an Bluten trcpischer <u>Nymphaea</u>--Arten. Planta 16:376-412.

_____. 1933. Zur Blutenbiologie Tropischer Nymphaea--Arten. II. Bor. als entscheidender. Faktor. Planta 18:641-650.

_____. 1935. Über den Einfluss von Barsäure auf Pflanzen, insbesondere Keinende Pollenkörner. Planta 23:264-283.

Sebanek, J. 1966. Interaction of IAA with synthetic and native growth regulators during transfer of ^{32}P into epicotyles of etioleted pea seedlings. Biol. Plant. Acad. Sci. Bohemoslovaca. 8(3):213-219.

Sells, B. H. 1964. Biochim. Biophys. Acta. 80:230 (cited from Sells, 1965).

_____. 1965. Puromycin: Effect on messenger RNA synthesis and β-galactosidase formation in Escherichia coli 15T. Science 148: 371-372.

Sisa, M. 1930. The germination test of pollen in some vegetable crops with special reference of the influence of pH of the media. J. Sci. Agri. Soc. Jap. 328:88-96.

Skoog, F., C. L. Schneider, and P. Malan. 1942. Interactions of Auxins and inhibition. Am. J. Bot. 29:568-576.

Smith, P. F. 1939. The influence of 3-IAA on pollen germination. Science 90:163-164.

Smith, D. L. 1967. The experimental control of inflorescence development in Carex. Ann. Bot. (Lond.) 31(121):19-30.

Snyder, W. E. 1949. Some responses of plants to 2,3,5-triiodobenzoic acid. Plant Physiol. 24:195-206.

Tanaka, K. 1964. The pollen germination and pollen tube development in Pinus densiflora Sief. et Zucc. IV. The effects of acidic growth inhibiting substances from Pinus pollen grains on the germination of Brassica seeds. Sci. Rep. Tohuku Univ. Ser IV. (Biol.) 30:21-25.

Thimman, K. V. 1935. On the plant growth hormone produced by Rhizopus suins. J. Biol. Chem. 109:279-91.

_____, and W. D. Bonner. 1948. The action of triiodobenzoic acid on growth. Plant Physiol. 23:158-161.

Tokeda, Y., S. Hayashi, H. Nakagawan, and F. Suzuki. 1960. J. Biochem. (Tokyo) 48:169 (cited from Sells, 1965).

Tukey, H. B. 1954. Plant Regulators in Agriculture. John Wiley & Sons, Inc. p. 10.

Tomisek, A., M. B. Reid, W. A. Short, and H. E. Skipper. 1957. Studies on the photosynthetic reactions. III. The effects of various inhibitors upon growth and carbonate fixation in Chlorella pyrenoidosa. Plant Physiol. 32:7-10.

Tumanove, I. I., and A. A. Lizandr. 1946. On the physiological action of triiodobenzoic acid upon plants. Bot. Z. USSR 31(3):13-21.

Van Overbeek, J., R. Blondean, and V. Harne. 1951. Trans-cinnamic acid as an anti-auxin. Am. J. Bot. 38:589-595.

_____, R. M. Muir, H. B. Tukey, and F. W. Went. 1954. Nomenclature of chemical plant regulators. Report by a committee of American Society of plant physiologists. Plant Physiol. 29:307-308.

Van Teighem, P. 1869. Recherchs physiologiques sur la vegetation libre du pollen et de l'ovule et sur la fecondation directe des plantes. Ann. Sci. Nat. Bot. V. 12:312-328.

Vasil, I. K., and N. Base. 1959. Cultivation of excised anthers and pollen grains. Indian Bot. Soc. Mem. 2:11-15.

_____. 1963. Studies on pollen germination of certain Leguminasae and Cruciferae. Beit. Biol. Pflanzer. 38:137-159.

Von Mohl, H. 1834. Beiträge Zur Anatomie und physiologie I. Ber. (cited from Brink, 1924d).

Waard, de J. and P. A. Florschütz. 1948. On the interaction of 2,3,5-triiodobenzoic acid and indole-3-acetic acid in growth processes. Proc. Kon. Ned. Akad. Wetemsch. Amsterdam. 51:1317-1320.

_____, and J. W. M. Roodenburg. 1948. Premature flower-bud in tomato seedlings caused by 2,3,5-triiodobenzoic acid. Proc. Kon. Ned. Akad. Wetensch. Amsterdam.

Walton, D. C. 1966. Germination of Phaseolus vulgaris. I. Resumption of axis growth. Plant Physiol. 41:298-302.

Warington, K. 1923. The effect of boric acid and borax on the broad bean and certain other plants. Ann. Bot. 37:629-672.

Weeraratne, V. 1965. Antiviral activity of triiodobenzoic acid. Life Sci. 4(20):1923-29.

Whiting, A. G. and M. A. Murray. 1948. Abscission and other responses induced by 2,3,5-triiodobenzoic acid in bean plants. Bot. Gaz. 109:447-473.

Wool, I. G., J. J. Castles, and A. N. Moyer. 1965. Regulation of amino acid accumulation in isolated rat diaphragm: Effect of Puromycin and insulin. Biochim. Biophys. Acta. 107:333:345.

Yarmolinsky, M. B., and G. L. DeLa Haba. 1959. Inhibition of Puromycin of amino-acid incorporation into protein. Proc. Natl. Acad. Sci. 45:1721-29.

Yasuda, S. 1934. Parthenocarpy caused by a stimulus of pollination in some plants of Solanacea. Agri. and Hort. 9:647-656 (English Summary, p. 656).

Zimmerman, P. W., and A. E. Hitchcock. 1942. Flowering habit and correlation of organs modified by triiodobenzoic acid. Contrb. Boyce Thompson Inst. 12:491-496.

_____, _____, and A. E. Prill. 1952. Substituted benzoic acids as growth regulators. Contrb. Boyce Thompson Inst. 16:419-427.

ACTION OF 2,3,5-TRIIODOBENZOIC ACID ON GERMINATION
AND GROWTH OF ORNITHOGALUM CAUDATUM AIT. POLLEN TUBES

by

ABDUS SADIQ

B.Sc., University of Peshawar, West Pakistan, 1959
M.Sc., University of Peshawar, West Pakistan, 1962

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Division of Biology
Botany

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1968

Fresh pollen of <u>Ornithogalum caudatum</u> Ait. and <u>Petunia hybrida</u> Vilm. were germinated in the absence or presence of 2,3,5-triiodobenzoic acid (TIBA) and germination percentage and pollen tube growth was studied. Goss (1962) basal media with modified hanging drop method for culturing these pollen were used. TIBA gave an increasing inhibition over control in germination and pollen tube growth in both species at a concentration of 20, 40, 60, 80 and 100 ppm. A 59% inhibition over control in germination percentage and 83% in pollen tube growth was observed at 100 ppm TIBA in <u>Ornithogalum</u> while at the same concentration (100 ppm) of TIBA 96% inhibition over control in germination and 87% in pollen tube growth was found in petunia. Effect of Puromycin was also studied on germination and tube growth of <u>Ornithogalum</u> pollen. Puromycin at the concentration range used (20 through 100 ppm) did not show any inhibition in this species.

The effect of combination of TIBA with indole-3-acetic acid (IAA), Trans-cinnamic acid (t-CA) and Puromycin on germination and pollen tube growth of <u>Ornithogalum</u> was studied. An interesting phenomenon with combination of TIBA and IAA was observed. Keeping TIBA concentration constant and using IAA at various concentrations it was found that the inhibition effect of TIBA was alleviated by IAA. This shows an antago-nism between TIBA and IAA. On the other hand keeping IAA concentration constant and using TIBA at different concentrations no such mechanism was found.

In a combination of t-CA and TIBA, it was found that TIBA did not alleviate the inhibitory effect of t-CA. The effect was supplementary.

The effect of combination of TIBA and Puromycin on germination and pollen tube growth of <u>Ornithogalum</u> was also studied. It was found that Puromycin did not alleviate the inhibitory effect of TIBA, suggesting the absence of interaction between these two compounds.

The concentrations of TIBA used in this study were similar to and often less than those used by others as field sprays to alter flowering. So this study indicates the possibility that some of the variations observed in the field may be due to the effects of TIBA in inhibiting pollen tube development, and therefore the normal functioning of pollen in fertilization.