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by

Evaristo Lazo

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Crop Production and Physiology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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Head of Major Department

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Dean of Graduate College

Iowa State University
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Ames, Iowa

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Effect of TIBA on the action of 2,4-D on plants

Evaristo Lazo

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The effects of 2,3,5-triiodobenzoic acid (TIBA) and 2,4-dichlorophenoxyacetic acid (2,4-D) and in particular their combined action on the growth, yield and morphology of plants were studied. Plants of soybeans (Glycine max Merrill), velvet leaf (Abutilon theophrasti Medic.) and oats (Avena sativa L.) were grown in the greenhouse and treated with a series of 2,4-D and TIBA doses in all factorial combinations. The growth of leaves and stems and the increase in fresh and dry matter above the treated leaf were used to estimate the effects of the treatments. Plants grown in nutrient solution in a conditioned room were treated with C¹⁴-2,4-D and TIBA. The effect of TIBA on the distribution of radioactivity was determined by means of radioautography and by counting plant extracts and residues. The effect of TIBA on the rate of excretion of radioactivity by the roots of plants treated with C¹⁴-2,4-D was studied in soybeans, velvet leaf, and oats grown in nutrient solution. The effects on growth and yields of corn hybrids, inbreds, and natural weed populations were evaluated in field plots.

Both TIBA and 2,4-D were inhibitory of growth and yield when applied alone, in concentrations above a certain minimum. Doses of 2,4-D under 1 µg/pl of 2,4-D were ineffective or slightly stimulatory on the growth of soybean and velvet leaf. The combined effects were

more than additive for doses of TIBA and 2,4-D that had small effects when applied alone. When each of them was strongly inhibitory the combination was less than additive. The marked toxic effects of high doses of 2,4-D were decreased by the addition of TIBA only to a limited extent. Oat was not consistently affected by TIBA or 2,4-D in terms of growth or deformations.

In the field, 2,4-D produced its expected effect on broad-leaved species and was not modified by the action of TIBA. Its effects on corn depended on the variety and date of application. TIBA did not affect 2,4-D effects on corn.

Autoradiographs of plants of soybeans and velvet leaf treated with C^{14} -2,4-D showed an inhibitory effect of TIBA on the translocation of radioactivity in a number of cases and no effect in others. Translocation out the treated leaf in oats was very small in all cases. The radioactivity counted in extracts and residues of different plant parts did not show any consistent effect of TIBA on the distribution of 2,4-D in any of the three species but the experiments had high coefficients of variability. The lethal effect of any dose of 2,4-D in the field or in the greenhouse was not increased by the addition of TIBA so negating the possible application of TIBA-2,4-D mixtures as a herbicide. Moderate doses of TIBA and/or 2,4-D induced an increase in the fresh and dry matter accumulated below the 2,4-D treated leaf.

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INTRODUCTION

The highest levels of crop productivity have been reached by a more complete utilization of the multiplicity of factors that affect the growth and development of the plants. The development and application of the concept of interaction has been of the greatest importance. The goal is to optimize the whole system of available resources and factors, soil, water microclimate, "management," genetic potential, "agri-chemicals," etc. according to established objectives.

The application of chemical substances in the form of fertilizers and pesticides to soils and plants is a general practice in modern agriculture all over the world. The study and application of pesticide mixtures was first developed in the field of insecticides (Summerford, 1954). An increasing number of reports are appearing recently on herbicide combinations (Beste and Schreiber, 1970; Colby et al., 1965; Staniforth, 1970; Sweet and Lynch, 1971). Fewer studies on combined effects of growth regulators in crop production are reported (Wort, 1966).

At Iowa State University, two field studies were recently conducted on the combined effects of 2,3,5-triiodobenzoic acid (TIBA) and 2,4-dichlorophenoxyacetic acid (2,4-D) on the growth and yields of soybeans (Afolabi, 1969; Johnson, 1970). The objective of the present study was to further explore the combined effect of both products. More species and a wider range of doses were studied. The evaluation of gross effects was the major objective. Some insight on the physiological nature of the combined effect also was attempted.

REVIEW OF LITERATURE

There are very few reports on studies similar to this thesis, namely the combined effects of 2,3,5-triiodobenzoic acid (TIBA) and 2,4-dichlorophenoxyacetic acid (2,4-D) on the growth and yield of whole plants. Therefore the literature reviewed here is related in different ways and degrees to my specific objective and is presented under a number of headings.

Action of 2,4-D in Plants

Gross effects

Two major types of application or objectives occur in the use of 2,4-D in agriculture. The most frequent and far most important is its use as a herbicide. A second application is the use of very low doses applied to some crop species with the objective of modifying their characteristic growth and development in favor of the productive yield (Wort, 1962; Wort, 1966; Afolabi, 1969; Johnson, 1970; Miller et al., 1962).

References to the use of 2,4-D as a straight herbicide will not be included in the present review. It would be a redundant and partial transcription of information already divulged in well-known textbooks (Audus, 1964; Crafts and Robbins, 1962; National Academy of Sciences, 1968).

Afolabi (1969) found in one experiment that doses of 2,4-D of 25 g/ha delayed maturity of soybeans by three days and reduced plant height from 103 down to 83 cm. The yield of seed was increased about 200 kg/ha or 8% above the untreated control. In other experiments he used 2.5 g/ha and 250 g/ha but did not include the 25 g/ha level which appeared to be a critical one. The extreme low and high doses were ineffective and

definitely detrimental respectively. Johnson (1970) conducted further field experiments on the effect of subherbicidal doses of 2,4-D on the growth and yield of soybean. An application of 22 g/ha that compares with Afolabi's 25 g/ha hardly reduced plant height by 2 cm and delayed maturity by 2 days. The effects on yield were from nil to 5% increase in a 3000 kg/ha crop. Thompson (1970) found that "subtoxic" doses of 2,4-D delayed leaf senescence in potato fields which resulted in increased yield of tubers. Actually he applied mixtures of 2,4-D plus some nutrients, so no evaluation of the true effect of each product is possible. West et al. (1960) found that 40 ppm of 2,4-D increased dry weight of cucumber seedlings. Miller et al. (1962) found that doses of 2,4-D up to 10 ppm increased the yield of beans and FeSO_4 nullified the detrimental effect of 100 ppm of 2,4-D.

Nature of 2,4-D action

It is generally accepted that the toxic action of 2,4-D is produced by its unaltered molecules. It is generally accepted that biochemical inactivation or degradation is due to decarboxylation, oxidation and/or cleavage of the ring structure in some way. Two relatively recent works have reported different results, however. Linscott et al. (1968) found that in alfalfa the inactivation of 2,4-D appeared to be associated with the lengthening of the side chain. Tutass (1967) found experimental evidences that monochloroacetic acid, derived from the metabolism of 2,4-D, was the actual active agent of 2,4-D toxicity.

Holm and Abeles (1967) studied the relationships among 2,4-D, ethylene, and nucleic acids metabolism in soybean seedlings. They found that both

ethylene and 2,4-D could cause swelling in the seedlings and increase RNA, DNA and protein content in the subapical region. From their results it appears that at least some of the 2,4-D effects are mediated by the effect on ethylene production. One of their experiments shows that 100 ppm of ethylene very well duplicated the effects of a 10^{-3} M dose of 2,4-D on seedling elongation, inhibition, and swelling.

Morgan and Hall (1962) determined a 26-fold increase in ethylene production by cotton plants treated with 2,4-D. No significant increases in ethylene could be determined in sorghum, a resistant plant, when treated with 2,4-D. The stimulation of the production of ethylene by 2,4-D appears not to be a direct one. No radioactive ethylene could be detected in plants treated with C^{14} labeled 2,4-D.

The action of 2,4-D on nucleic acid metabolism

Chrispeles and Hanson (1962) determined large increases of RNA content in the microsomal and soluble fractions of soybean hypocotiles treated with 10^{-4} M 2,4-D. They proposed that one action of 2,4-D is the renewal of nuclear activity of the tissues, leading to an increased synthesis of RNA and cell proliferation. The cytochemical basis of the herbicidal action of 2,4-D would be therefore a sort of uncontrolled reversion of some tissues to a meristematic condition. West et al. (1960) found that herbicidal doses of 400 and 800 ppm increased the contents of RNA and protein in cucumber seedlings on a per gram of fresh weight basis but apparently not in absolute amounts. Key (1969) reviewed numerous experimental findings on the relationships between hormones, including 2,4-D, and the metabolism of the nucleic acids. From them he concluded that

Possibly the simplest interpretation of these observations, when taken together, is that the hormones are involved in the regulation of the synthesis of specific RNAs (transcriptional control). These RNAs would then serve as templates for the synthesis of the proteins required for the physiological response in question. The evidence for such a role for the hormones is at best indirect and fragmentary at present.

Key et al. (1966) investigated the relationships between 2,4-D-induced growth aberrations and its effects on the metabolism of nucleic acids. They found that cell division and elongation were inhibited in the apical region but cell division was increased and led to root formation at the base of the hypocotyl. Their results are therefore consistent with the hypothesis that the herbicidal effect of 2,4-D is due to the induction of disorganized growth of the tissues.

The auxin-like action of 2,4-D Studies by Holm and Abeles (1967)

strongly support the concept that 2,4-D is or acts like auxins. They found that applications of 2,4-D to soybean hypocotyls increased the evolution of ethylene and that applications of ethylene in turn mimicked the effects of 2,4-D. It was found that 1 ppm of ethylene or 10^{-4} M 2,4-D reduced hypocotyl elongation by 50%. 10 ppm of ethylene or 10^{-3} M 2,4-D reduced elongation 100%, and caused marked swelling of the hypocotyl and increased the content of RNA, DNA and proteins. A 15% CO₂ atmosphere nullified the effects of 1 ppm of ethylene.

Van Overbeek (1959) also discussed the similarities and differences between 2,4-D and 3-indoleacetic acid (IAA), the normal auxin existing in plants. 2,4-D acts also as an auxin but it does not induce IAA-oxidase, and it is more stable in the plant. From these characteristics it results that an abnormally high level of auxin takes over the plant

when 2,4-D is applied. The failure of the IAA-oxidases to regulate the activity of 2,4-D initiates a general perturbation of the patterns of growth.

Other modes of action of 2,4-D Hallman (1970) investigated the effects of solutions of 2,4-D applied to leaves of Phaseolus vulgaris. He used microhistology and electron microscopy techniques to observe the epidermis and the chloroplasts. He was able to observe and photograph very striking changes in the chloroplasts: disappearance of starch granules, rupture of the outer membrane, disorganization of the thylakoid structure. The symptoms described were observed only in leaves treated with 2,4-D in the presence of light and as soon as four hours after the application. He did not observe any major modification of the cuticle, and entry appeared uniformly distributed all over the area covered by the drop. From his results the author suggests that the chloroplast could be the prime site of 2,4-D action. Actually the effect on chloroplasts could well be mediated by events occurring sooner than four hours after treatment.

Crafts (1961) pointed out that many processes studied in relation to the action of the herbicides may well have no direct causal relationship. They are only a remote consequence mediated by other steps we had been unable to detect. The effects of 2,4-D in plants appear to be multiple as reported by the National Academy of Sciences (1968): cell enlargement, mainly in one direction; epinasty; abnormally enhanced growth; reversion of vascular differentiation which apparently leads to a functional collapse of the translocation in the phloem.

Moreland (1967) also pointed out in his concluding remarks to the lack of distinction or separation between the primary or direct effects and those that are secondary or indirect. He also notes the fact that the detectable symptoms of the action of a biologically active compound can occur far away from the actual site of action within the plant. These types of phenomena make especially complicated the interpretation of experiments with whole plants. It is probably a major reason to prefer working with simplified systems like part of organs or tissues.

Factors Affecting the Activity of 2,4-D

Penetration and translocation

Two obvious factors that will affect the response of plants to a biologically active compound applied externally is the actual portion of it that enters into the plant and the amount that finally reaches the region where its action or effect is produced. The variability of results from experiment to experiment so frequently found can be better understood (and it will make us more cautious) if we consider the number and variety of factors affecting the final activity of a compound upon its application to the plant. Some sample works will be cited.

Sargent and Blackman (1961) studied factors affecting the entry of 2,4-D in leaves of beans. They found that young leaves, eight days old, a relatively low water status, surface agents, low pH, and light were factors favoring the entry of 2,4-D. Q_{10} was 2.3 to 2.8 when temperatures were between 3° and 37°. The entry also was proportional to the stomatal density in the light. The abaxial face absorbed more than the adaxial one. Addition of IAA increased entry of 2,4-D, and TIBA

decreased it in this case. Amounts penetrated into the leaf disc were directly linear with concentration in the solution from 0.1 to 200 ppm. The experimental conditions used by these authors were very different from field conditions. They consisted in "sticking" a small glass cylinder to a leaf disc, and applying a volume of 2,4-D solution in the tube during a period of time. Volumes of liquid applied were large enough as to make the decrease in concentration due to absorption insignificant during the duration of the experiment. The larger absorption by the abaxial face in light is explained in part by the larger stomatal density on that side of the leaf. The effects of IAA and TIBA would indicate that the absorption is somehow an active process, either directly or indirectly, by the rapid binding of 2,4-D into the tissues which would help to keep a favorable gradient for penetration. The National Academy of Sciences (1968) cites the following factors that affect activity of compounds applied to the soil: adsorption, volatilization, pH, microbial activity and leaching.

Bryan et al. in 1950 reported a study of the effects of temperature on the absorption of 2,4-D and found Q10s of the order of 2.0 or larger, indicating the presence of a metabolic control of the absorption. They also demonstrated the increased toxicity of a given dose of 2,4-D by addition of surface active agents, especially nonionic wetting agents. Sugars increased the effect of 2,4-D only when applied at low doses of 0.5 to 1.0%. Barrier (1956) also found Q10's about 2.0, suggesting that absorption of 2,4-D was an active process. Translocation of 2,4-D lagged behind absorption by two hours, suggesting the existence of

two independent processes. The role of surfactants or adjuvants can be one of more significance than simply lowering the surface tension (Parr and Norman, 1965). They make a strong point about the need for including two controls in the experiments, namely one control receiving the surfactant and another one without it, in order to detect the effect of the surfactant itself on the plant.

I feel that the method of measurement can significantly affect the results obtained in studies of absorption of compounds. To estimate the amount penetrated into the leaf, some authors wash the leaf with an "adequate" solvent after a predetermined period of time since the application (Blacklow, 1966). The amount collected in the "washings" is measured and subtracted from the total applied. The balance is accounted for the absorption. It may overestimate the amount absorbed because direct losses from the plant surface are not considered apart. From what has been reported on the characteristics of leaf surfaces, I cannot visualize a clear-cut, well-defined line of separation or interphase between "absorbed" and "unabsorbed" states, but a continuum of molecules. Those retained in the heterogeneity of the outer layers of the leaf would be arbitrarily washed out or retained (and eventually penetrate into the leaf) depending on the severity of the washing procedure and/or the characteristics of the leaf surface. Therefore the "absorbed" and "unabsorbed" fractions, in many cases, appear poorly defined in practice.

The fate after penetration and selectivity

The physical-chemical fate of the molecule of 2,4-D after penetration into the plant has been extensively pursued during the last twenty

or twenty-five years. Radiolabeled 2,4-D and chromatography have been two major tools for that pursuit. Radioactive 2,4-D is usually labeled with C^{14} in the carboxylic or in the ethylene carbons. Most of the studies have, with variable degrees of modifications and sophistication, followed the following scheme. Radioactive 2,4-D is applied to a suitable part of the plant and a time lapse allowed for the product to act. Samples are taken from different plant parts. Radioactivity is counted. Chromatography of the plant extracts and their radioassays indicate whether the radioactivity remains in the unaltered molecule of 2,4-D or is in some derived compounds. Refinements in the radiocounting and chromatographic techniques have permitted the use and detection of minute amounts of materials (Newman, 1970). Autoradiography is another important tool to study the distribution of a labeled molecule into a plant. The method consists of exposing sensitive films to the radiation emitted from plants or parts treated with a radiolabeled sample of the compound under study. The position and intensity of the impression give an estimate on the pattern and intensity of translocation. Even if it is hardly more than a qualitative technique, it has been extensively used, particularly by Dr. Craft's group at California (Crafts and Yamaguchi, 1968). It appears as an important contribution in the elucidation of mechanisms and ways of herbicide translocation, as well as in the elucidation of differences among species (Crafts and Yamaguchi, 1968). Crafts (1961) outlined some general principles on the movement of 2,4-D in whole plants, based on his studies. Plant tissues have the ability to accumulate 2,4-D. The first site in the path of foliarly

applied 2,4-D is the chlorenchyma of the treated leaf. Parenchyma along the translocation path also binds 2,4-D. The higher the dose, the farther the movement. A marked gradient from source to sink is a strong determinant of 2,4-D movement. The age of the treated leaves and their relative position in the plant also affect the pattern of translocation. For example, spraying only the top leaves in a perennial would not allow the chemical to reach the roots and kill the plant.

Hay and Thimann (1956) conducted studies on the fate of 2,4-D in bean seedlings grown in nutrient solution. The amount translocated was proportional to the dose applied up to 75 μ g per plant. Higher doses appeared to affect the tissues involved in translocation. Similar amounts of 2,4-D or derivatives were detected in phloem and xylem. However, cutting a ring in the phloem resulted in a complete stop of 2,4-D transport. Sucrose was found to activate transport of 2,4-D in the dark. Translocation appears to be a relatively inefficient mechanism, especially in monocots. Studies by Fang and Butts (1954) showed that the proportion of radioactivity translocated from the treated leaf to the rest of the plant in a period of five days was at most 9% in corn and only 2% in wheat.

Freed and Montgomery (1969) have recently reviewed the metabolism of 2,4-D in plants. Reactions of hydroxylation, decarboxylation and conjugation have been determined for 2,4-D applied to plants. Its inactivation in the presence of riboflavin also has been demonstrated. The metabolism of the side chain results in the formation of chlorinated phenols. The lack of ability for β -oxidation of the side chain by some crop species like alfalfa gave a basis for selectivity.

Blacklow (1966) in his review grouped the compounds obtained from 2,4-D-treated plants in six types: extractable unaltered 2,4-D, free metabolites, bound metabolites, conjugates of 2,4-D, products of decarboxylation, and products derived due to lengthening of the side chain. In considering the fraction of extractable 2,4-D, the most important thing is probably the relative amount translocated into the sensitive tissues. Authors cited elsewhere in this review report cases where only a tiny percentage of the compound moved out the treated leaf. The fraction that is not extractable with ethanol or mild hydrolysis constitutes the bound metabolites. The free metabolites are those readily extracted with alcohol or ether. Under the group of conjugates of 2,4-D are considered the derived compounds that are ether insoluble or non-acidic, and for which indications exist that they are 2,4-D bound to sugars or proteins.

Because the principles of and reasons for the selective action of herbicides have both been logically searched in the area of the physical-chemical fate of the compound upon application, both aspects will be reviewed together. The term "herbicide selectivity" as defined by the American National Academy of Sciences (1968) applies "to the use of a chemical to kill individual plant species in a mixed plant population without harming or more than slightly affecting other plants." It appears to be a practical, working definition. Perhaps one could be more generic and say that selectivity is a differential effect of a given treatment on plant species in a given situation.

Biochemical differences have been proposed and in many cases demonstrated experimentally as one major reason for selective action of 2,4-D. Extensive studies comparing the fate of 2,4-D in tolerant oats versus susceptible soybeans and sunflowers were conducted by Rakitin and coworkers (1966). They found that, three days after application, about 50% of the absorbed 2,4-D was in a bound nonextractable form in oats while only 2.6% was bound in sunflowers. Crosby (1964) studied the nature of 2,4-D metabolites in bean plants. He found that the ether extracted substance was the unaltered 2,4-D molecule. The ether insoluble fraction yielded 2,4-D and other chlorinated compounds upon acid hydrolysis, but no 2,4-dichlorophenol or its derivatives could be detected. He noted that tracer technique alone, using carboxyl- or ethylene carbon labeled 2,4-D, gives us indications about the fate of the side chain but not about the ring structure, and chromatographic detection of chlorinated compounds would contribute to a more complete idea.

Jaworski and Butts (1952) studied the metabolism of 2,4-D in bean plants. They found that the decrease of 2,4-D content in the plant was very marked by the fifth day after application. It was closely associated with the formation of another compound ("unknown 1") which was ether insoluble and yielded back 2,4-D upon acid hydrolysis. The similarity of results using 2,4-D labeled either in the carboxylic or in the ethylene carbon appeared to indicate that decarboxylation did not occur readily in this experiment. Morgan and Hall (1963) compared the metabolism of 2,4-D in cotton and sorghum. They found that the decarboxylation (evolution of

$C^{14}O_2$ from 2,4-D) was much faster in cotton than in sorghum. A large proportion of C^{14} appeared as "2,4-D-complex 3" in sorghum whereas in cotton most of it remained associated with the fraction of unaltered 2,4-D. Chkanikov and Pavlova (1966) compared the relationships of 2,4-D to plant proteins between tolerant and susceptible species. No 2,4-D was found bound to any of the major protein fractions in beans and sunflowers, whereas in oats and wheat, concentrations of 2,4-D of 225 and 164 $\mu g/100$ g protein were determined respectively. Resistance to toxic 2,4-D appeared, at least in part, associated with the ability for conjugation with proteins.

Rakitin et al. (1966) conducted comparative studies on the fate of 2,4-D in tolerant oats and the susceptible dicots sunflowers and soybeans. They consistently found that in oats the largest portion of 2,4-D was bound in inactive forms. Soybeans and sunflowers, in contrast, excreted more than 50% of the applied 2,4-D out the roots to the nutrient solution. Although the dicots were able "to get rid" of a significant amount of the compound, actually the molecule of 2,4-D was in the plant in a very mobile or active state.

Luckwill and Lloyd-Jones (1960) studied the fate of 2,4-D in susceptible and tolerant varieties of grapes, and found that the rate of decarboxylation was significantly higher in the tolerant variety, and accounted for 50% of the applied 2,4-D.

O'Brien (1968) studied the four possible points in the fate of 2,4-D where selectivity between beans and oats could possibly be manifested, namely spray retention, absorption, translocation and metabolism of the

compound at the target site. He concluded that in oats the lack of toxicity was associated with its inability to translocate or export 2,4-D from the treated leaf. He could not associate this lack of translocation with either larger phloem destruction or a failure in the translocation of general assimilates out the leaf.

Kiermayer (1964) suggested that the generally greater susceptibility of the dicots to 2,4-D could be associated with the fact that this group has more cambial tissue associated with the vascular system, whereas in the monocots the phloem is surrounded by tissues more highly differentiated and consequently less sensitive to 2,4-D.

Blacklow (1966) investigated the possible causes of the different susceptibility of two strains of Lotus corniculatus to 2,4-D. He found that the more tolerant variety yielded twice as much 2,4-D after acid hydrolysis of the ether-nonextractable fraction. Selectivity to other herbicides also exists and has been studied in a similar manner (Stoller, 1969; Slife et al., 1958).

Extensive studies comparing the absorption of 2,4-D by the roots of tolerant cereals and susceptible dicots were carried out by the Oxford group (Blackman, 1961). Characteristic differential patterns and rates of absorption were found in both groups of species. The tolerant species sustained a steady rate of accumulation during 33 hours, whereas the susceptible ones showed a steeper rate of initial accumulation up to a peak reached after four to eight hours. Then a net loss of 2,4-D from the plant back into the nutrient solution occurred.

In general, it is apparent that degrees of tolerance to 2,4-D were associated with different mechanisms in different species and/or experimental situations. The most consistently reported phenomenon appears to be the ability of tolerant monocots to bind the compound in an inactive form in contrast with a much greater activity in the dicots, in spite of its ability to excrete significant amounts of 2,4-D out the plant. The cases where the selectivity is determined by differential retention of the spray, differential absorption or external factors were not included in the present review.

The Action of TIBA on Plants

The effects of TIBA on plants were reviewed by Greer (1965). Afolabi (1969) summarized part of the review. Johnson (1970) included a few additional references more recently reported.

The following effects have been reported once or more associated with applications of TIBA to different species: induction of flowering from vegetative buds in tomato, increased flowering in plants previously induced, increased growth of axillary buds, inhibition of apical dominance, delayed maturity in soybeans when sprayed before flowering, hastened maturity of soybeans when applied late, increased number of pods set, decreased plant height and lodging in soybeans. Its effects on the seed yield of soybeans are apparently associated with increased pod setting and plant resistance to lodging. These effects on yield have varied from nil to a moderate 15-20% increase (Greer and Anderson, 1965). Aberg (1953) suggested three dose-dependent effects of TIBA on plant growth. Very low doses act like weak auxin itself or perhaps by a

synergistic effect on the residual endogenous IAA of the plant tissue.

- Higher doses are manifested by an antiauxin effect. Its effect is reversed by external applications of IAA or 2,4-D. Finally TIBA may exert plant toxicity when large amounts are applied. This toxicity appears unrelated to the antiauxin effect. Whitting and Murray in 1948 conducted studies on the response of beans to TIBA. The effects depended markedly on the mode of application. A 2% TIBA in lanolin paste applied to decapitated apices produced only small tumors localized near the treated area. Applications in the forms of "ring" or sprays resulted in morphological effects in other parts of the plant. Snyder (1949) found that concentrations of 5 ppm of TIBA were able to depress rooting and stimulate bud development in cuttings of Coleus blumei Beuth. Krause and Boke (1968) found that doses of 50 ppm of TIBA altered the normal correlation between the growth of the veins and the mesophyll in soybean leaves. Deformed leaves resulted as a consequence. He suggests that TIBA acted by interfering with the normal pattern of auxin distribution in the leaf. Ghorashi et al. (1969) studied microscopically the effects of TIBA on the structure of leaves and petioles as well as macroscopic morphological modifications. They found that TIBA produced more branching, shorter stems, asymmetric distribution of vascular tissue, poorer distribution of leaf veins, smaller leaflets, tetrafoliated leaves, fused vascular strands, and abnormal differentiation of tissues. The symptoms were apparent only in organs developed after the application of TIBA. Niedergang-Kamien and Skoog (1956) attributed to TIBA the inhibition of polar transport of IAA in tobacco stem sections. Bud development

in the upper part of the sections was increased as a consequence. Gorter (1949) found that 20 ppm of TIBA in a nutrient solution greatly interfered with the development of root hairs. Instead, an unorganized mass of tissue and cellulose was formed. Ohki (1968) carried out nutrient culture studies to determine effects of root-applied TIBA on the absorption of nutrients and growth of soybeans. Typical signs of TIBA effects were noticed in the leaves when 5 and 10 ppm TIBA were applied. In the roots, visible morphological effects were caused by doses as low as 0.1 ppm. The only nutrients affected were phosphorus and iron. There was a 25% increase in the absorption of P and a decrease in Fe content of the tops of plants treated with TIBA. Works reported by Libbert (1959) and Keutzer and Libbert (1961) indicated that TIBA is able to block the transport of other substances in addition to IAA. Gibberellin was one of the inhibited substances. To these authors it appeared that TIBA was able to inhibit the general transport of energy-requiring substances. Experiments by Vardar in 1968 indicated that TIBA decreased the transport of IAA by 20% but the total uptake of IAA by oats hypocotyls was actually increased. This result is to some extent coincident with Winter (1967). Keitt (1968) reported his works on acropetal transport of auxin and reviewed others. He found that TIBA also caused reduction in the acropetal movement of auxin, although the effect was less drastic than on the basipetal transport. Blackman and Sargent (1959) reviewed several studies on the action of TIBA in plants. They concluded that TIBA can show either auxin-like or antiauxin effects depending on the experimental conditions. Their own work on the absorption and translocation of TIBA

in Lemna minor showed that the uptake of the compound increased up to a peak during the first two to five hours, depending on the concentration applied. Thereafter, a net loss back to the nutrient solution occurred. This behavior was very similar to that of 2,4-D in similar conditions.

The fate of TIBA after absorption has been much less studied than its visible effects. Sant' Anna et al. (1970) investigated the distribution of I^{131} -labeled TIBA into the soybean plant. The radioactive sample was applied to a group of plants. They were successively harvested at intervals and the radioactivity in different organs counted. 45% of the total activity had been lost 48 hours after treatment. They found positive correlations between meristematic or growth activity of a plant organ and its ability to accumulate radioactivity. Up to 7% of the total applied radioactivity and concentrations up to .56 ppm of I^{131} were detected in the seeds even 60 days after the treatments had been applied. The relative increase of radioactivity in the pods as they grew would indicate a retranslocation of TIBA retained in older organs. Almost fantastic speeds of translocation to the roots and to the pods were reported. Measurable radioactivity was detected in those organs at "time zero."

Polarity and Apical Dominance

Because of the auxin-like properties of 2,4-D and the effects of TIBA on growth correlation in plants, it appears pertinent to consider work done in the field of polar transport and apical dominance.

Leopold and de la Fuente (1968) reviewed studies on polar auxin transport. As a form of an active process, transport should be characterized

by the following properties (Danielli, 1954). It is driven by metabolic forces and is capable of accumulation against a concentration gradient; it has velocities greater than diffusion, shows selectivity and, because it involves some sort of chemical attachment to sites of transport, it should show a concentration saturation. From studies conducted by Hertel and Leopold (1963) and by Christie and Leopold (1965) it is apparent that auxin transport involves a secretory process by the cells. They consider that the concept of polarity has recently changed from an absolute to a relative one. As it is visualized by these authors, polarity is a sort of balance of basipetal minus acropetal movement of a substance. Polarity appears now to be a dynamic rather than fixed parameter of the tissues; namely, polarity appears to decrease as the tissue ages. It also has been found that polarity increases as the length of the section of tissue considered increases. This correlation is made a major reason in favor of the hypothesis that polarity of a segment of tissue is made up of the successive differential secretions between the opposite extremes of each individual cell. A strong polar movement would result from the successive differentials between "top" and "bottom" of thousands of cells, even if within each single cell only a minute differential exists. Another work from Leopold's group, Leopold and de la Fuente (1968), reports that velocity and polarity of transport appear to be two distinct magnitudes, driven by different mechanisms. Goldsmith (1968) reviewed the polar transport of auxins in higher plants. She considers that the three major characteristics defining transport are: active, basipetal, independent of doses applied, and on the length of the piece of tissue considered. From the fact that the effects of inhibitors are stronger

when applied to the basal end, it appears that uptake is a passive phenomenon and only exit or secretion is the really active step. She thinks that acropetal movement is mostly by diffusion. De la Fuente and Leopold (1970) investigated the characteristics of auxin transport in stem sections of sunflowers. Their results suggest that the transport of auxin can be limited by the pool size of mobile auxin or by the capacity or availability of transport sites. Capacity for transport appeared largely independent of the amount of auxin accumulated in the tissue. McCready (1963) compared the polar transport of IAA and 2,4-D, and found that both products behave in a similar manner. The major difference is that 2,4-D has a translocation speed of only 1 mm/hr, whereas IAA was translocated at 6 mm/hr. In addition, 2,4-D sustained a steady rate of transport longer than IAA. According to Blackman, the polar system results in 2,4-D-"saturation" at very low concentrations. It seems that, for most practical situations, the phloem and the xylem are the major ways for the movement of 2,4-D in plants. The integrity of the vascular system is instrumental for an effective action of 2,4-D (Crafts, 1961). Vardar (1967) reviewed the historical evolution and the current ideas on the phenomenon of apical dominance. Three groups of ideas on the control of growth were considered: (a) hypothesis not considering auxin as the agent of apical dominance, (b) different views that do consider auxin the major control and (c) "recent" theories, involving interaction of several factors. Before the existence of plant hormones was demonstrated, it was believed that somehow the apical bud was dominant in its demand for nutrients. The lateral buds could not

grow because of nutritional limitations. Even in 1957 Gregory and Veale found that plants of Linum branched abundantly if provided with adequate amounts of nitrogen and carbohydrate, and that applications of IAA to the decapitated main stem did not have any control of branching in plants grown in these conditions. The control of apical dominance by hormonal factors has been most accepted since 1925 when Snow demonstrated the transmissibility of the inhibitory effect from a grafted plant. Basically the idea is that growth response to auxin is very sensitive to concentration or activity. There is a linear positive response at low levels, an optimum is reached, and the effect is negative or inhibitory at supraoptimal activities. The apical growing point would produce auxin levels that are supraoptimal for the lateral buds. Yet a point of argument is whether the control is exerted by a direct translocation of a supraoptimal amount of auxin to the action site or is an indirect one via feedback inhibition of precursors, or by controlling the synthesis of specific inhibitors of lateral buds. The latest hypothesis on the mechanism of apical dominance was initiated by Wickson and Thimann (1960). The hypothesis was summarily stated by Thimann (1965) saying that "Apical dominance depends on an interact between two hormones, auxin and kinetin." Kinetin appears synthesized in sites in the lateral buds and, depending on its level, it may counteract the auxin effect and "release" the lateral buds for growth. Phillips (1969) also published a recent review on apical dominance.

Interactions

Generalities

In a sense, all biological processes are interaction phenomena, for life, as it is visualized or sensed by man, appears to be something more than the adding up or putting together of a number of physico-chemical phenomena. The intimate mechanics of an interaction phenomenon is difficult to understand beyond the consideration of two or at most three factors. We seldom can find a second order interaction discussed in scientific articles.

Although the terms synergism, antagonism and interaction are widely used and people generally agree about their respective meanings, a brief statement will be made to better understand their use along the rest of the discussion. By interaction we understand that the response produced by two or more factors acting together is "different" from the addition of the individual effects of such factors acting singly. The "significance" of such difference will depend on the accuracy of the experimental conditions, on the model known or assumed to fit the nature of the responses to these factors, and on the objectives or application of the investigation. The first and third considerations appear obvious. Further comments are needed to make more clear how the model of action assumed affects the significance of a given interaction.

Audus (1954) critically discussed the methodological aspects involved in the study of antagonistic and synergistic compounds. He points out the oversimplification of equating vital processes with simple biochemical reactions which are nicely controlled by enzyme systems. In a living

cell, the system is amazingly variable and complex. Another point stressed by Audus is the need of a correct design of the experiments and proper statistical treatment of the data, in order to have a reliable estimation on the "value" of the data obtained. He considers it an important and grave fallacy when only the experiments with acceptably low coefficients of variability are reported. Actually, a false idea about the uniformity of results and the reliability of the technique is given in doing so. When we apply two interacting substances externally to a plant and measure their effects on growth, yield, morphology or even on physiological processes, we are measuring largely final effects. Descriptive and practical as they may be, these observations may not give much of an insight on the very nature and site of the interaction between both compounds.

Models for interaction of compounds active in plants

Two major alternatives are usually adopted. Many authors simply assume the additive model i.e. if factor A inhibits growth by 20% and factor B inhibits growth by 30%, then A + B together will produce a 50% decrease if they are additive, more than 50% if they are synergistic and less than 50% if they are antagonistic.

Gowing (1960) discussed criteria for evaluating synergism which were further developed by Colby and coworkers (Colby et al., 1965; Colby, 1967; Colby and Feeny, 1967). Gowing suggested that the reason for synergistic action of two similar compounds could be that the two compounds were of similar action, with affinity for the same sites for toxic action, and also those for detoxification. Then, if the least toxic of two compounds

has however the greatest affinity for the site of detoxification, the "proportional" partition of the two compounds will result in larger amounts of the most toxic compound being available for toxic action, because it will be largely spared at the detoxification sites. He also gave consideration to the fact that active compounds have particular dose-response curves. Their combined effects may largely depend on the points (doses) of their respective response lines taken in consideration. He also noted that the practical criteria and conditions prevailing in field plot experiments could well be not the most adequate ones for studies on the principles of combined action of chemicals on plants. For example, the "required" dose for 50% kill is considered more accurate criterion for comparative studies of biologically active compounds. We seldom would feel satisfied with such performance of a herbicide or insecticide in the field.

Colby (1967) is the only author, to my knowledge, who has recently given attention to the "mathematical" aspect of synergism and antagonism of herbicides, since after Gowing (1960). His method to detect synergism or antagonism between two compounds consists basically in computing an "expected" relative growth with respect to the untreated control, to be caused by the combined application of the compounds, based on the "observed" relative growths obtained with the same doses of such a compound when applied separately. The expected relative growth E can be expressed by

$$E = x y \quad (1)$$

where x and y are the relative growths with the doses p and q of the products A and B , respectively, when they are applied singly. E is the "expected" growth or yield associated with the same doses p and q of the products, applied together. Synergism is said to exist when the values actually observed or measured are smaller than E , in the case of an inhibitor, or larger than E if the effects are stimulatory. Actually, Colby discussed his model only with respect to inhibitors.

To me the formula developed by Colby implies that the products act separated in time or else they act on different mechanisms of the plant; and it also implies that the relative growth under a given dose of single produce is proportional to the growth potential of the plant were it not treated.

Nitsch and Nitsch (1961) adopted without hesitation a simple additive model in their work reported on synergists of natural auxins and gibberelins. They point out that synergism can well be an indirect or secondary relationship. They suggest that some compounds like chlorogenic acid and glutathione may be synergistics of auxins by their direct inhibitory action on IAA oxidase. Van Overbeek (1966) also presents the example of caffeic acid which appears as an auxin synergist by its inhibitory effect on the activity of IAA-oxidase.

The distinction between Colby's and the additive model appears irrelevant in the case when active herbicides or other toxic substances are combined with substances that are not biochemically active themselves. Here, any modification induced by the inactive compound on the effects of the active ones can be interpreted as synergistic or antagonistic, independently of the model assumed.

Interactions involving TIBA

Most of the studies in this category refer to the effect of TIBA on the activity of IAA and other auxin-like compounds. Thimann and Bonner (1948) found that concentrations of TIBA as low as 0.2 ppm were strongly synergistic with auxin but it was inhibitory at 25 ppm. The effects were measured by the split pea stem test (Mitchell and Livingston, 1968). It was found that, in general, the addition of TIBA enabled small amounts of IAA to cause disproportionate responses in growth. Audus and Bakhsh (1961) studied the effect of TIBA on the rooting activity of IAA. Based on their results, they proposed that the anti-auxin effect of TIBA was because TIBA induced the activity of IAA-oxidase. This does not appear to be a complete explanation, at least, because TIBA also was active against 2,4-D, which is not known to be regulated by IAA-oxidase. Niedergang-Kamien and Leopold (1957) studied the effect of TIBA on auxin transport and physiological processes in stem sections of sunflowers. Doses of TIBA as low as 10^{-5} M produced 88% inhibition of IAA transport and consequently on extension growth. However, no TIBA effect on respiration was apparent. This would indicate a specific effect of TIBA on translocation, independent from effects on respiration. Data on respiration in this article appear to me quite strange--so doubtful. Hay (1956) studied the effects of TIBA and 2,4-D on the rate of polar transport of auxin. He used the "stem section between agar blocks" technique to measure transport and bioassay to measure auxin activity. His results indicated that the transport of IAA to the receiver agar block was greatly inhibited by either TIBA or 2,4-D. Actually, only a very small proportion of the total IAA applied

in the donor was translocated into the receiver even in the controls without TIBA. Winter (1967) reported one of the most elegant (simple) and critical works on the effect of TIBA on the activity of IAA. He used the same technique that did Hay (1956), but he was able to measure the contents of IAA at the end of the experimental period that were present in the donor block, in the receiver block and in the stem section itself. He demonstrated that under his experimental conditions the total uptake of IAA by the tissue was not significantly affected by TIBA. The amount of IAA excreted out the tissue into the receiver agar was reduced by the addition of TIBA to only about 30% with respect to the untreated control. The fraction of absorbed-nontranslocated IAA was uniformly distributed along the coleoptyl section and was recovered as the unaltered molecule of IAA upon extraction with diethyl ether. Christie and Leopold (1965) had already shown that the effect of TIBA is greater on the exit than on the entry of IAA into the section of tissue. Niedergang-Kamien and Skoog (1956) reported the effects of TIBA on the auxin-induced differentiation of organs in pieces of tobacco stems. Addition of TIBA modified the distribution of active auxin. Buds and roots were formed in any part of the tissue when TIBA was added. Newman (1970) reported an apparently detailed and accurate study on the kinetic aspects of auxin transport as affected by TIBA. The use of a C^{14} detector with an anti-coincidence circuit, and counting times in the order of several hours allowed him to detect, as significant, differences as small as 0.1 CPM. He again found that the effect of TIBA was the inhibition of basipetal transport. He also found indications that TIBA interferes with IAA in situ.

Interactions between TIBA and 2,4-D

One of the earliest references to interaction between TIBA and 2,4-D is Frans (1955). His data show that both TIBA and 2,4-D were inhibitory to soybean growth. The inhibition was less than additive when both compounds were applied together. Sargent (1968) reported that the effect of TIBA on the uptake of 2,4-D by isolated leaves of *Phaseolus* was sophisticatedly dependent on the experimental conditions. He found that TIBA could increase the uptake of 2,4-D only if both products were applied simultaneously, under illumination, and to young leaves. This example well illustrates the "delicate" nature of biochemical interactions and how easy opposing results can be obtained by simply overlooking some of the experimental conditions. Widholm and Shaffer (1969) at the Growth Sciences Center, Libertyville, Illinois, carried out studies on the combined effects of TIBA and 2,4-D on several processes in sections of soybean hypocotyls. They found that 10 ppm of TIBA alone was slightly inhibitory on fresh weight growth but did not affect the significant stimulation in growth produced by 5 ppm of 2,4-D. 100 ppm of TIBA almost nullified the 2,4-D stimulation. 2,4-D at 5 and 20 ppm sustained a steady increase of accumulation in the tissue during the first four hours of treatment and a reversal trend showing net output from the tissue thereafter. The addition of TIBA at 50 ppm nullified that tendency to release 2,4-D back to the medium. Both TIBA and 2,4-D increased the uptake of oxygen. Their combined effect appeared less than additive. 5 ppm of 2,4-D alone increased the synthesis of DNA and RNA by 25% and the addition of 100 ppm of TIBA nullified the effect. No data on the

effects of TIBA alone were given. In general, it appeared again that TIBA may counteract the biological activity of 2,4-D but probably not its absorption and movement into the plant. Afolabi (1969) conducted one field experiment where the combined application of doses of TIBA and 2,4-D was markedly depressive on soybean yields. Whereas both increased yields with respect to the control when applied alone.

Interactions of 2,4-D and other compounds

McRae et al. (1953) studied and theoretically discussed the interacting effects of IAA, 2,4-D and naphthalene acetic acid (NAA) on the elongation of oat coleoptile sections. They concluded that the three auxins, being structurally related, compete for the same sites to form a "regulator-substrate" complex which is finally the active factor for growth. Based on the "optimum-type" of dose response curve usually found for auxins, they concluded that an interaction between two growth factors can well be either positive or negative, depending on the doses considered. Their own experimental results fairly well substantiate their theory.

Hull (1967) cites several cases in which the simultaneous application of ammonium salts increased the effectiveness of phenoxy herbicides. Ammonium sulfate, nitrate, or phosphate increased toxicity of 2,4-D on Sonchus arvensis L. and Polygonum aviculare L. in one of the cases reported. James et al. (1970) reported on the interaction between herbicidal carbamates and 2,4-D. Polygonum hepaticifolium L. and Amaranthus retroflexus L. were severely affected by 75 or 150 g/ha of 2,4-D

respectively, but no toxic symptoms were apparent when isopropyl-m-carbamate (chloroprophan) at 1.2 kg/ha also was applied, indicating an antagonistic action. Parallel studies with labeled 2,4-D indicated no effect of chloroprophan, 2-methoxy-2,6-dichlorobenzoic acid (dicamba), or 4 amino-2,5,6-trichloropicolinic acid (pichloram) on the uptake and translocation of 2,4-D. Their results suggest, by elimination, some unknown biochemical interaction between those compounds and 2,4-D. Beste and Schreiber (1970) also have reported antagonistic action between 2,4-D and the carbamate ethyl N,N-diprophylthiocarbamate (EPTC). Both products were applied at highly inhibitory levels. Results were consistent in showing antagonistic action on both top and root growth, according to Colby's method (Colby, 1967). A statistical treatment was not reported however. The authors suggest two possible explanations for the antagonistic action between EPTC and 2,4-D, although none of them are specifically supported by their results. 2,4-D may enhance the metabolic detoxification of EPTC or else 2,4-D and EPTC may balance or compensate their respective effects on nucleic acid metabolism in the plant.

Korolev and Voitekhova (1967) hypothesized that 2,4-D is usually more toxic on young plants because the young plants contain high amounts of some products synergistic to 2,4-D. They made homogenates from leaves of Raphanus spp. and oats and added different amounts of these filtered homogenates to 2,4-D solutions. The mixtures were up to eight times more toxic than 2,4-D alone when applied to sunflower seedlings. The effect of the homogenates applied alone was not reported. Lucas and Hammer (1947) also had found that onion extracts greatly increased 2,4-D toxicity, whereas the extract alone did not show any significant effect.

Agbakoba and Goodin (1970) reported results of a study on combined applications of 2,4-D and pichloram. They applied each of the compounds labeled with C^{14} , each one alone or both together, but only one of them labeled in each case. They found that the relative amount of 2,4-D translocated to the growing tip doubled when in presence of pichloram. Conversely, pichloram decreased 75% its relative translocation to the growing tip due to addition of 2,4-D and more of it remained in the roots. The authors do not report on visible effects in the plants.

Livingston et al. (1954) and Fulton and Payne (1956) in the same group carried out experiments on the individual and combined effects of 2,4-D and maleic hydrazide on the protein and free amino acids content of potatoes, sugar beets and beans. They found that maleic hydrazide increased the content of leucine, isoleucine, valine, and γ -aminobutyric acid; 2,4-D increased all amino acids in one experiment and decreased some in another case. Combined effects were mostly additive, and the results in general varied greatly with the environment and dates. Ennis and Boyd (1946) found that using 0.5% solutions of polyethylene glycol (Carbowax 1500) as a carrier enhanced the action of 2,4-D on kidney beans, in comparison to the aqueous solutions. Although not specifically reported, carbowax alone was probably ineffective on plants. Nolla (1948) reported that mixtures of 2,4-D with diesel or aromatic oils were more effective weed killers than any of the individual compounds, in sugar cane fields.

Davis and Bovey (1968) briefly reported on interactions among 1,1'-dimethyl-r,r'-bipyridinium salt (paraquat), pichloram and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). In many cases the interactions

found were with the effect of a compound on the uptake or translocation of the other one. The uptake of herbicides was actually controlled largely by environmental factors. The experiments were carried out on cotton, beans, and mezquite.

Hardcastle and Wilkinson (1970) tested numerous herbicide combinations on germinating rice seedlings under laboratory conditions. They used doses of each product capable of 50% inhibition of growth and applied them in all possible binary combinations. Trichloroacetic acid (TCA) was synergistic with 3-(p-chlorophenyl)-1,1-dimethylurea (monuron), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron), 2,2-dichloropropionic acid (dalapon), and 3-amino-1,2,4-triazole (amitrole). Dalapon and monuron were also synergistic. Antagonistic responses were obtained when maleic hydrazide or 2,4-D were combined with the substituted ureas, dalapon or amitrole. 2,4-D and TCA also were antagonistic. The variety and number of interactions found by the authors suggest an ample field for speculation and research.

Colby et al. (1965) compared the effects of diverse herbicide combinations on crabgrass (Digitaria sanguinalis), velvet leaf (Abutilon theophrastii) and tomato (Lycopersicum sculentum). They found that the addition of 50 g/h of paraquat allowed one to reduce the dose of 3'-chloro-2-methyl-p-valeritoluidide (solan) from 4 down to 2 kg/ha. Velvet leaf was resistant to either sodium-2,4-dichlorophenoxyethyl sulfate (sesone) or to dimethyl-2,3,5,6-tetrachloroterephthalate (DCPA), but quite susceptible to their combined application. Paraquat and solan were antagonistic when applied to tomatoes, and a suggested explanation

was that solan inhibits photosynthesis while paraquat needs active photosynthesis in order to be more activated in the plant. Synergistic effects were obtained almost only at low doses. They found particularly promising the possibility of improving selectivity by the use of mixtures. Their results were obtained in tomato and strawberry crops. Sweet and Lynch 1971 recently reported some effective applications of mixtures of herbicides showing synergism at Cornell University. Amounts as low as 320 g/ha 2-chloro-4-ethylamino-6-isoprophylamino-S-triazine (atrazine) and 2-chloro-2',6'-diethyl-n-(meroxymethyl) acetanilide (alachlor) were highly effective and consistent for weed control in sweet corn. Alachlor plus atrazine also were synergistic against Japanese millet. Corn appeared indifferent to the combination of alachlor plus atrazine.

GENERAL PROCEDURES AND METHODS

Experiments were carried out in the field, in the greenhouse and in a conditioned growth room. The plant species in one or more experiments were corn (Zea mays L.), oats (Avena sativa L.), soybean (Glycine max Merrill) and velvet leaf (Abutilon theophrasti Medic.). Natural populations of multiple weed species were obviously present in the field experiments and were the major component of weed yields. Table 1 summarizes the abbreviations to be used along this discussion.

Table 1. Abbreviations used to designate the different experiments according to their type and species grown

Type of experiment	Species grown	Abbreviation
Field experiments	Natural species and corn	F ^a
Greenhouse experiments	Oats	GO
Greenhouse experiments	Soybeans	GS
Greenhouse experiments	Velvet leaf	GV
Radiotracer experiments ^b	Oats	RO
Radiotracer experiments	Soybeans	RS
Radiotracer experiments	Velvet leaf	RV

^a A number subscript will designate different experiments of the same type.

^b All radiotracer experiments were carried in a conditioned growth room.

Greenhouse Experiments

Growing the plants

Plants were grown in the greenhouse in 10 cm (4 in) diameter earth pots or disposable food containers of polystyrene of similar size. No differences in the growth of seedlings were noticed in the different containers. A mixture 1:1:1 of sand, peat and loam soil was used. A fertilizer dose equivalent to 200 kg/ha of nitrogen, 88 kg/ha of phosphorus and 186 kg/ha of potassium was thoroughly mixed with the soil before potting. An excess of seeds were planted and the number of seedlings thinned to a uniform number per pot. Two plants of soybeans, three of velvet leaf and four to six of oats were the number of plants usually grown on each pot. Soybean seeds were enclosed in a humid atmosphere for 24 hours before planting. Velvet leaf seeds were treated during one minute in boiling water. This treatment resulted in uniform germination, consistently higher than 90%. The planting procedure consisted in partly filling the pots up to the depth of planting, applying abundant irrigation and letting it "settle" for a few hours. Then the seeds were laid on the moist surface and covered with additional soil containing little moisture. A light compacting of the soil was applied. Tap water was used for irrigation. Temperatures varied from 20°C. at night to 27°C. during the day. Occasional increases up to 30°C. occurred. A first group of experiments were conducted with no supplementary light. Most of the experiments reported, however, received supplementary light from incandescent lamps during the evenings and cloudy days. The intent was to obtain 15 hours illumination and 1200 foot candles minimum intensity.

Treating the plants

The growth stage of the plants when the treatments were applied varied somewhat from experiment to experiment but the most uniform group of pots were chosen for each individual experiment. In general, soybeans had developed a fully expanded first trifoliate leaf and the second trifoliate was between initial expansion or was half size. Velvet leaf was expanding the third or fourth true leaf above the cotyledonary pair. Oats were usually treated when the second leaf was fully expanded and the third one had shortly emerged from the whorl.

The application of 2,4-D was in general made to the youngest fully expanded leaves. Applications were made in 0.01 or 0.02 ml drops over the leaf surface near the petiole in soybean and velvet leaf and about one third length from the base of the lamina in oats. Variable doses were generally obtained by using different concentrations, and by different volumes in two cases.

TIBA was applied as a mixture with 2,4-D in a single application in the earlier experiments of 1969. Then I found that method cumbersome and it was also suspected that chemical reactions of both products could affect the true effect of the chemicals on the plants. All the following experiments received TIBA as a spray over all of the plant. It was applied with a plastic atomizer bottle. These bottles deliver a fine mist and were tested as acceptably uniform in the volume per stroke delivered (.5 ml/stroke in the unit I used). Pots receiving the same doses of TIBA within an experiment were sorted at random in a square arrangement and sprayed with a uniform number of strokes of the atomizer

from several directions so as to obtain a "heavy dew" but without reaching run-off. It was apparent that spraying "in block" was more uniform, faster and spray-saving than treating each individual pot.

TIBA was applied early in the morning followed by 2,4-D early in the afternoon in some cases. In others, 2,4-D was applied the following day. The separation was 18 to 20 hours and included one night in these cases. Preliminary experiments never show any effect of the relative timing of both of these products. Distilled water and Tween 20 at 0.02% were used to prepare the solutions. The pH of 2,4-D solutions was adjusted between 3.5 and 4.0. Blank pots sprayed with water plus Tween 20 were included in most of the experiments. They did not give any measurable effect in terms of yield or deformation, respect the untreated controls. The dimethylamine salt of 2,3,5-triiodobenzoic acid in aqueous 2.5% solution (lot IMC-6889) and the dimethylamine salt of 2,4-D with 49% of actual 2,4-D were the stocks utilized for all the experiments. Different concentrations applied are always expressed in terms of actual 2,4-D or TIBA.

The pots corresponding to one experiment were set in randomized blocks or in completely randomized arrangement, depending on space availability. In all cases the pots were moved two or three times within the arrangement in an effort to minimize effects of location within the bench.

Assessing effects

The major criterion to assess toxic effects was the growth above the treated leaf. All the following attributes were measured in some

of the experiments and only part of them in others: number of new expanded leaves, fresh and dry weights of leaves, and stems accumulated above the 2,4-D-treated leaf, elongation of the stem and leaf area. Also degrees and frequency of leaf deformation, epinasty, swelling of the stems, and cracking and discoloration of the epidermis were visually estimated in soybean and velvet leaf. The period from the date of application of the treatments to the date of final harvest varied from two to four weeks in different experiments but it was three weeks in most cases. Fresh weight determinations were made by weighing the samples within one hour from the initiation of the harvest. While waiting, samples were kept in a closed plastic container. Samples were then dried at 70°C. for 48 hours (72 hours for stems) before weighing for dry weight. Leaf area was measured in an electronic planimeter from Patton Industries, Stepney, South Australia. The yields of dry and fresh matter are expressed in grams or milligrams per pot. Heights, lengths and leaf number are also expressed as summations of all plants in one pot.

Radiotracer Experiments

Generalities

Three types of experiments were conducted using plants growing in a conditioned room. The conditioned room itself was located at the ground floor of the Agronomy Department. It is 4.0 by 1.8 m in size. It is equipped with an air conditioner and thermostatic controls. A bank of fluorescent tubes, 50% of them cool white type and 50% daylight type delivered 1100 foot-candles at the surface of the bench and 1600

foot-candles at the top of plants 25 cm high. Temperature was set at 27°C. during the illuminated period of 16 hours and at 21°C. during the dark period of 8 hours and these conditions were maintained the same along all the work.

Growing plants in nutrient solutions

Seeds were germinated in rolled paper towels, the rolls standing up on a cylindric glass jar with some water at the bottom. When seedlings had elongated one or more roots and the cots (or first leaf in oats) had expanded sufficiently, selected seedlings were transferred to 450 ml glass jars. The jars were lined with aluminum foil to avoid light incidence on roots. The roots were "passed through" holes drilled in the metallic lid and supported erect by surrounding the stem with a foam stopper cut in halves and plugged through the hole in the lid, holding the stem in the middle. I found that rolled towels is a much better method to obtain seedlings for nutrient culture than the also recommended germination in trays with wet "vermiculite." Using paper towels well developed and uniform seedlings of either species were obtained and all the operations were much simpler and time and space required were definitely less than with the vermiculite trays. We also found that the plastic foam plugs were a very favorable substitute for the cotton or glass wool plugs in terms of handling, durability, and uniformity. When no special requirements of porosity or sterility exist they can be made by hammering a cork borer through any piece of foam of adequate thickness.

Seedlings were initially transferred to half strength Hoagland solution. Successive renewals of the solution were made at least

weekly with full strength solution. Iron chelate was substituted for iron citrate. Two experiments were grown in polystyrene food containers filled with sand and irrigated with Hoagland solution so as to keep adequate moisture.

Stocks of TIBA and 2,4-D

We utilized an experimental sample (lot IMB-3889) of a 2.5% aqueous solution of the dimethylamine salt of TIBA and 2,4-D as the crystallized acid radiolabeled with C^{14} in the carboxylic carbon and with a specific activity of 1.02 mc per millimole.

Treating the plants

The method of application of TIBA and 2,4-D did not vary much from those described before in the greenhouse experiments. We tried initially the use of lanolin rings as a help to hold in place the drop of 2,4-D. I found that, if the leaves are fairly horizontal, a 0.01 or 0.02 ml drop of 1:1 water-alcohol solution could be applied fairly safely and save the extra plant manipulation and time involved in applying the rings. TIBA was always sprayed 2 to 4 hours before 2,4-D. A few variations will be cited in the specific experiments. The doses of radiolabeled 2,4-D used are expressed in μg per plant and concentrations were varied accordingly as to include the desired doses of 0.01 ml. Application of 2,4-D was made in 0.01 ml drops to the right lower quarter of soybean leaflets or velvet leaves or along the middle third of the lamina in oats. The pH of the solutions was adjusted between 3.0 and 3.5 for each experiment. All applications of TIBA and 2,4-D were made in the

light and a period of at least five hours of illumination followed the application. The treated leaf in oats was held horizontal during the application of the treatment and the following 24 hours. A rectangle of plexiglass was positioned at adequate height alongside the row of jars and the leaf to be treated was bent and held laying over the plexiglass with masking tape. The rest of the plant was kept in its normal position.

Objectives and types of experiments

The general objective was to determine the effect of TIBA on the fate of 2,4-D or its derivatives in the plant. C^{14} -radiolabeled 2,4-D was applied to plants receiving a previous spray of TIBA versus others receiving a spray of distilled water plus 0.02% Tween 20 only. According to the method or fraction of C^{14} measured, three types of experiments were carried out: (a) autoradiography, (b) extractable and residual radioactivity in different plant parts, and (c) excretion of radioactivity out the roots to the nutrient solution.

Autoradiography These experiments were carried out to study the general distribution of radioactivity after application of radio-labeled 2,4-D. We followed common techniques, as described by Crafts and Yamaguchi (1964) and others. At the times required after treatments, plants were taken out the jar and the different parts cut apart. Roots were blotted. All plant parts were pressed flat between layers of paper and aluminum sheets and put to dry immediately at 90°C . for 2-3 hours. Then the temperature was lowered down to 70°C . and left drying for 48 hours. I could not use the most recommended technique of freeze-drying

but, by immediate separation of plant parts, I think that major alterations of the distribution in vivo were avoided. Dry plant parts were mounted as "exploded views" in sheets of Whatman paper 25 x 20 cm with regular glue. There was no other reason than "availability" for preferring Whatman paper. Kodak nonscreen medical X-ray films 25 x 20 cm were faced in direct contact with the mounted plants. Exposure lasted for two weeks. Many films were exposed together in a "sandwich package" with layers in the following order (from the bottom): aluminum plate 2 mm thick - film - plant mounting facing it - a pad of old newspapers or other soft uniform material - another sheet of aluminum, etc. The aluminum sheet provided a rigid plane base for the film and the soft pad made more uniform the pressure of contact between film and plant materials which were of variable thickness in different parts. Films were processed personally at the photographic room of the Agronomy Department. Following recommendations in Crafts and Yamaguchi (1968), the time of development was shortened from 5 to 3 minutes. Untreated plants were included besides the treated ones. None of them yielded any detectable impression. This made me confident that the impressions obtained were caused by true radioactivity only. Pictures of the autoradiographs included in this thesis were prepared by the Photo Service of Iowa State University.

Extractable and residual radioactivity in different plant parts

At the established time, one to six days in different after treatment experiments, the plants were harvested, immediately separated into the different parts, weighed and stored frozen at -20°C . until the

extractions could be performed (usually within a week after harvesting). The parts analyzed were the tops developing above the 2,4-D-treated leaf, the tops below it, and the root in some of the experiments. The treated leaf was taken apart and was not extracted. The extraction was performed by homogenizing each sample with 15 ml 95% ethyl alcohol in a Virtis homogenizer at 20,000 rpm during three minutes. The homogenate was centrifugated 15 minutes at 4000 x g. The supernatant was then collected and the pellet was resuspended in alcohol and recentrifugated twice successively. All the supernatants were collected together and the volume let reduce to 20 ml on warm plate under a ventilated hood. One or two ml from this concentrated extract (depending on the experiments) was transferred to stainless steel planchettes, by successive additions of small amounts to the center of the planchettes. Disposable hypodermic syringes were used for transferring the samples. The extract was continuously evaporated and dried by keeping the planchettes over the hot steam plate. The samples were counted in a gas flow counter (Model M186 of Nuclear Chicago) and detector M5 with manual changer. The background counted with an empty planchette was 45 counts per minute (cpm). Later we had access and used a model with automatic changer and 22 cpm average background. Usually a fixed number of counts per sample was recorded for each sample, and the variable time recorded. The background counting was subtracted and the results finally expressed in net counts per minute (ncpm).

Excretion of radioactivity by roots of C¹⁴-2,4-D-treated plants

The plants were handled and the treatments applied in a manner similar to the experiments already described. The plants were transferred to fresh solutions immediately before application of the treatments. At the end of the experimental period, six or seven days, the plants were transferred and the used solution was "extracted" with diethyl ether. Three successive partitions with 100 ml of ether each were practiced on the 400 ml volume of each solution. All the ether was collected and quantitatively transferred to planchettes. They were counted to a fixed number of counts. The fixed number of counts varied from experiment to experiment for a compromise had been reached between precision and time, depending on the radioactivity present in the samples.

Field Experiments

A group of experiments with the objective of exploring the possible interaction of TIBA with the action of 2,4-D on weeds and corn in field conditions was established in the growing season of 1969. The experiments were replicated in three locations. Two of them were near Ames and the other was near Madrid, 30 km southwest of Ames. One of the locations near Ames gave poor and erratic yields, due to excess of moisture on part of the plots. It is not considered in the present thesis. Three types of experiments were carried out.

Experiment F1

The effects of 2,4-D and TIBA on weeds and corn was tested. A wide range of doses of both products in all their factorial combinations were

sprayed on uncultivated corn field plots. Each plot was 10 m long and three inter-row spaces 1 m wide each. The two central rows of corn and the weeds in between were the harvested plot. The corn was seeded in soil normally prepared for Iowa conditions and fertilized with 200 k/ha of nitrogen and 44 kg/ha of phosphorus. The timing of the major operations are summarized in Table 2. The treatments of TIBA and 2,4-D were applied as mixtures with a motorized sprayer that delivered 180 l/ha of solution at approximately 3 kg/cm² of pressure. The dimethylamine salts of both TIBA and 2,4-D were used. The doses of actual ingredients in g/ha will appear in the tables in the section on results. The early harvest of weeds consisted of two .5 m² subsamples harvested in the

Table 2. Dates of planting, harvests and application of treatments in the field experiments of 1969

Operations	Dates				
	Ames		Madrid		
	F1	F2	F1	F2	F3
Planting	5/12	5/12	5/14	5/14	5/14
Early spraying	6/13	6/13	6/14	6/14	6/15
Late spraying	-	-	-	-	7/5
Early weed harvest	7/2	7/3	7/8	7/7	-
Final weed harvest	8/4	8/5	8/6	8/6	-
Corn harvest	10/4	10/4	10/7	10/7	10/9

interrow space in different points of each plot. The material harvested was immediately sorted into grasses and broad-leaved species. The number of individual seedlings in this last group was counted. Both samples were weighed in the field (fresh weight yield). All the samples were dried during 48 or 72 hours at 70°C. and then the dry weight (D.W.) yield was recorded. The final weed yield was taken from 5 m length of inter-row space, to make a 5 m² sample. Similar procedures to those given for the earlier harvests were followed. The corn ear yields and the stands were taken from two 5 m rows where the weeds had not been harvested earlier. The ear corn yields were taken after one week of drying at 70°C.

Experiment F2

In this experiment a few selected doses of 2,4-D and TIBA were applied in corn plots receiving or not receiving mechanical cultivation, and with or without application of 2-chloro-N-isopropylacetanilide (alachlor) as a pre-emergence treatment. All the variables were applied in all possible combinations and, like in Experiment F1, replicated twice both at Madrid and Ames. The rest of the procedures were like those in Experiment F1.

Experiment F3

The effects of relatively high doses of 2,4-D and combinations with TIBA on the growth and yield of inbreds and hybrids of corn were tested under normal cultural conditions, including fertilization and mechanical weed control. Eight different 2,4-D doses and TIBA combinations were sprayed at two different dates (Table 2). The design was a split-split

plot with date of spraying as main plot, chemicals as subplots and corn entries as sub-subplots. Each subplot was a single row 3.3 m long. Evaluation of the effects were in terms of grain yield and percent shelling; percent barrenness, double eared plants, and scores of visible toxic symptoms: stem bending and breakage, abnormal bracing roots, and abnormal leaves.

So far I have described the general procedures utilized in the major groups of experiments carried out. Variations appearing in specific experiments will be cited along the chapter on results.

RESULTS

Greenhouse Experiments

Soybeans

Three successive experiments with soybeans were carried out in 1970. Table 3 summarizes the significance of the F ratios for the main effects of TIBA and 2,4-D and for their interaction on the attributes of growth and yield measured. Table 4 summarizes the coefficients of variability obtained for the same attributes in the three experiments.

Experiment GS1 TIBA was sprayed four hours in advance and 2,4-D was applied as five 0.01 ml drops of each solution to the first trifoliated leaf. The plants were harvested 21 days after the treatments were applied. Table 5 summarizes the mean yields per treatment for the different attributes measured. Table 6 presents the effects on the quantitative attributes in the form of percent yields with respect to the untreated control. Both products, either alone or in combination, were strongly inhibitory on all the attributes. 2,4-D alone appeared relatively more depressive on the accumulation of dry matter and fresh matter than on the development of new leaves and on the elongation of the stem. TIBA also was strongly inhibitory. It almost arrested any new elongation or dry matter accumulation. Its effect on the number of leaves appeared less marked, or even stimulatory at low doses. The growth of vigorous branches from upper nodes in some plants was perhaps the cause of the increased number of leaves at 50 ppm of TIBA. To analyze the nature of the interaction TIBA \times 2,4-D, which was statistically significant for all attributes in this experiment, Table 7 was prepared. It shows the

Table 3. Significance of F ratios for the main effects and interactions of 2,4-D and TIBA on different soybean plant characteristics in three greenhouse experiments^a

Experiment	Factor	d.f.	Leaves			Stems			Deformation		
			Number	F.W.	D.W.	Length	F.W.	D.W.	Branching	Cracked stems	"2,4-D" leaves
GS1	TIBA (A)	3	**	**	**	**	**	**	**	N.S.	**
	2,4-D (B)	6	**	**	**	**	**	**	**	**	**
	A x B	18	**	**	**	**	**	**	**	**	**
	Error	56									
GS2	A	2	**	**	**	**	**	**	**	**	**
	B	5	**	**	**	**	**	**	**	**	**
	A x B	10	**	**	**	**	**	**	**	**	**
	Error	54									
GS3	A	2	**	**	**	**	**	**	**	**	**
	B	5	**	**	**	**	**	**	**	**	**
	A x B	10	**	N.S.	N.S.	**	**	**	**	**	**
	Error	54									

^a * = significant at 0.05 P level, ** = significant at 0.01 P level, N.S. = non-significant at 0.05 P level. These notations in the following tables also have the same meanings.

signs of the differences between the "observed" yields of each TIBA-2,4-D combination and the "expected" yields according to Colby's formula (Colby, 1965). The combined effects for number of leaves were, in general, antagonistic. 2,4-D increased the number of leaves, and the antagonistic effect of TIBA is indicated by the predominance of negative signs in the combined applications. For the yield attributes to which both 2,4-D and TIBA were inhibitory when applied alone, their combined effect was less

Table 4. Coefficients of variability of the plant attributes measured in greenhouse experiments with soybeans

Attribute	Experiments		
	GS1	GS2	GS3
Leaf number	4.4	19.8	11.3
Leaf F.W.	7.2	26.5	18.8
Leaf D.W.	6.8	26.3	19.0
Stem length	4.1	17.2	17.7
Stem F.W.	6.2	18.9	21.5
Stem D.W.	5.6	18.8	22.4
Branching	11.0	11.6	20.0 ^a
Cracked stems	11.6	9.4	14.9 ^a
"2,4-D" leaves	11.5	11.6	9.6 ^a

^a Coefficients based on the transformed variable $TY = \sqrt{Y + 1/2}$.

Table 5. Experiment GS1. Effects of TIBA and 2,4-D on growth and yield attributes of soybean plants. All values refer to plant parts grown above the 2,4-D-treated leaf and are averages of three replications. All figures are totals per pot with three plants.

TIBA ppm	2,4-D ppm	Leaves			Stems		
		Number	F.W. gm	D.W. mg	Length mm	F.W. gm	D.W. mg
0	0	14.7	7.02	1490	289	3.88	547
0	10	17.0	5.54	986	279	2.85	358
0	40	16.7	6.33	1159	282	3.39	409
0	80	15.7	4.38	744	297	2.60	342
0	200	15.3	3.25	577	331	1.85	260
0	600	13.7	1.92	341	144	.96	115
0	1800	14.0	2.26	424	161	1.05	133
50	0	18.0	2.37	453	87	.49	75
50	10	12.3	1.76	357	99	.80	124
50	40	15.0	1.79	346	75	.44	67
50	80	11.7	2.16	409	146	1.16	171
50	200	9.3	1.12	219	81	.64	98
50	600	9.7	.88	175	69	.52	68
50	1800	9.7	.99	205	77	.65	91
200	0	7.7	1.63	380	48	.24	47
200	10	4.0	1.42	315	114	.61	107
200	40	4.0	1.50	362	91	.55	108
200	80	3.3	1.43	286	125	.63	113
200	200	3.3	1.02	197	110	.67	117
200	600	2.0	.71	142	66	.46	87
200	1800	2.7	.83	187	77	.57	117
400	0	5.7	1.08	241	44	.21	43
400	10	3.7	1.19	223	110	.48	77
400	40	4.3	1.02	228	62	.28	52
400	80	3.3	1.29	253	121	.77	119
400	200	2.3	.90	181	94	.52	93
400	600	2.0	.70	148	76	.48	91
400	1800	2.0	.60	138	71	.56	99

Table 6. Experiment GS1. Relative effects of TIBA and 2,4-D on the growth of soybean tops above the treated leaf (each figure is an average of three observations)

TIBA ppm	2,4-D ppm	Leaves			Stems		
		Number	F.W. mg	D.W. mg	Length mm	F.W. mg	D.W. mg
0	0	100	100	100	100	100	100
0	10	116	79	66	96	73	65
0	40	114	90	78	98	87	75
0	80	107	62	50	103	67	62
0	200	104	46	39	114	48	47
0	600	93	27	23	50	25	21
0	1800	95	32	28	56	27	24
50	0	122	34	30	30	13	14
50	10	84	25	24	34	21	23
50	40	102	25	23	26	11	12
50	80	80	31	27	50	30	31
50	200	63	16	15	28	16	18
50	600	66	13	12	24	13	12
50	1800	66	14	14	27	17	17
200	0	52	23	25	17	6	9
200	10	27	20	21	39	16	20
200	40	27	21	24	31	14	20
200	80	22	20	19	43	16	21
200	200	22	15	13	38	17	21
200	600	14	10	10	23	12	16
200	1800	18	12	12	27	15	21
400	0	39	15	16	15	5	8
400	10	25	17	15	38	12	14
400	40	29	15	15	21	7	10
400	80	22	18	17	42	20	22
400	200	16	13	12	32	13	17
400	600	14	10	10	26	12	17
400	1800	14	9	9	25	14	18

Table 7. Experiment GS1. Nature of the differences between observed and expected growth and yields of soybean tops under different TIBA-2,4-D combinations. Effects of single applications of TIBA or 2,4-D were compared with respect to the untreated control. A double sign (++) or --) represents differences larger than 15%.

TIBA ppm	2,4-D ppm	Leaves		Stems	
		Number	F.W.	Length	F.W.
0	0	0	0	0	0
0	10	++	--	-	--
0	40	+	-	-	-
0	80	+	--	+	--
0	200	+	--	+	--
0	600	-	--	--	--
0	1800	-	--	--	--
50	0	++	--	--	--
50	10	--	-	+	+
50	40	+	-	-	+
50	80	--	+	++	++
50	200	--	0	-	+
50	600	--	+	+	+
50	1800	--	+	+	+
200	0	--	--	--	--
200	10	--	+	++	+
200	40	--	0	++	+
200	80	--	+	++	+
200	200	--	+	++	+
200	600	--	+	++	+
200	1800	--	+	++	+
400	0	--	--	--	--
400	10	--	+	++	+
400	40	--	+	+	+
400	80	--	+	++	++
400	200	--	+	++	+
400	600	--	+	++	+
400	1800	--	+	++	+

inhibitory than expected, as indicated by the predominance of positive signs in columns for fresh weights and stem elongation. The apparent contradiction between the stimulation of leaf number and inhibition of leaf weight by 2,4-D or TIBA resulted from the formation of much smaller leaves in the treated plants. All leaves larger than 2 cm diameter were collected. Part of the additional leaves in treated plants originated in lateral branches. The effects of TIBA-2,4-D combinations on the morphology of the plants are summarized in Table 8. It shows that 2,4-D alone also induced a moderate amount of branching. A much larger amount of lateral branching was associated with doses of TIBA. The combined effects on branching appears antagonistic, particularly with doses of 2,4-D at 80 ppm or larger.

The brownish discoloration, deep cracking of the epidermis and twisting of stems (and petioles in some cases) were very noticeable effects of 2,4-D and TIBA-2,4-D combinations in most of the experiments with soybeans. The term "cracked stems" will be used to name together this group of symptoms. The mean values in Table 8 suggest a synergistic effect of TIBA and 2,4-D on this characteristic. TIBA alone did not produce any symptom. When applied alone, only doses of 600 ppm or more 2,4-D induced cracked stems, whereas only 80 ppm of 2,4-D were required to induce incipient effects when combined with TIBA. Doses of 2,4-D from 10 to 200 ppm induced typical deformations in leaves produced after the applications. Larger doses did not produce any visible symptom on the new leaves, as shown in Table 8. With the higher doses of 2,4-D applied the apices were severely damaged or killed, and a number of

Table 8. Experiment GS1. Effects of TIBA and 2,4-D on branching and visible signs of toxicity in soybeans (each figure is an average of three replications and is expressed as total number per pot)

TIBA ppm	2,4-D ppm	Branching	2,4-D leaves	Cracked stems
0	0	0	0	0
0	10	1.7	5.7	0
0	40	.7	5.0	0
0	80	2.7	6.3	0
0	200	1.7	4.0	0
0	600	1.7	0	2.0
0	1,800	0	0	1.7
50	0	6.7	0	0
50	10	5.3	.7	0
50	40	7.0	1.7	0
50	80	4.3	1.7	0
50	200	1.3	0	1.3
50	600	1.3	0	2.0
50	1,800	1.3	0	2.0
200	0	5.3	0	0
200	10	5.3	2.0	0
200	40	5.0	0	0
200	80	3.3	1.3	0
200	200	2.0	.7	1.0
200	600	.3	0	2.0
200	1,800	1.0	0	2.0
400	0	6.3	0	0
400	10	3.3	1.3	0
400	40	5.7	.7	0
400	80	6.0	1.0	.3
400	200	1.7	0	1.3
400	600	2.3	0	1.3
400	1,800	2.3	0	2.0

small new leaves grew from lateral branches. The effect of TIBA was definitely inhibitory on the appearance of 2,4-D symptoms in leaves. Table 3 also shows a statistically significant main effect of TIBA, which effect is not apparent from the actual values in Table 8 where TIBA alone does not show an effect at all! It is probably an artifact from a differential effect of the various levels of TIBA on the effect of 2,4-D. (What does a main effect mean when a significant interaction exists?)

Experiment GS2 This experiment was established with similar objectives and under similar conditions as those in Experiment GS1. The number of doses of both TIBA and 2,4-D were reduced, and the highest level of 2,4-D was increased to 2,400 ppm, with .06 ml/plant applied to the first and second trifoliated leaves. The plants were harvested 17 days after treatment. The mean yields of parts above the treated leaf are summarized in Table 9. The statistical significance of the results and their coefficients of variability appear in Tables 3 and 4, respectively. The mean values in Table 9 show that TIBA was strongly inhibitory of both leaf and stem yields. 2,4-D also was inhibitory on yield attributes. The 200 ppm level of 2,4-D approached 50% inhibition, when applied alone, for most of the attributes. TIBA at 50 ppm induced inhibitions larger than 50% on matter accumulation and elongation of the stem. The interaction between TIBA and 2,4-D was again statistically highly significant for all attributes measured. To illustrate the nature of the interaction, the differences of observed minus expected yields are summarized in Table 10 for the quantitative attributes. When 50 ppm of

Table 9. Experiment GS2. Effects of TIBA and 2,4-D on soybean attributes (like in Tables 5 and 8)

TIBA ppm	2,4-D ppm	Leaves			Stems		
		Number	F.W. mg	D.W. mg	Length mm	F.W. gm	D.W. mg
0	0	6.25	4977	943	351	4.03	431
0	10	5.75	4177	750	334	3.64	388
0	50	6.00	3877	703	289	3.15	346
0	200	4.00	2522	447	188	2.09	246
0	600	3.75	2730	457	221	1.90	215
0	2400	2.75	1317	245	145	.97	114
50	0	5.50	1862	414	98	.77	136
50	10	3.00	1637	326	91	.68	109
50	50	3.25	1685	327	116	.97	159
50	200	2.25	1117	197	93	.70	97
50	600	1.50	727	135	61	.46	61
50	2400	1.00	222	44	46	.26	40
200	0	2.75	715	158	46	.38	89
200	10	3.25	1287	261	75	.60	106
200	50	2.50	1310	234	151	1.01	137
200	200	1.75	597	102	87	.63	83
200	600	1.50	260	54	55	.45	74
200	2400	.50	80	20	42	.22	45

Table 10. Experiment GS2. Relative yields associated with different levels of TIBA and 2,4-D when applied alone (signs of the differences of observed yields minus expected yields associated with TIBA plus 2,4-D combinations, according to Colby's formula)

TIBA ppm	2,4-D ppm	Leaves		Stems	
		Number	F.W.	Length	F.W.
0	0	100	100	100	100
0	10	92	79	95	90
0	50	96	74	82	80
0	200	64	47	54	57
0	600	60	48	63	50
0	2400	44	26	41	26
50	0	88	44	28	32
50	10	-- ^a	0	0	0
50	50	--	0	+	+
50	200	--	0	+	+
50	600	--	-	0	0
50	2400	--	-	0	0
200	0	44	17	13	21
200	10	+	+	+	+
200	50	0 ^b	+	++	+
200	200	0	+	+	+
200	600	0	-	+	+
200	2400	-	-	+	+

^a A double sign indicates differences larger than 20%.

^b Differences smaller than 3% are considered as no difference.

TIBA were combined with any level of 2,4-D the number of leaves resulted in less than expected, thus indicating a synergistic action. Observed and expected values were practically similar at the highest level of TIBA. The comparison between observed and expected yields of leaf dry weight indicates a tendency towards antagonism or additivity at lower levels of 2,4-D and towards synergism at high doses of 2,4-D. Yields were actually very low at these higher doses and, according to their high coefficients of variability, they appear of no practical relevance. The effects on stem elongation appear antagonistic. The inhibition was less than expected in 8 out of 10 combinations of TIBA plus 2,4-D. And there were no differences in the other two. The combined effects on the accumulation of dry matter in the stem were also less than additive for all combinations including the highly inhibitory dose of 200 ppm of TIBA. Combinations of 2,4-D with 50 ppm of TIBA were additive or less, and all of them were small differences. In general, it appeared that when 2,4-D was combined with a dose of TIBA producing only slight inhibition the combined effect was more than additive. When both TIBA and 2,4-D were highly inhibitory, their interaction was negative and the yields were larger than expected. Fresh weights of leaves and stems were closely and directly correlated with dry weights and showed the same behavior.

The deformative effects are summarized in Table 11. The production of lateral branches was induced only by TIBA. The interaction with 2,4-D was negative. TIBA-induced branches were produced only at zero or low levels of 2,4-D. TIBA itself appeared to be supraoptimal at

Table 11. Experiment GS2. Effects of TIBA and 2,4-D on the number of lateral branches, number of leaves with 2,4-D toxicity symptoms and number of stems with discolored, cracked cortex (like in Table 8)

TIBA ppm	2,4-D ppm	Branching	2,4-D leaves	Cracked stems
0	0	0	0	0
0	10	0	2.0	0
0	50	0	3.5	0
0	200	0	3.3	0
0	600	0	2.8	0
0	2,400	0	1.3	1.5
50	0	8.0	0	0
50	10	6.5	0	0
50	50	2.8	0	0
50	200	0	0	0
50	600	0	0	1.3
50	2,400	0	0	1.8
200	0	2.8	0	0
200	10	3.3	0	0
200	50	0	0	0
200	200	0	0	1.0
200	600	0	0	2.0
200	2,400	0	0	2.0

the 200 ppm level, which yielded less branches than 50 ppm. The appearance of "2,4-D" leaves was another instance for antagonistic action between TIBA and 2,4-D. No deformed leaves were produced in plants receiving TIBA. The toxic effect of 2,4-D on the discoloration and cracked epidermis of the stem was enhanced by TIBA. Only the 2,400 ppm level of 2,4-D was active on the stems when applied alone. When 200 ppm of TIBA were added, some degree of stem damage was apparent at only 200 ppm of 2,4-D.

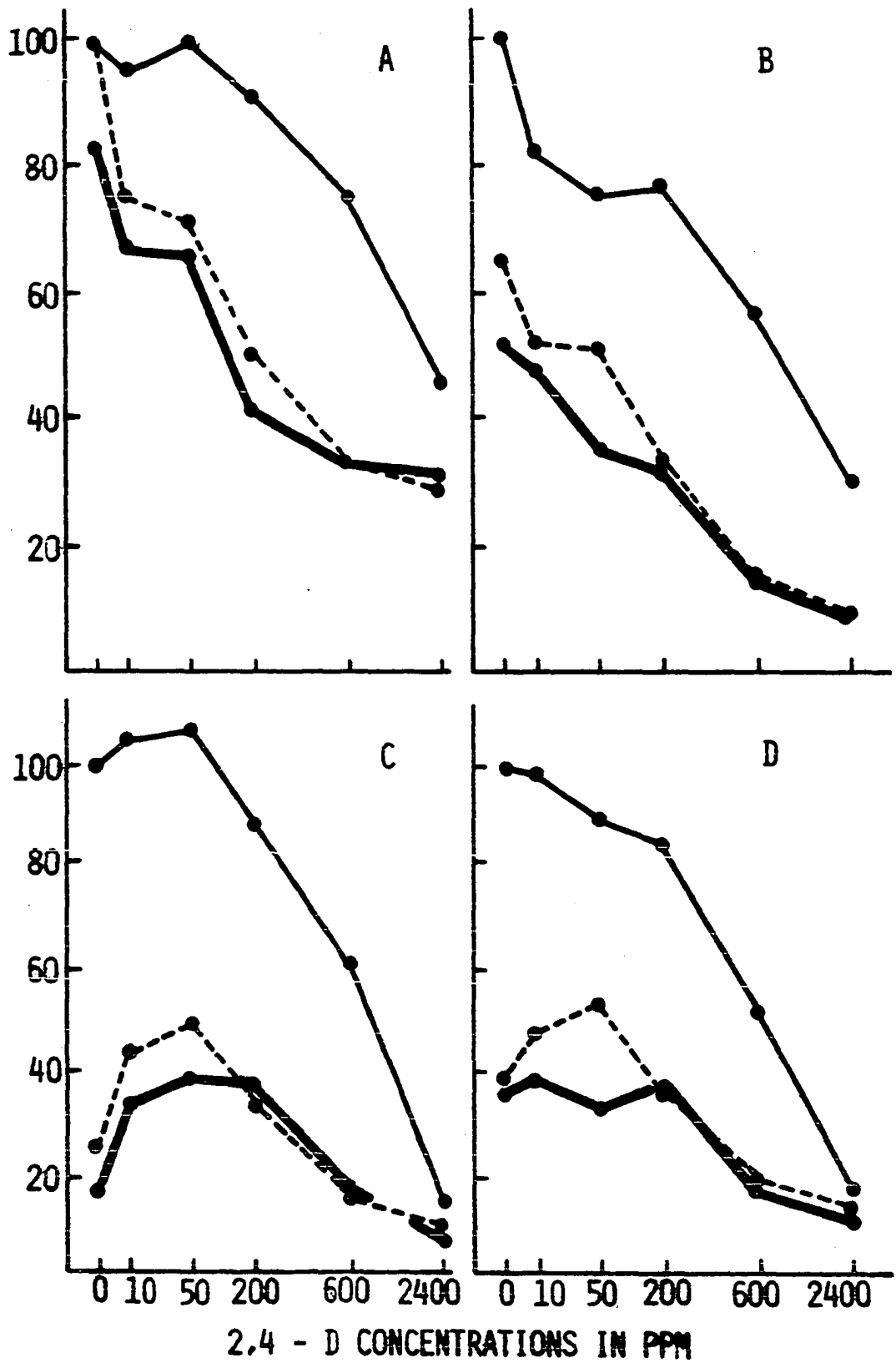
Experiment GS3 This experiment was carried out in the greenhouses of the Agronomy Department. The plants had the first trifoliated leaf fully expanded when the treatments were applied. The first internode above the first pair of leaves appeared relatively etiolated for plants at that stage. The procedures of application and harvest were those used in Experiment GS2. Each plant received four 0.01 ml drops of the corresponding 2,4-D solution. The plants were harvested 24 days after the application of the treatments. Table 12 summarizes the mean yields per treatment for the different quantitative attributes measured. Statistical significances and the coefficients of variability were presented in Tables 3 and 4 respectively. The highest doses of 2,4-D and TIBA caused yield depressions larger than 50% when applied alone, except for the number of leaves, which was reduced below 50% only by 2,400 ppm of 2,4-D. TIBA, when applied alone at 100 ppm, did not affect the number of leaves, and decreased the number only by 17% when applied at 400 ppm. The combined effect was synergistic between TIBA and intermediate doses of 2,4-D, as shown in Figure 1a. Both TIBA and 2,4-D were

Table 12. Experiment GS3. Effects of TIBA and 2,4-D on growth and yield attributes of soybeans (expressions like in Table 5)

TIBA ppm	2,4-D ppm	Leaves			Stems		
		Number	F.W. mg	D.W. mg	Length mm	F.W. mg	D.W. mg
0	0	6.00	4335	897	352	3890	469
0	10	5.75	3631	736	375	4009	467
0	50	6.00	3342	670	381	3390	424
0	200	5.50	3255	691	313	2925	398
0	600	4.50	2482	508	214	1752	241
0	2400	2.75	1392	269	55	571	75
100	0	6.00	2394	583	88	864	182
100	10	4.50	1950	466	153	1219	224
100	50	4.25	2050	469	173	1440	250
100	200	3.00	1466	301	115	1115	167
100	600	2.00	729	136	52	657	83
100	2400	1.75	429	94	36	360	64
400	0	5.00	2017	470	61	702	165
400	10	4.00	1914	429	115	999	185
400	50	4.00	1457	311	138	886	145
400	200	2.50	1480	294	136	1159	173
400	600	2.00	721	134	60	552	71
400	2400	2.00	384	87	25	305	50

Figure 1. Experiment GS3. Relative effects of TIBA and 2,4-D on the number of leaves (A), leaf dry weight (B), stem elongation (C) and stem dry weight (D) (the yields of the untreated control equals 100)

—— 2,4-D alone
- - - 2,4-D plus 100 ppm TIBA
———— 2,4-D plus 400 ppm TIBA



depressive on the weight of leaves. The combined effect was of the additive type, as indicated by the lack of statistical significance (Table 3) and the parallelism of the lines in Figure 1b. The marked inhibition produced by TIBA alone, even at 100 ppm, also is noticeable in Figure 1b. Both the main effects and the interaction of TIBA and 2,4-D on the growth and yield of stems were statistically significant. Particularly marked were the strong depressions caused by both levels of TIBA when applied alone. 2,4-D showed a more gradual dose-response curve with slight effects at 200 ppm or less. Only 600 and 2,400 ppm were markedly inhibitory. The interaction of both products is depicted in Figures 1c and 1d for stem elongation and stem dry weight respectively. It is shown that the effect of TIBA itself was much more inhibitory than that of 2,4-D, but when both were combined the effects were less than additive in terms of degree of inhibition induced. This convergence of the plots at high degrees of inhibition are in part an artifact. Some amount of plant material and some elongation of the stem above the treated leaf were already present when the treatments were applied. It is this "carry-over" yield which probably accounts for most of the yields measured in those plants receiving very toxic levels of TIBA and 2,4-D. When the growth after treatments tends to be zero, the actual yields recorded will correspond closely with the "carry-over" yields which were very similar in all the pots. An improvement in future experiments will be the previous estimation of growth above the treated leaf and the adjustment of all the final yields accordingly. The mean effects on lateral branching and stems and leaf deformations

appear in Table 13. 2,4-D alone did not induce any lateral branching in this experiment, but it negatively affected the frequency of TIBA-induced branching. Both 100 and 400 ppm of TIBA induced similar degrees of branching. Leaf deformation of "2,4-D" type showed a curvilinear response to increasing doses of 2,4-D. When it was applied alone the number of 2,4-D leaves increased up to a dose of 200 ppm and lower numbers were induced by doses of 600 and 2,400 ppm. TIBA antagonistically interacted with 2,4-D. Both 100 and 400 ppm of TIBA significantly decreased the number of "2,4-D" leaves induced by any level of 2,4-D. The effect of the highest dose of 2,4-D was completely arrested by TIBA. Only 600 and 2,400 ppm of 2,4-D were able to induce brown-cracked stems in this experiment, as shown in the last column of Table 13. The addition of TIBA appeared to increase the activity of the 600 ppm level of 2,4-D. 2,400 ppm were able to affect all the treated plants either with or without TIBA. The interactions on these attributes appeared statistically highly significant in Table 3.

The nitrate content in leaves above the treated leaf was determined in this experiment. The four replications harvested in the usual manner to determine leaf yields were pooled in two pairs of samples. The dried material was ground through mesh 40 and analyzed for nitrates according to the method described by Johnson and Ulrich (1954). The results are summarized in Table 14 and the analysis of variance in Table 15. The effect of 2,4-D was highly significant and the interaction TIBA \times 2,4-D was significant. Figure 2a shows that combinations of low doses of 2,4-D with TIBA gave the lowest content, whereas the addition of TIBA to high levels of 2,4-D resulted in the highest content of nitrate in leaves.

Table 13. Experiment GS3. Effects of TIBA and 2,4-D on branching from lateral buds and visible symptoms of toxicity in leaves and stems of soybeans (like in Table 8)

TIBA ppm	2,4-D ppm	Branching	2,4-D leaves	Cracked stems
0	0	0	0	0
0	10	0	3.25	0
0	50	0	3.25	0
0	200	0	3.50	0
0	600	0	2.25	0.75
0	2,400	0	0.75	2.00
100	0	6.50	0	0
100	10	5.50	2.00	0
100	50	4.75	2.00	0
100	200	1.50	0.75	0
100	600	0	0	2.00
100	2,400	0	0	2.00
400	0	6.00	0	0
400	10	5.00	1.75	0
400	50	3.50	2.00	0
400	200	2.00	0.50	0
400	600	0.25	0	1.75
400	2,400	0	0	2.00

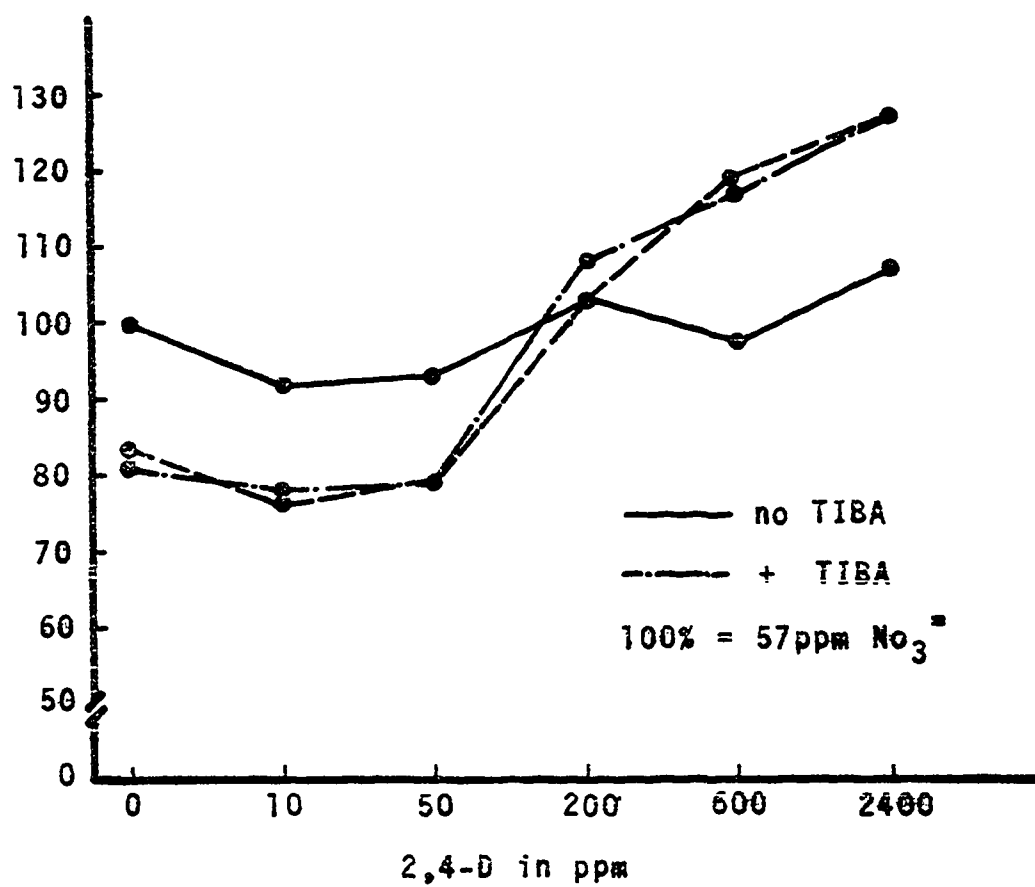
Table 14. Experiment GS3. Nitrate content in soybean leaves as affected by TIBA and 2,4-D (figures are expressed in ppm of dry weight and are averages of two replications)

TIBA ppm	2,4-D in ppm						Ave.
	0	10	50	200	600	2400	
0	57.0	55.3	52.8	59.0	55.5	61.3	56.8
100	47.5	43.5	45.5	59.0	68.0	72.3	56.3
400	39.5	45.0	45.5	65.8	67.5	72.5	56.3
Ave.	48.0	47.9	47.9	61.2	63.6	68.9	

Table 15. Analysis of variance of the effects and interaction of TIBA and 2,4-D on nitrate content in soybean leaves

Source	d.f.	F ratio
TIBA	2	.7 N.S.
2,4-D	5	14.2**
TIBA × 2,4-D	10	2.9*
Error	14	----
C.V.	9.6%	

Figure 2a. Experiment GS3. Effects of TIBA and 2,4-D on the nitrate content of soybean leaves



Velvet leaf

A series of experiments was conducted in the greenhouses of the Botany and Agronomy Departments during 1970. The general conditions were similar to the ones described for the experiments with soybeans. Table 16 summarizes the statistical significance of the effects of TIBA, 2,4-D and their interactions on the major parameters measured. Table 17 summarizes the coefficients of variability with which the different characteristics were measured in this series of experiments.

Experiment GV1 In this experiment, velvet leaf plants, after developing the fourth true leaf, were treated. TIBA was sprayed over all the plant and 2,4-D was applied at 0.04 ml per plant of each solution to the third leaf. Table 16 shows that the interaction between TIBA and 2,4-D was statistically significant only for stem length and lateral branching. The main effects of both TIBA and 2,4-D were highly significant for most of the attributes measured in this experiment. Table 18 summarizes the mean yields of leaves and stems harvested 22 days after the treatments. The effect of TIBA on the number of leaves produced appears significant at the 5% level (Table 16). However, TIBA showed practically no effect when applied alone. 2,4-D did not show any effects on the number of leaves at doses up to 200 ppm, and it was depressive at higher levels. When combined with TIBA, the stimulatory levels of 2,4-D were even more stimulatory and the highest doses were in turn more inhibitory when TIBA was added. The small size of most of the differences and the relatively large coefficients of variability

Table 16. Significance of F ratios for the main effects and simple interactions between TIBA and 2,4-D on the yield and growth attributes of velvet leaf

Experiment	Factor	Leaves			Stems			Branching	Swollen stems	"2,4-D" leaves
		Number	F.W.	D.W.	Length	F.W.	D.W.			
GV1	TIBA (A)	*	**	*	**	**	**	**	N.S.	*
	2,4-D (B)	**	**	**	**	**	**	**	**	**
	A × B	N.S.	N.S.	N.S.	**	N.S.	N.S.	**	N.S.	N.S.
GV2	TIBA (A)	*	**	*	**	**	**	**	**	N.S.
	2,4-D (B)	**	**	**	**	**	**	**	**	**
	A × B	*	*	N.S.	**	**	N.S.	**	**	**
GV3	TIBA (A)	*	*	*	**	**	*			
	2,4-D (B)	**	**	**	**	**	**			
	A × B	N.S.	N.S.	N.S.	**	N.S.	N.S.			

Table 17. Coefficients of variability percent for different attributes measured in greenhouse experiments with velvet leaf

Attribute	Experiments		
	GV1	GV2	GV3
Number of leaves	17.9	15.2	15.8
Leaf F.W.	23.3	27.6	27.3
Leaf D.W.	24.2	26.1	27.4
Stem length	16.6	27.8	13.2
Stem F.W.	21.3	33.9	25.2
Stem D.W.	22.3	31.8	26.7
Branching	23.6	21.8	--
Swollen stems	18.6	27.9	--
"2,4-D" leaves	38.7	14.4	--
Stem length ^a	--	10.6	--
Stem F.W. ^a	--	17.6	--
Stem D.W. ^a	--	18.1	--

^a Lengths and weights in the part of the plant below the treated leaf (roots were not included).

Table 18. Experiment GV1. Effects of 2,4-D and TIBA on growth and yield attributes in velvet leaf (each value is an average of three replications and is expressed on a per pot containing four plants basis)

TIBA ppm	2,4-D ppm	Leaves			Stems		
		Number	F.W. gm	D.W. mg	Length cm	F.W. gm	D.W. mg
0	0	31	6.29	1202	74.7	5.27	591
0	10	33	5.71	1058	67.3	4.58	501
0	40	32	5.42	988	62.3	4.39	469
0	80	32	3.82	712	49.7	3.74	443
0	200	35	4.37	746	48.3	3.97	444
0	600	26	1.35	275	26.0	2.21	295
0	1800	16	.63	190	21.3	1.47	185
50	0	33	5.53	1027	63.3	4.60	514
50	10	35	5.96	1020	65.7	4.54	465
50	40	36	5.47	974	59.3	4.63	509
50	80	36	4.13	720	45.3	3.35	356
50	200	32	2.78	467	35.3	2.55	312
50	600	21	1.04	265	26.3	1.96	256
50	1800	13	.64	168	23.7	1.92	237
200	0	31	5.22	972	50.7	3.69	408
200	10	35	5.54	1016	50.3	3.90	406
200	40	33	4.56	812	48.3	3.23	331
200	80	46	4.63	819	44.0	3.26	357
200	200	44	3.67	657	41.0	2.95	357
200	600	21	1.02	207	22.3	1.76	224
200	1800	22	1.20	266	23.0	1.67	227
800	0	32	4.42	853	39.0	2.85	294
800	10	34	4.87	945	39.7	2.91	331
800	40	32	3.18	607	37.0	2.04	259
800	80	46	3.48	627	33.0	2.43	271
800	200	39	2.80	511	31.7	2.27	278
800	600	26	1.16	246	20.7	1.60	198
800	1800	22	.72	218	21.3	1.64	198

could impede statistical significance of this apparently synergistic effect. It was fairly consistent when the mean values were considered (Table 18). Columns 5 and 6 of Table 19 show a majority of positive differences, as indicating some degree of antagonistic or less than additive action of TIBA and 2,4-D on the yield of dry matter in leaves. Deviations from the expected values were, in general, very small (less than 10%). The effects on the stem attributes, namely length and fresh and dry matter yields, all followed the same trend of response to treatments. Both TIBA and 2,4-D produced highly significant reductions in growth and yield when applied alone (Figure 2b). Intermediate and high levels gave reductions down to 50% or less than the normal growth. The interaction was statistically significant only for stem length, but the trends were similarly consistent and in the same direction for the three stem attributes considered. This can be realized by comparing columns 6, 7 and 8 in Table 18. In all but three cases yields were larger than "expected", indicating that somehow the inhibitory actions of TIBA and 2,4-D were antagonistic. The majority of the deviations of the observed yields from the "expected" ones were between 5 and 15% of relative yield with respect to the untreated control.

The deformative effects of TIBA and 2,4-D on velvet leaf were evaluated by their effects on the growth of lateral branches, number of "swollen" stems and "2,4-D" leaves. Mean values of these observations are summarized in Table 20.

The "swollen" stem symptom was characteristically observed in plants of velvet leaf treated with relatively high doses of 2,4-D.

Table 19. Experiment GV1. Observed and "expected" relative yields in velvet leaf plants treated with combinations of 2,4-D and TIBA

TIBA ppm	2,4-D ppm	<u>Leaf number</u>		<u>Leaf D.W.</u>		<u>Stem length</u>		<u>Stem D.W.</u>	
		Obs. ^a	Exp. ^b	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
0	0	100	---	100	---	100	---	100	---
0	10	106	---	88	---	90	---	85	---
0	40	103	---	82	---	83	---	79	---
0	80	103	---	59	---	66	---	75	---
0	200	113	---	62	---	65	---	75	---
0	600	84	---	23	---	35	---	50	---
0	1,800	52	---	16	---	28	---	31	---
50	0	106	---	85	---	85	---	87	---
50	10	113	113	85	75	88	76	79	74
50	40	116	110	81	70	79	71	86	69
50	80	116	110	60	51	61	56	60	65
50	200	103	102	39	53	47	55	53	65
50	600	68	89	22	20	35	29	43	43
50	1,800	42	55	14	13	32	24	40	27
200	0	100	---	81	---	68	---	69	---
200	10	113	106	85	71	67	61	69	58
200	40	106	103	68	66	65	57	56	55
200	80	148	103	68	48	59	45	60	52
200	200	142	113	55	50	55	44	60	52
200	600	68	84	17	18	30	24	38	34
200	1,800	71	52	22	13	31	19	38	22
800	0	103	---	71	---	52	---	50	---
800	10	110	110	79	62	53	47	56	42
800	40	103	106	50	58	50	43	44	39
800	80	148	106	52	42	45	35	46	37
800	200	126	116	42	44	42	34	47	37
800	600	84	87	20	16	28	18	33	25
800	1,800	71	53	18	11	28	15	33	16

^a Observed values.

^b Expected values.

Figure 2b. Simple and combined effects of TIBA and 2,4-D on growth characteristics of velvet leaf (each point is an average of three or four replications; the yield of the untreated control equals 100)

———— 2,4-D alone
- - - - 2,4-D plus TIBA

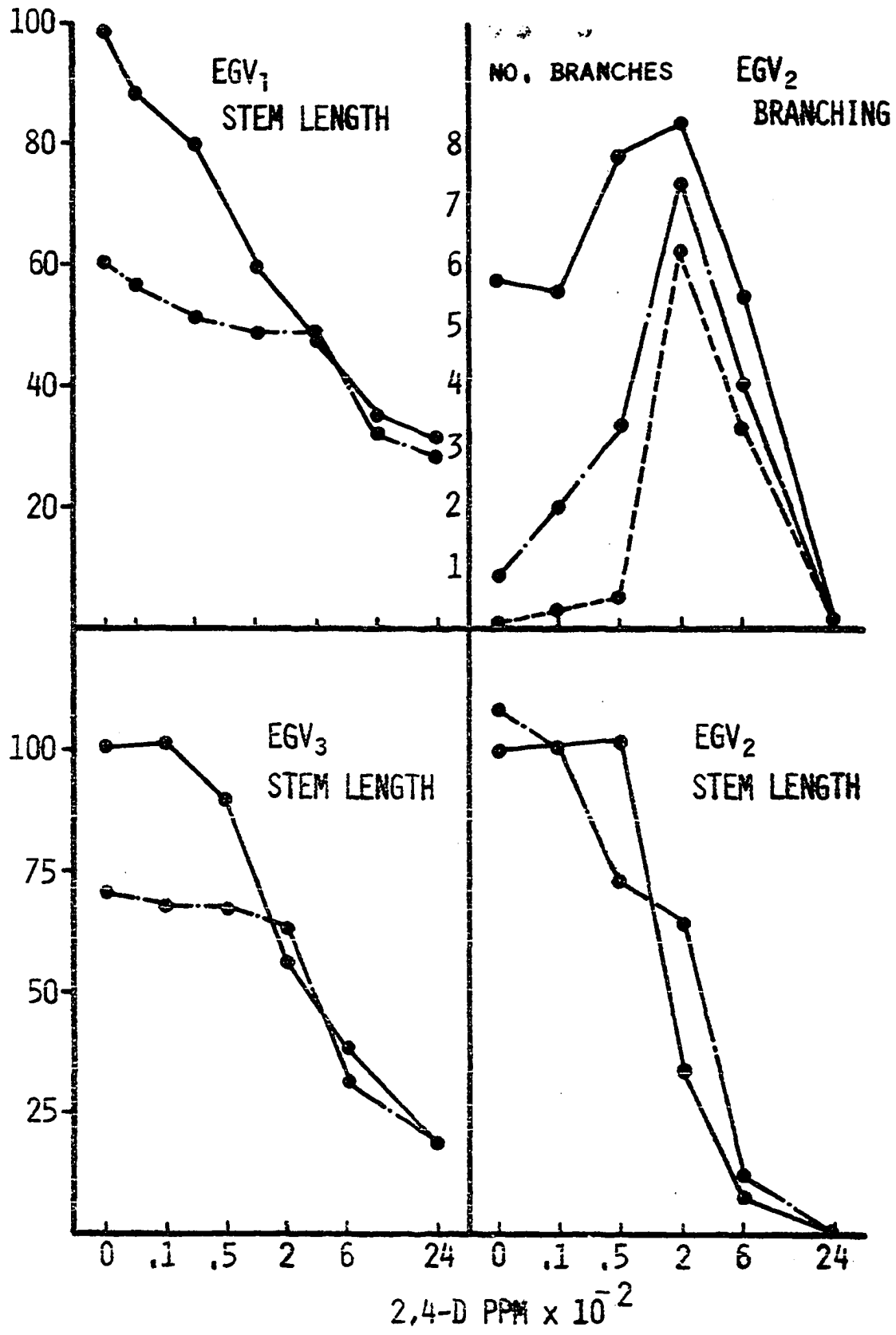


Table 20. Experiment GV1. Effects of TIBA and 2,4-D on the growth of lateral branches and toxic symptoms in stems and leaves of velvet leaf (each figure is an average of three replications)

TIBA ppm	2,4-D ppm	Lateral branches	Swollen stems	2,4-D leaves
0	0	0	0	0
0	10	0	0	5.0
0	40	0	0	7.0
0	80	0	.7	12.7
0	600	5.7	3.7	7.7
0	1,800	1.0	4.0	2.0
50	0	0	0	0
50	10	0	0	2.0
50	40	1.3	0	12.0
50	80	4.0	.3	13.0
50	200	3.0	1.7	14.7
50	600	4.3	3.3	4.0
50	1,800	3.3	4.0	2.0
200	0	0	0	0
200	10	2.0	0	0
200	40	3.3	0	4.7
200	80	7.0	0	13.0
200	200	7.0	.7	16.7
200	600	3.7	3.3	1.7
200	1,800	2.3	2.7	4.0
800	0	3.7	0	0
800	10	2.0	0	0
800	40	4.0	0	0
800	80	8.0	.3	7.0
800	200	6.3	1.7	9.7
800	600	3.3	3.3	2.3
800	1,800	4.7	4.0	1.7

Elongation from the apex was arrested. A several-fold increase in the cross section of stem down to where the first bottom leaves are inserted occurred. The color also changed from normal green to a pale yellow. Increase in diameter appeared to be caused by massive production of non-vascular tissue. No microscopic studies were carried out to ascertain this point, however. All these three characteristics could be measured only with very large coefficients of variability, as shown in Table 17. Under these circumstances no significance tests between specific pairs of means is attempted.

The effects of both TIBA and 2,4-D on lateral branching, as well as their interaction, appeared highly significant. Table 20 shows that 600 ppm of 2,4-D or 800 ppm of TIBA were required to initiate growth of lateral branches when either of the chemicals was applied alone to the plants, while it took only 40 ppm of 2,4-D plus 50 ppm of TIBA or 200 ppm of TIBA plus only 10 ppm of 2,4-D to induce some degree of branching when they were applied together. A synergistic type of action at low and medium doses of both products appeared to account for the significance of the interaction in the analysis of variance. The highest dose of 2,4-D showed again a definite inhibitory action. The formation of "swollen" stems appeared to be purely a 2,4-D-induced effect, as shown by data in column 4 of Table 20, and the non-significant F values for TIBA and 2,4-D interaction in Table 16. The incipient symptoms were associated with a dose of 40 ppm of 2,4-D and practically all the plants appeared affected by the highest rate of 1,800 ppm. The effect of 2,4-D on leaf deformation was highly significant. It showed an optimum type

of dose response curve, with incipient effect at the lowest level of 10 ppm, a maximum at about 200 ppm and decreasing responses as doses increased above 200 ppm. The main effect of TIBA also appeared significant, although no 2,4-D symptoms were noticed as produced by TIBA alone (Table 20, column 5). The F value for the interaction between TIBA and 2,4-D on leaf deformation was computed as nonsignificant (Table 16). However a trend is apparent in Table 20. Doses of 2,4-D were inhibited in their effects as increasing doses of TIBA were added to them. This apparently synergistic effect was particularly marked at low doses of 2,4-D.

Experiment GV2 This experiment was carried out under similar conditions as Experiment GV1. The number of doses of each chemical was reduced. The highest dose of 2,4-D was increased up to 2,400 ppm. The treatments were applied to three plants per pot and harvested 17 days later. At harvest, plants were cut at ground level and the top was split at the insertion of the third leaf, which was the treated one. The mean yields per treatment of plant attributes above the treated leaf are summarized in Table 21. Their analyses of variance and coefficients of variability were included in Tables 16 and 17 respectively. Table 22 shows the nature of the differences of observed minus expected yields for leaf and stem attributes which showed statistical significance in the analysis of variance (Table 16). The number of expanded leaves appeared significantly affected by 2,4-D alone. It was highly depressive at doses of 200 ppm or more. TIBA alone produced no marked effect on the number of leaves, although its main effect appears

Table 21. Experiment GV2. Effects of TIBA and 2,4-D on the growth and yield attributes of velvet leaf, 17 days after treatment (yields correspond to the plant parts above the treated leaf; each figure is an average of four replications and are expressed on a per pot basis)

TIBA ppm	2,4-D ppm	Leaves			Stems			Branches	"2,4-D" leaves	Swollen Stems
		Number	F.W. gm	D.W. mg	Length mm	F.W. mg	D.W. mg			
0	0	14.3	3.65	600	270	2065	180	0	0	0
0	10	14.8	3.40	543	280	2096	188	.3	5.0	0
0	50	14.3	3.14	482	275	2095	214	.5	9.5	.25
0	200	7.3	.86	138	85	938	105	6.3	4.3	2.50
0	600	5.8	.52	100	20	272	32	3.3	.8	2.75
0	2400	3.3	.22	44	0	0	0	0	0	2.75
100	0	15.8	4.04	687	291	2311	206	.8	0	0
100	10	16.3	3.86	651	271	2065	190	2.0	.8	0
100	50	14.5	2.72	434	197	1491	158	3.3	6.8	.25
100	200	12.0	1.99	302	176	1465	153	7.3	6.3	1.00
100	600	5.3	.46	80	25	220	27	4.0	1.3	2.75
100	2400	3.3	.18	40	0	0	0	0	0	3.00
400	0	14.5	2.86	516	201	1336	140	5.8	0	0
400	10	14.5	2.75	476	182	1235	128	5.5	1.8	0
400	50	13.3	2.15	382	158	976	127	7.8	4.3	.25
400	200	11.5	1.67	271	125	851	106	8.3	5.3	2.50
400	600	6.5	.63	103	39	303	33	5.5	2.8	2.75
400	2400	3.3	.14	53	0	0	0	0	0	2.75

Table 22. Experiment GV2. Comparisons between observed and expected yields under different TIBA plus 2,4-D combinations (like in Table 19)

TIBA ppm	2,4-D ppm	<u>Leaf number</u>		<u>Leaf F.W.</u>		<u>Stem length</u>		<u>Stem F.W.</u>	
		Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
0	0	100	---	100	---	100	---	100	---
0	10	103	---	93	---	104	---	101	---
0	50	100	---	86	---	102	---	101	---
0	200	51	---	24	---	31	---	45	---
0	600	41	---	14	---	7	---	13	---
0	2,400	23	---	6	---	0	---	0	---
100	0	110	---	111	---	108	---	112	---
100	10	114	114	106	103	100	112	100	114
100	50	101	110	74	95	73	110	72	114
100	200	84	56	54	26	65	34	71	51
100	600	37	45	13	16	9	8	11	15
100	2,400	23	25	5	7	0	0	0	0
400	0	101	---	78	---	74	---	65	---
400	10	101	105	75	73	67	77	60	66
400	50	93	101	59	67	58	76	47	66
400	200	80	52	46	18	46	23	41	29
400	600	45	41	17	11	5	5	15	8
400	2,400	23	23	4	5	0	0	0	0

significant in Table 16. The interaction, even though statistically significant, was completely erratic in nature to judge from values in Table 22 columns 3 and 4. Differences between observed and expected values varied from negative to positive without any explainable trend. The coefficient of variability for the number of leaves was not larger than others found in this group of experiments. The yield of fresh weight in leaves was decreased by 2,4-D at any dose. TIBA alone was slightly stimulatory at 100 ppm and definitely depressive at 400 ppm. The interaction between both products was just significant statistically. The mean yields in Table 21, column 4, and the signs of the differences between observed and expected yields in Table 22 show again an erratic distribution, with no apparent trend. Significant interactions occurred but they varied from highly synergistic to highly antagonistic in other cases. It appears that whenever an exaggerated inhibition is produced by either TIBA or 2,4-D alone, the combined effects are antagonistic. They show synergism only when small effects are produced by each product applied alone. The yield of leaf dry matter showed similar results to that of fresh matter, and the interactions did not reach statistical significance. 2,4-D was increasingly inhibitory from the lowest dose and TIBA yielded slight increases and inhibitions at 100 and 400 ppm respectively.

The effects on stem growth above the treated leaf showed high significance of the main effects of 2,4-D and TIBA, and their interaction except on dry matter yield. The elongation of the stem was practically unaffected by 2,4-D at 50 ppm, was strongly decreased by 200 and 600 ppm, and completely arrested by the heaviest application

of 2,400 ppm of 2,4-D (Figure 2). TIBA was also slightly stimulatory at 100 ppm and markedly depressive at 400 ppm, when applied alone. The action of TIBA appeared antagonistic of that of 2,4-D in any circumstance, i.e. it depressed stimulatory effects of 2,4-D, as well as made less pronounced the inhibition caused by the higher levels of 2,4-D applied. This trend was apparent for the low level of TIBA (slightly stimulatory) as well as for the highest dose, which was inhibitory itself. Dry and fresh yields presented very similar responses to those found for stem elongation, as shown in Table 21, columns 7 and 8, respectively. Again doses of 2,4-D up to 50 ppm were slightly stimulatory; 200 and 600 ppm were strongly depressive and 2,400 ppm completely arrested any growth of the main stem. TIBA also produced stimulation and inhibition at 100 and 400 ppm respectively. Again TIBA was antagonistic to 2,4-D, in the same sense as it was reported for stem elongation.

The visible effects on branching and deformations of leaves and stems were all statistically significant, as shown in Table 16, except for TIBA which obviously is not expected to induce signs of 2,4-D toxicity!

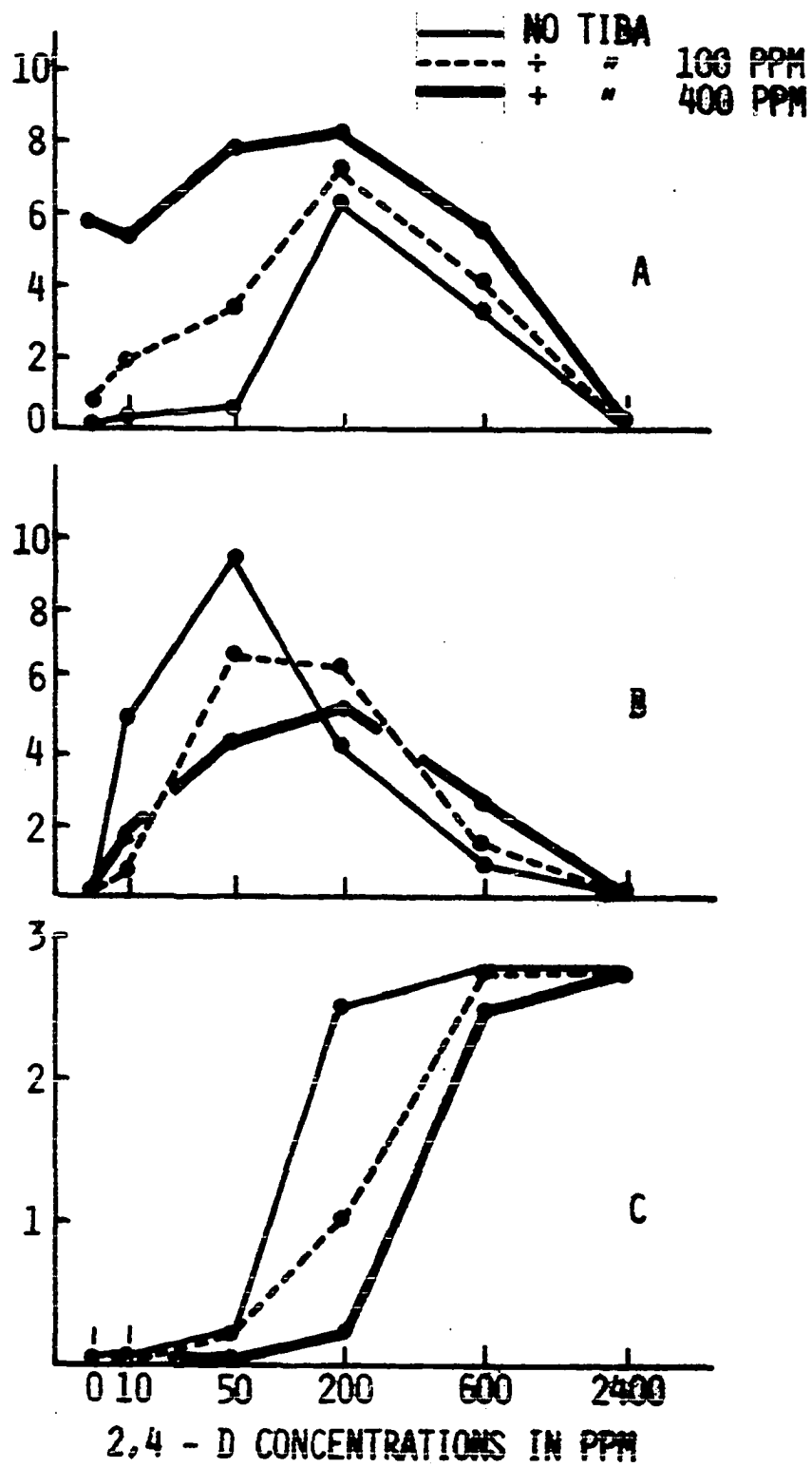
Both 2,4-D and TIBA induced lateral branches to grow when applied alone. 200 or 600 ppm of 2,4-D, and 400 ppm of TIBA were the most active single doses (Figure 3a). 2,4-D at 2,400 ppm completely arrested any lateral growth when applied either alone or with TIBA. Significant interaction resulted from the apparent synergism between TIBA and low doses of 2,4-D shown in Figure 3a. 10 and 50 ppm of 2,4-D combined

Figure 3. Some visible effects of TIBA and 2,4-D on velvet leaf (each observation is an average of four replications and is expressed in number per pot containing 3 plants)

A = number of lateral branches

B = number of "2,4-D" leaves

C = swollen stems



with 100 ppm of TIBA produced more than additive number of branches. The high dose of TIBA also appeared synergistic with 50 ppm of 2,4-D. At the most potent level of 2,4-D, namely 200 ppm, the effect of TIBA appears less than additive. Probably this lack of additivity or synergism was caused by a limited potential of the plants for branching, whether determined by internal or environmental factors. The number of leaves showing "2,4-D" deformation increased up to doses of 50 or 200 ppm, depending on the level of TIBA combined with the 2,4-D; 600 ppm of 2,4-D produced markedly less "2,4-D leaves" and the highest dose did not produce any symptoms. This phenomenon will be considered in the discussion of results. Both doses of TIBA were antagonistic to the toxicity of low doses of 2,4-D but did not affect markedly the highest levels, as shown in Figure 3b. The production of "swollen stems" by 2,4-D and the antagonistic effect of TIBA on its activity is shown in Figure 3c. Again TIBA was antagonistic to the effects of 2,4-D and decreased the effects produced by doses of 2,4-D up to 200 ppm. At higher 2,4-D applications, TIBA was ineffective (or the doses used were insufficient), and practically all the plants were affected by 2,4-D.

The means of stem length and weight in the region below the insertion of the treated leaf are shown in Table 23. 2,4-D alone resulted in slight increases or slight inhibition of elongation when applied alone at low and high doses respectively. TIBA alone hardly produced an increase in length and it was not statistically significant. The interaction TIBA by 2,4-D was not significant either, and most of

Table 23. Experiment GV2. Effects of TIBA and 2,4-D on the elongation of stems and dry and fresh matter accumulation in the lower tops (below the treated leaf)--expressions like in Table 21

TIBA ppm	2,4-D ppm	Stem length mm	Top F.W. mg	Top D.W. mg
0	0	161	1,918	290
0	10	178	2,404	354
0	50	176	2,472	377
0	200	159	3,316	506
0	600	154	3,161	471
0	2,400	148	1,876	304
100	0	170	2,101	318
100	10	174	2,131	326
100	50	170	2,534	391
100	200	163	3,105	448
100	600	163	3,206	473
100	2,400	143	1,708	289
400	0	163	2,373	378
400	10	149	2,117	338
400	50	153	2,565	421
400	200	151	3,092	488
400	600	184	3,379	490
400	2,400	120	1,482	209

the treatment combinations did not deviate from the expected values by more than 10%. The yields in fresh and dry matter appear, as usually expected, closely and directly correlated and both yielded similar statistical analysis. The only significant F values found were for the main effects in both cases. 2,4-D was increasingly stimulatory at doses up to 200 ppm and the response was decreasing for the higher levels of 600 and 2,400 ppm when applied alone. TIBA also increased the accumulation of fresh and dry weight. Even though its main effect appeared statistically nonsignificant in Table 16, it was consistent and produced up to 30% increase in dry matter with respect to the untreated control, when applied alone. The trend of the combined effect was of antagonistic type. TIBA and 2,4-D did not add their stimulatory actions when combined. Actual yields of treatment combinations varied from slightly smaller to slightly higher than the yield of corresponding 2,4-D alone (5% or less).

Experiment GV3 This experiment also was established to further observe the combined effects of doses of TIBA and 2,4-D on the yields, growth and deformation of leaves and stems in velvet leaf. Similar procedures to those used in Experiment GV2 were followed. The plants had up to the third true leaf fully expanded when the treatments were applied. They were harvested 24 days later. Actually only two out of four replications were harvested and analyzed at this time. The other two replications were repotted and left growing for observations for a longer term and for a late harvest. They will be reported as a separate experiment. The mean values of the characters observed are summarized

in Table 24. In this experiment the effects of TIBA were statistically significant in four of the attributes measured and highly significant in the other two. The main effect of 2,4-D was highly significant on all of them. The interaction TIBA \times 2,4-D appeared significant only for the effect on stem elongation (Table 16).

The number of leaves expanded after treatment was markedly depressed by 2,4-D at doses of 200 ppm or more. TIBA at the doses applied induced a similar small decrease that is shown additive to the effect of 2,4-D by the parallelism of the plots in Figure 4a. Some degree of synergism however is apparent between TIBA and the low dose of 50 ppm of 2,4-D. The yields of dry matter and fresh matter were usually closely related in all the experiments. Figures 4b and 4d illustrate the effects of TIBA and 2,4-D on the yields of dry matter in leaves and stems respectively. Again there was some indication of a synergistic effect at low doses of 2,4-D without reaching statistical significance. At high doses of 2,4-D, all growth was practically arrested and the plots "with" and "without" TIBA had similar yields.

The only significant interaction detected in this experiment was the one on the elongation of stems above the treated leaf, as shown in Figure 4c. It appeared that combinations of 400 ppm of TIBA with 10 or 50 ppm of 2,4-D were synergistic. All the attributes tended to give very similar low yields at high doses of 2,4-D, whether applied alone or in combination with TIBA. It is illustrated by the convergence of all the plots in Figure 4, at high 2,4-D levels. Rather than a true antagonism, this convergence seems to be an artifact. At these highly

Table 24. Experiment GV3. Effects of 2,4-D and TIBA on growth and yield of velvet leaf (figures are averages of two replications)

TIBA ppm	2,4-D ppm	Leaves			Stems		
		Number	F.W. mg	D.W. mg	Length mm	F.W. mg	D.W. mg
0	0	18.5	5747	1157	546	4915	538
0	10	19.0	6004	1103	552	4847	516
0	50	19.0	4437	0858	496	3577	472
0	200	14.5	1720	0343	315	2535	370
0	600	6.5	587	0136	205	1630	216
0	2400	2.5	184	0047	92	680	86
100	0	16.5	4962	982	377	2995	382
100	10	17.5	4370	854	392	3205	418
100	50	14.0	2415	476	362	2115	295
100	200	13.5	1567	286	340	2480	343
100	600	4.5	515	89	165	1625	182
100	2400	4.5	150	57	87	547	67
400	0	16.5	4372	862	377	3005	386
400	10	18.0	4262	863	337	2740	358
400	50	12.0	2240	425	322	2010	289
400	200	13.0	1995	361	352	2232	301
400	600	4.5	287	56	160	1072	133
400	2400	4.0	290	74	90	704	79

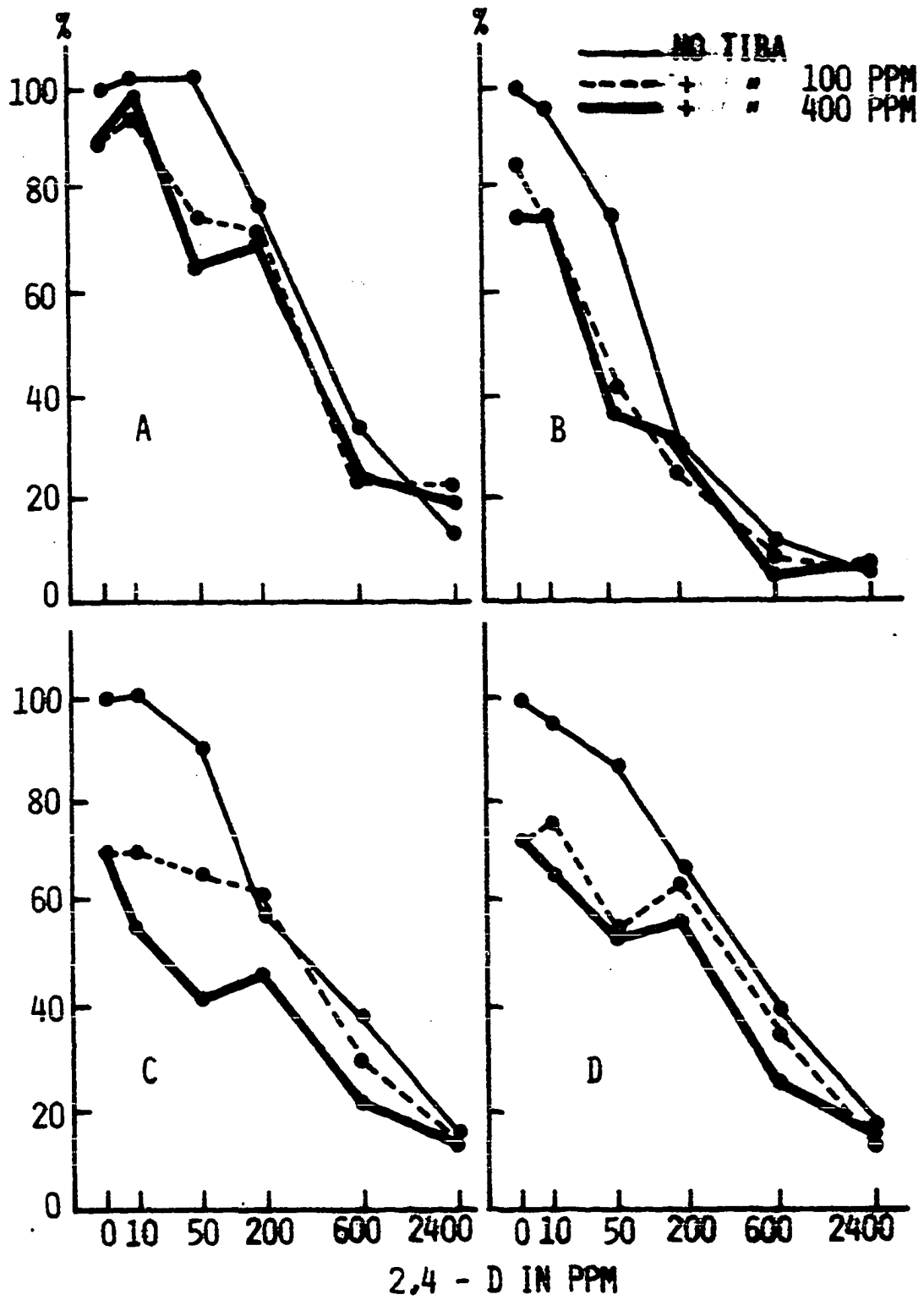
Figure 4. Experiment GV3. Effects of TIBA and 2,4-D on the relative growth and yields of leaves and stems in velvet leaf

A = number of leaves

B = dry matter in leaves

C = stem elongation

D = dry matter in the stems



toxic doses any further apical growth was practically arrested. The small amount of plant material already present and formed before the treatments exerted their toxic action is what in practice accounts for this phenomenon.

Experiment GV4 Two replications under the same conditions as Experiment GV3 treated on December 5, 1970, were left to grow until February 12, 1971, when Figures 5, 6, and 7 were taken. It is apparent in Figure 5 that 10 ppm of 2,4-D did not produce any appreciable effect at any level of TIBA. (Compare plants 01, 11 and 21 at top, center and bottom, respectively.) Synergism was manifested by 50 ppm of 2,4-D with 100 ppm of TIBA, as noticeable by the marked growth inhibition of plant 12, center, as compared with 02, top. TIBA appeared antagonistic to levels of 2,4-D of 200 ppm or more 2,4-D. The growth was visibly poorer with the higher levels of 2,4-D alone than when TIBA was previously applied. Figures 6 and 7 show that the growing points were killed at these high doses of 2,4-D. What appears associated with the addition of TIBA is a more vigorous regrowth from lower lateral buds. (Observe the increased growth of plants from left to right in Figure 7.)

Experiments in 1969 A preliminary group of experiments were carried out in the greenhouse in 1969. The experimental conditions were similar to the ones already described. Their results did not differ in anything fundamental from the ones obtained later. Figure 8 best exemplifies the general trend found in our research in the greenhouse on dose-response and combined effects of TIBA and 2,4-D on soybean and velvet leaf. At low doses of 2,4-D, which are however inhibitors, TIBA

Figure 5. Effects of TIBA and 2,4-D on the growth of velvet leaf

Levels of 2,4-D, from left to right, are 0, 10, 50, 200, 600 and 2,400 respectively. (Level 0 is present in the center part only.) Levels of TIBA, from top to bottom, are 0, 100 and 400 ppm respectively. On each pot the left digit indicates level of TIBA and the right one indicates levels of 2,4-D.

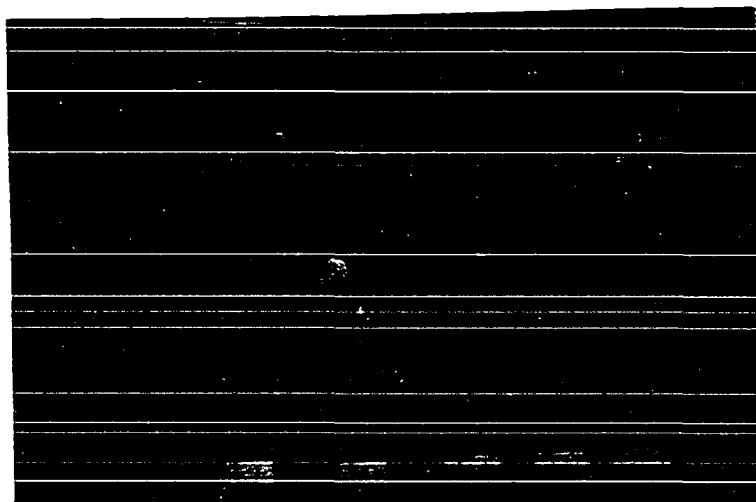
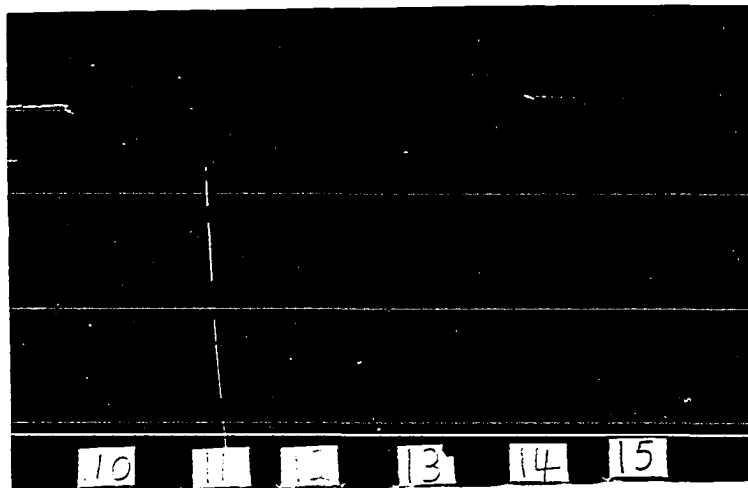
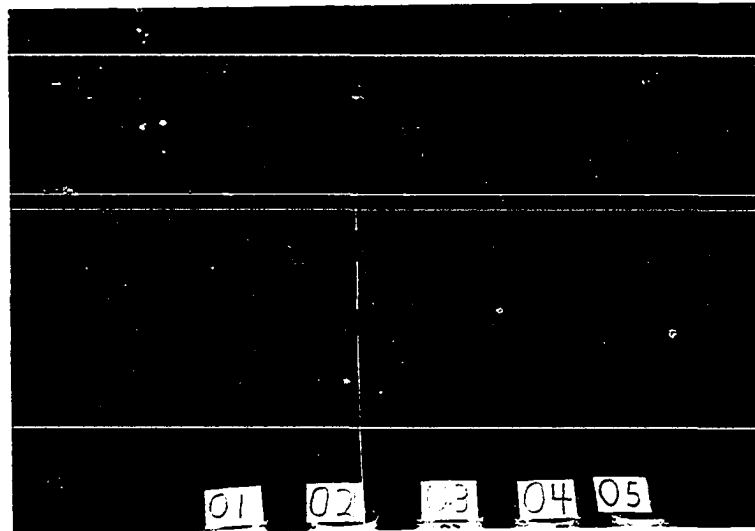


Figure 6. Experiment GV4. Effect of 2,4-D at 600 ppm on velvet leaf seedlings. The growing points and young leaves were killed. Vigorous regrowth from lower lateral buds took place.

Figure 7. Experiment GV4. Effect of additional TIBA on the regrowth of plants receiving 600 ppm of 2,4-D

04 - no TIBA

14 = plus 100 ppm of TIBA

24 = plus 400 ppm of TIBA

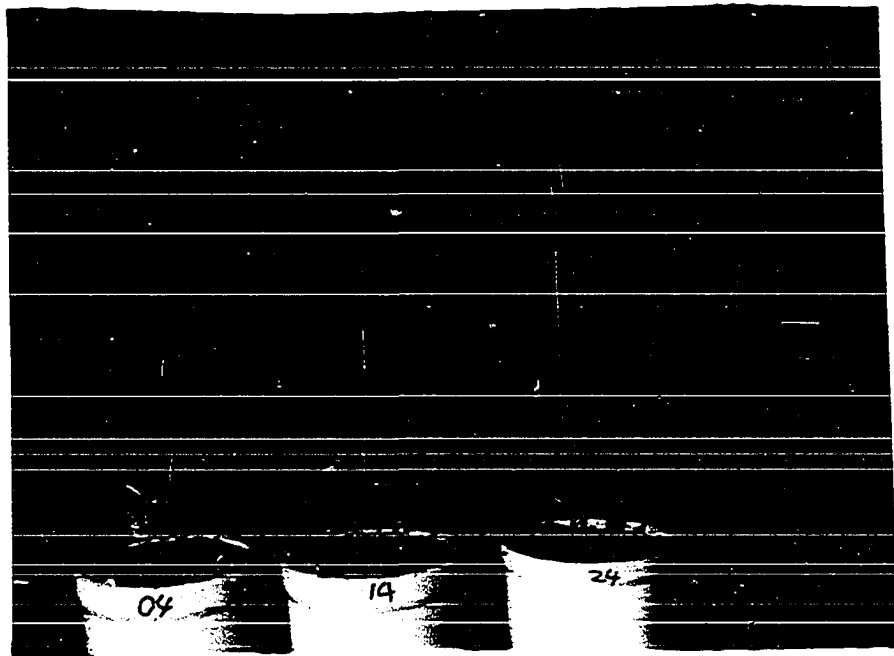
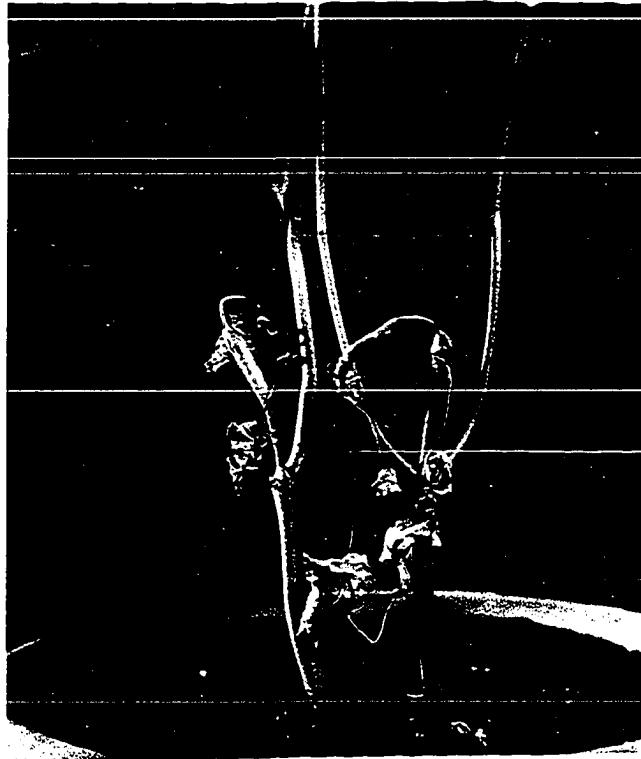


Figure 8. Relative effect of TIBA and 2,4-D on the yields of plant tops and roots of velvet leaf (each point in the figure is an average of four replications)

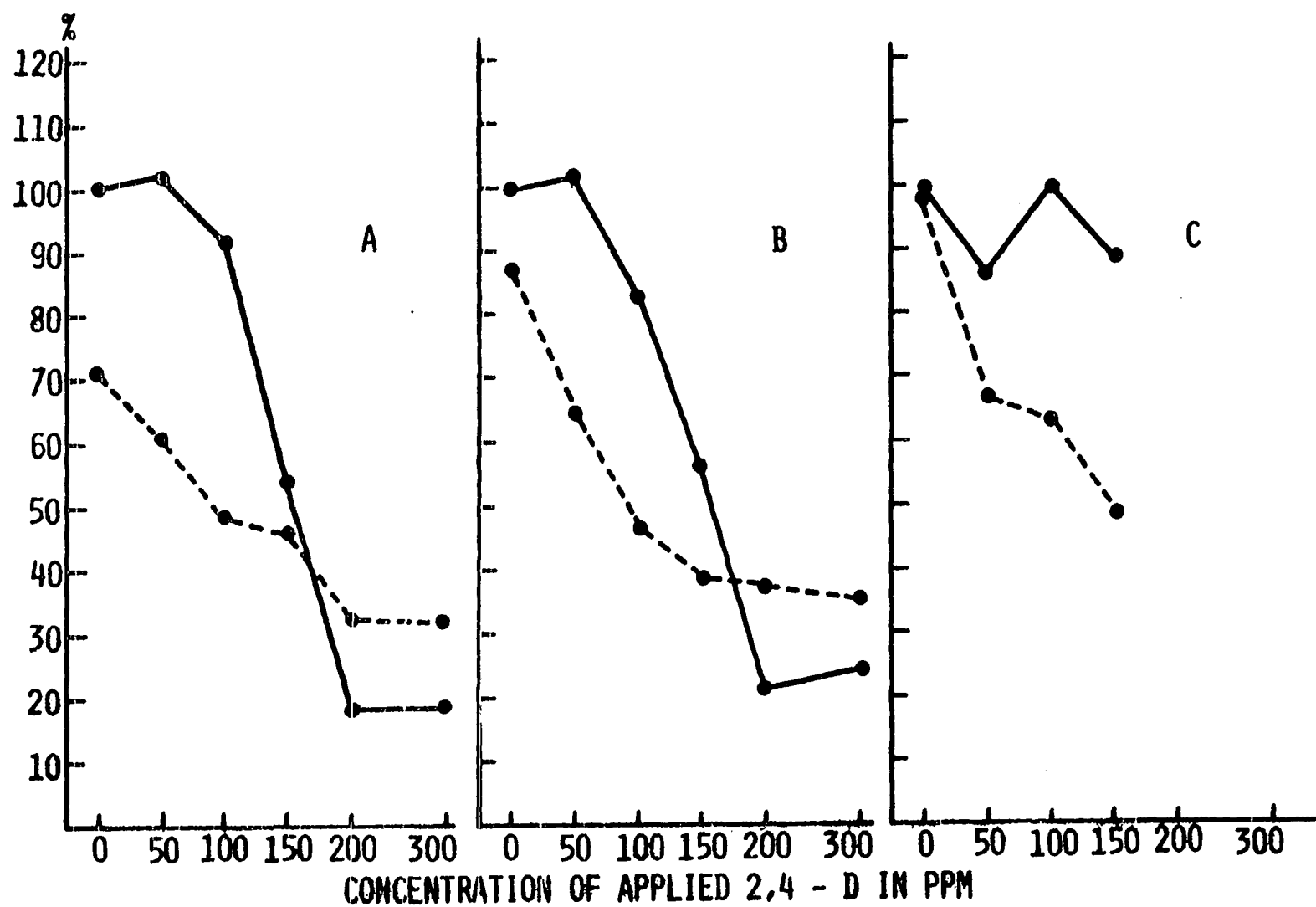
———— 2,4-D alone

- - - - 2,4-D plus TIBA (average of 50, 100,
and 150 ppm doses)

A = top fresh weight

B = top dry weight

C = root dry weight



added up its own inhibition and eventually some degree of synergism was apparent. At higher doses of 2,4-D, the effect of additional TIBA tended to make less drastic the highly toxic effects of 2,4-D. A true antagonistic effect was apparent in some cases.

Oats

The experiments with oats were carried out with similar objectives and under similar conditions to those with soybeans and velvet leaf. A major difference was the mode of application of 2,4-D, which was sprayed over all the leaves with an atomizer.

Experiment G01 The leaf tips of oat seedlings germinated November 3, were clipped November 14, and the TIBA-2,4-D treatments were applied two days later. The plants were unfolding the second leaf at this time. TIBA was sprayed 24 hours before 2,4-D. The plants were harvested 16 days after the treatments were applied. Tables 25 and 26 summarize the significance of the F ratios and the coefficients of variability respectively. Table 27 contains the means of the different attributes measured in this experiment.

Both TIBA and 2,4-D depressed the yields progressively with increasing doses. The interaction between TIBA and 2,4-D, which was highly significant for top yields, is illustrated in Table 28. It reveals that the combined inhibitory levels were less than additive in all but one of the TIBA-2,4-D combinations. No other interactions were apparent in this experiment. Another effect of 2,4-D was the production of "onion-like" leaves. These leaves elongated but they failed to emerge and expand normally. The frequency of these leaves increased along

Table 25. Significance of F ratios for the effects of TIBA, 2,4-D and their interaction on growth and yield attributes in oats

Experiment	Source	Attributes						
		Top F.W.	Top D.W.	Leaf number	Shoot height	2,4-D leaves	Tillers number	Tillers D.W.
G01	TIBA (A)	**	**	N.S.	**	N.S.	--	--
	2,4-D (B)	**	**	N.S.	N.S.	**	--	--
	A X B	**	**	N.S.	N.S.	N.S.	--	--
G02	TIBA (A)	N.S.	N.S.	--	N.S.	--	N.S.	N.S.
	2,4-D (B)	**	**	--	**	--	**	**
	A X B	N.S.	N.S.	--	N.S.	--	**	N.S.

Table 26. Coefficients of variability for different attributes measured in greenhouse experiments with oats

Variate	Experiments	
	G01	G02
Top F.W.	16.6	13.8
Top D.W.	15.5	13.0
Leaf number	17.8	47.5 ^a
Shoot height	9.4	13.0
2,4-D leaves	16.5	--
Number of replications	4	6

^a Number of tiller per pot.

with larger doses of 2,4-D, and the response was not affected by TIBA. The elongation of the shoot appeared significantly affected by TIBA only, but the inhibition was only 10% with respect to the untreated control even under the highest dose of TIBA. In general, the effects on yield and growth of oats were relatively less marked than in soybeans and velvet leaf, under comparable conditions and concentrations, even though oats received larger amounts of total 2,4-D, because the whole plants were sprayed.

Experiment G02 In this experiment, much larger doses of both TIBA and 2,4-D were applied in an attempt to induce some significant degree of growth and/or yield response in the tolerant oats. Both products were sprayed as usual and six replications for each treatment

Table 27. Experiment G01. Effects of TIBA and 2,4-D on the yield and growth attributes of oats. (each figure represents an average of four replications, as total yield per pot)

TIBA ppm	2,4-D ppm	Tops yield		Leaf number	2,4-D ^a leaves	Shoot ^b length cm/plant
		F.W. g	D.W. mg			
0	0	7.16	797	22.0	.00	18.0
0	25	5.90	696	20.5	.25	20.0
0	100	4.64	548	17.7	1.75	18.2
0	400	4.43	534	18.2	4.25	18.0
0	1600	3.79	464	17.0	4.00	16.0
50	0	5.32	625	21.7	.00	16.5
50	25	5.05	601	20.5	.25	17.5
50	100	4.86	595	20.0	2.25	17.5
50	400	4.65	555	19.0	3.75	17.7
50	1600	3.81	492	19.7	4.50	18.2
200	0	5.75	677	22.2	.00	16.7
200	25	3.50	439	15.7	.50	17.5
200	100	4.36	532	19.0	1.75	16.2
200	400	4.13	503	17.7	4.50	17.5
200	1600	3.11	399	16.7	4.25	16.5
800	0	3.98	516	17.7	.00	16.2
800	25	4.59	529	22.0	.25	16.5
800	100	4.29	533	20.7	2.75	17.5
800	400	4.64	550	21.0	4.75	16.5
800	1600	3.47	450	19.2	4.75	15.7

^a They appear as "onion-like" leaves, which elongate but fail to unfold normally.

^b As measured from ground level up to the base of the uppermost lamina.

Table 28. Comparisons between observed and expected yields of oat plants treated with TIBA plus 2,4-D combinations

TIBA ppm	2,4-D ppm	Top yields F.W.		
		Observed	Expected	O - E ^a
0	0	100		
0	25	82		
0	100	65		
0	400	62		
0	1600	53		
50	0	74		
50	25	70	61	+
50	100	68	48	+
50	400	65	46	+
50	1600	53	39	+
200	0	80		
200	25	49	66	-
200	100	61	52	+
200	400	58	50	+
200	1600	43	42	+
800	0	56		
800	25	64	46	+
800	100	60	36	+
800	400	65	34	+
800	1600	48	29	+

^a Signs of the differences of observed minus expected yields.

were established. Table 29 presents the average results of the attributes measured, relative to the untreated control, and the differences with respect to the expected value according to Colby's model. Applications of 2,4-D or TIBA resulted in slight to moderate increases in total dry matter in main tops, except for the highest doses of 2,4-D. When both TIBA and 2,4-D were applied in combination, the effects were less than additive. The largest number of tillers were produced by the untreated control. 2,4-D alone was markedly inhibitory, TIBA alone also was inhibitory at the highest dose. The combined effect was less inhibitory than expected. These results on tillering are not very reliable due to the large coefficient of variability and to the fact that the plants were relatively crowded in the pots, which inhibited the potential capacity for tillering. Shoot height appeared affected only by the highest dose (25,000 ppm) of 2,4-D and practically no manifestation of synergism or antagonism was apparent. The effects on the yield of dry matter in the tillers were closely related to the effect on the number of tillers. Both products were depressive and the combined effect was antagonistic. The actual yields of dry matter in the tillers were at most 15% of the yield of the main stems and leaves.

Experiment G03 This experiment was actually conducted in nutrient solution. The plants were utilized for the study of excretion of radioactivity in Experiment R02. At the end of the second week after application of the treatment, the plants were separated in main tops, roots and tillers, dried and weighed. Table 30 summarizes the yields of dry matter, the root : shoot ratios and the number of tillers.

Table 29. Experiment G02. Effect of 2,4-D and TIBA on the relative growth of main tops and tillers in oats (each figure is an average of six replications)

TIBA ppm	2,4-D ppm	main tops D.W.	O - E ^a	Tiller number	O - E	Shoot height	O - E	D.W. in tillers	O - E
0	0	100		100		100		100	
0	1000	110		67		95		25	
0	5000	117		56		88		17	
0	25000	80		0		63		0	
1000	0	111		96		109		78	
1000	1000	123	0	93	+	103	0	41	+
1000	5000	109	-	67	+	91	-	21	+
1000	25000	88	-	18	+	73	+	5	+
5000	0	115		71		103		78	
5000	1000	117	-	71	+	96	0	35	+
5000	5000	106	-	51	+	96	+	16	+
5000	25000	86	-	7	+	66	0	2	+

^a O - E equals the signs of the differences between the actual yields observed minus the yields expected according to Colby's model for the combined treatments.

Table 30. Effects of TIBA and 2,4-D on the yield of roots and tops, their ratio and frequency of tiller in oats (each value is the average of three replications)

TIBA ppm	2,4-D μg/pl	<u>D.M. yield mg/plant</u>			Root/shoot ratio	<u>Tillers</u>
		Top	Roots	Total		Number/plant
0	5	1430	253	1683	.18	2.0
0	50	1179	157	1336	.13	3.0
200	5	1059	112	1071	.11	4.7
200	50	1130	124	1254	.11	5.3

Table 31. Experiment G03. Significance of F ratios of the main effects and interactions of TIBA and 2,4-D on the growth and yield characteristics of oats, and coefficients of variability

Factor	d.f.	Shoots	Roots	Total	Root/shoot ratio	Tillers
TIBA (A)	1	10%	*	*	*	**
2,4-D (B)	1	N.S.	N.S.	N.S.	N.S.	N.S.
A × B	6	N.S.	10%	10%	N.S.	N.S.
C.V. %	-	14.2	26.6	14.1	15.7	29.5

Table 31 summarizes the statistical significance and the coefficients of variability. Both 2,4-D and TIBA decreased the yields of dry matter when applied alone. Their effects were relatively more marked on the roots. The application of TIBA and 2,4-D together resulted in even smaller root : shoot ratios, so indicating a relatively greater effect on the roots than on the tops. The addition of TIBA increased the number of tillers per plant with respect to the plants receiving 2,4-D alone. Again the combined effects appeared less than additive at the higher dose of 2,4-D. The small number of replications and treatments and relatively large coefficients of variability make the data in this experiment of low reliability. They do indicate a trend, however.

Field Experiments

A series of experiments with the objective of measuring the effects of TIBA on the action of 2,4-D in weeds and corn were carried out in two locations near Ames and in a third location, Madrid, 30 km S.W. of Ames. Three types of experiments were conducted during the growing season of 1969.

Experiments with uncultivated plots

One experiment with two replications each were established at each of the locations mentioned above and according to the procedures described in the chapter on materials and methods. One of the experiments near Ames was abandoned due to poor growth of some plots. The other two will be presented separately for different conditions were present in Ames and Madrid.

Experiment Fl, Ames The yields of grassy and broad leaved (BL) weeds one month after treatment as well as the final yields of weeds and corn are presented in Table 32. The statistical significances and the coefficients of variability for both Ames and Madrid are summarized in Table 33. At Ames, a heavy stand of broad leaved weeds, mostly smart weed (Polygonum spp.), was predominant since the beginning of the growing season. All treatments which included 2,4-D decreased the yield of broad leaved weeds when measured three weeks after spraying. The small amount of grasses present did not vary consistently with any treatment. TIBA did not show any additional effects either on grasses or broad leaved weeds, or they were masked by larger variations due to heterogeneity of the natural weed stand. Considering the yields of mature weeds (final harvest) it is noticeable how the growth of grasses, after the sprays were applied, compensated in large extent the lack of growth of the broad leaved species which were most affected by 2,4-D. Comparison of columns 5 versus 6 in Table 32 indicates a close negative relationship. Some indication appears in column 6, suggesting that TIBA could further increase the inhibition of growth of smart weed when combined with the intermediate levels of 2,4-D, although some "wild" values are also present. The yield of corn, which appeared mostly dependent on stand, was relatively higher at the intermediate doses of 2,4-D. Where 2,4-D was not applied, there was a significant decrease of the stand associated with the very vigorous growth of smart weed. At 1,200 g/ha, 2,4-D was apparently toxic enough to increase breakage and/or directly depress the yield of corn.

Table 32. Experiment Fl--Ames. Effects of TIBA and 2,4-D on the yields of grasses, BL weeds and corn in uncultivated plots (all values are in grams of dry matter per plot, and averages of two replications; corn stands are expressed in plants per plot)

2,4-D g/ha	TIBA g/ha	Final harvest					
		Early harvest		Weeds		Corn	
		Grasses	Broad- leaved	Grasses	Broad- leaved	Stand pl/plot	Ear yields
0	0	15.5	189.5	75	1507	15.0	1847
0	50	20.5	178.5	20	1532	19.0	2591
0	100	37.0	190.5	0	1470	22.0	2973
0	200	16.0	119.0	25	1324	23.5	3335
200	0	40.0	103.5	670	335	26.5	4072
200	50	43.5	84.5	1052	80	25.0	4027
200	100	25.5	65.0	790	145	23.5	3381
200	200	68.5	93.5	1130	106	28.0	4400
400	0	59.0	63.0	1172	120	23.5	3847
400	50	21.0	92.0	1070	33	30.5	5376
400	100	15.5	56.0	680	49	30.0	5376
400	200	54.0	47.5	1177	20	31.5	4859
600	0	70.0	72.0	1267	45	27.0	4067
600	50	28.0	97.0	1020	60	29.5	4629
600	100	32.5	93.5	882	31	29.0	4708
600	200	72.0	39.0	1045	65	26.0	3709
1200	0	43.5	35.0	875	62	24.5	3031
1200	50	20.5	48.5	1115	15	24.0	3936
1200	100	60.0	67.5	1190	20	28.5	3507
1200	200	49.0	63.5	944	45	27.0	4576

Table 33. Significance of F ratios for the effects of TIBA and 2,4-D on three weeks after treatment and final yields of weeds and corn, in uncultivated plots

Experiment	Factor	Variates					
		Early yields		Final yields			
		Broad-leaved	Grass	Broad-leaved	Grass	Corn	
						Stand	Yield
F1 Ames	TIBA (A)	*	N.S.	N.S.	N.S.	N.S.	N.S.
	2,4-D (B)	**	*	**	**	**	**
	A x B	10	N.S.	N.S.	N.S.	N.S.	N.S.
	C.V. %	24.6	49.0	37.6	15.4	18.2	20.5
F1 Madrid	TIBA (A)	10	N.S.	**	N.S.	N.S.	N.S.
	2,4-D (B)	**	N.S.	**	**	**	**
	A x B	10	N.S.	**	N.S.	N.S.	N.S.
	C.V. %	11.4	24.4	38.0	8.6	13.1	13.0

Experiment F1, Madrid The mean yields of corn and weeds in this location are summarized in Table 34. The major difference with respect to the site at Ames was the moderate to marked predominance of grasses, mainly Setaria spp., instead of smartweed. The population of broad leaved species was more varied in Madrid than in Ames. It included variable proportions of pigweed (Amaranthus retroflexus L.), lambsquarter (Chenopodium album L.), velvet leaf and smartweed. The grasses appeared compensating largely for the weeds killed by 2,4-D. At the time of final harvest a massive growth of grasses had "taken over," and all the broad leaved species had practically disappeared from the 2,4-D treated plots, as shown in column 6 of Table 34. Obviously, there was no place for synergistic action of TIBA. Stand and ear yields of corn again appeared to be the highest at low or medium doses of 2,4-D applied. With no 2,4-D, there was a significant growth of broad leaved weeds present which somehow appeared to make a stronger competition than grasses on corn. The highest amounts of 2,4-D were clearly damaging for the stand and, consequently, on ear yield.

Experiment F2 The overall effects of 2,4-D and its combination with TIBA on the yields of weeds and corn are summarized in Table 35, for both locations at Ames and Madrid. Table 36 summarizes the statistical significance of different factors considered in these experiments. The results were similar to those obtained in Experiment F1, for the locations and the conditions were similar. At Ames, the addition of TIBA appeared to increase the early effect of 2,4-D at low doses and

Table 34. Experiment F1--Madrid. Effects of TIBA and 2,4-D on the yields of weeds and corn in uncultivated plots (expressions like in Table 32)

2,4-D g/ha	TIBA g/ha	Final harvest					
		Early harvest		Weeds		Corn	
		Grasses	Broad- leaved	Grasses	Broad- leaved	Stand pl/plot	Ear yields
0	0	82.5	178.5	799	366	28.5	5626
0	50	95.0	136.5	550	500	33.5	5242
0	100	118.5	79.0	498	79	37.0	7056
0	200	134.0	56.5	957	100	34.0	6736
200	0	153.0	16.0	997	0	31.5	6915
200	50	172.5	13.5	925	0	32.0	6337
200	100	206.0	2.5	1077	0	36.0	6733
200	200	185.0	13.0	990	0	38.0	7419
400	0	140.0	7.0	790	0	34.5	7742
400	50	184.0	0.5	1110	0	34.5	7262
400	100	172.5	6.5	1082	0	36.5	7825
400	200	135.0	2.0	1035	0	37.5	7629
600	0	185.0	0	975	0	28.5	6198
600	50	162.5	0	925	0	30.5	6527
600	100	197.5	0	1182	0	31.0	5500
600	200	177.5	0	1165	0	29.5	5739
1200	0	142.5	0	1305	0	20.5	3840
1200	50	200.0	0	1490	0	17.0	3088
1200	100	190.0	0	1502	0	19.5	3598
1200	200	187.5	0	1407	0	16.0	3187

Table 35. Experiment F2. Effects of 2,4-D and TIBA on the yields of weeds and on the stand and ear yield of corn (each figure is an average of eight observations taken across pre-emergence herbicide and mechanical cultivation variables)

TIBA g/ha	2,4-D g/ha	Early yields ^a		Final yields			
				Weeds		Corn	
		Grass	Broad- leaved	Grass	Broad- leaved	Stand	Ear yield
<u>Ames</u>							
0	0	11.0	141	11	848	24.9	5064
0	200	12.5	58	432	104	34.4	6991
0	400	17.2	50	409	32	32.6	6620
100	200	17.1	27	378	54	32.9	6374
<u>Madrid</u>							
0	0	47.4	55.5	299	139.5	39.2	8486
0	200	75.1	0.0	538	2.5	33.5	7423
0	400	60.0	0.3	633	1.6	23.4	4822
100	200	58.8	1.5	462	4.0	34.7	7267

^a All yields are expressed in gm/plot of dry matter.

Table 36. Experiment F2. Significance of F ratios for the main effects of mechanical cultivation, ramrod in pre-emergence and post-emergence applications of 2,4-D and TIBA (only interactions including post-emergence treatments are included)

Source	d.f.	Final yields					
		Early yields		Weeds		Corn	
		Grass	Broad-leaved	Grass	Broad-leaved	Stand	Ear yield
<u>Ames</u>							
A ^a	1	**	**	**	**	**	**
B	1	**	**	**	N.S.	N.S.	*
C	3	N.S.	**	**	**	**	10%
A x C	3	N.S.	*	**	**	**	*
B x C	3	N.S.	N.S.	**	N.S.	N.S.	N.S.
A x B x C	3	N.S.	N.S.	*	N.S.	N.S.	N.S.
C.V. %	-	31.0	15.2	10.8	31.5	14.0	21.5
<u>Madrid</u>							
A	1	**	N.S.	**	*	**	**
B	1	**	N.S.	*	N.S.	**	**
C	3	N.S.	**	**	**	**	**
A x C	3	N.S.	**	10%	**	N.S.	10%
B x C	3	N.S.	N.S.	N.S.	N.S.	N.S.	10%
A x B x C	3	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
C.V. %	-	25.8	63.5	19.8	85.0	16.6	13.0

^a A = mechanical cultivation, B = ramrod in pre-emergence, C = 2,4-D and 2,4-D plus TIBA applications in post-emergence.

consistently repeated the same increased the effectivity of the mixture with respect to 2,4-D alone in the final harvest of broad-leaved weeds. Again no effect of TIBA was apparent on the control of broad-leaved species at Madrid for the lowest level of 2,4-D almost completely eliminated them. The decrease in stand and associated yield of corn was associated with different treatments at the two sites. At Ames, the lowest stand and yield occurred in the untreated plots. Except for those receiving mechanical cultivation, all these plots were heavily covered by vigorous smartweed. Plots receiving alachlor in preemergence kept a weed free band over the row for about three weeks. Then the unchecked growth of neighbor plants "took over" and soon formed a full-closed canopy. Even though the soil close to the corn stalks remained free from weeds, the result of the neighbor weed plants was equally detrimental to the crop. At Madrid the amount of broad-leaved plants was smaller even in the untreated plots and did not significantly affect the stand and yields. The most significant losses of stand and yields were again, as in Experiment F1, caused by the higher doses of 2,4-D. This effect on corn and the almost complete killing of broad-leaved weeds in many plots indicated that similar doses were more toxic at Madrid than at Ames. The stand of 47 plants per plot (Table 37) in cultivated unsprayed plots is abnormally high and probably was caused by a faulty planter. Yields of ear corn closely followed the variations in stand. The yields of broad-leaved weeds generally presented extremely high coefficients of variability which make the results poorly reliable. In Ames, where we had a dense and almost pure stand of

Table 37. Effects of post emergence applications of 2,4-D in relation to mechanical cultivation on the yield of weeds and stand and ear yields of corn (yields are expressed as gm/plot D.M. and the stand in plants per plot; post emergence treatment are the average of two levels of 2,4-D and treatment included 2,4-D plus TIBA)

Experiment location	Factor	Weeds				Corn			
		Grass		Broad-leaved		Stand		Ears	
		No cult.	Plus cult.	No cult.	Plus cult.	No cult.	Plus cult.	No cult.	Plus cult.
Ames	no 2,4-D	0	23	1593	106	14	36	1853	8274
	+ 2,4-D	733	80	102	21	31	36	5527	7796
Madrid	no 2,4-D	586	12	278	1	31	47	6328	10645
	+ 2,4-D	1065	24	1	5	26	35	5216	7672

smartweed, the C.V. was 15.2%. At the final harvest, after the interaction with the treatments and the compensatory growth of grasses, the C.V. increased to 31.5%. At Madrid, where the population of BL species was more sparse and heterogeneous, the C.V.'s were as high as 63.5 and 85.0%. This indicates that the site and/or the method of evaluation by harvesting total dry matter per plot were not adequate. Counting the number of surviving plants was equally or even more variable. The interaction that appeared to be statistically significant for more characteristics measured at both sites was between mechanical cultivation and postemergence applications of 2,4-D or 2,4-D plus TIBA. Table 37 indicates the orientation of that interaction. In uncultivated plots, the application of postemergence herbicide resulted in a large increase in the yield of grassy weeds, associated with a good control of broad leaved species. In the cultivated plots all weed yields were very small, with no major possibilities for the herbicide to have an influence on weed growth.

Experiment F3 This experiment was established only at Madrid, with two replications. The effects of 2,4-D and its date of application are summarized in Table 38. In the last date the plants were high enough in the hybrids plots so as to make difficult a perfectly uniform spray over the tops. To make more clear the illustration, we "pooled" together all 2,4-D treatments in Table 38. No additional effects of TIBA were apparent, and all levels of 2,4-D gave a similar type of effect, even though with some degree of variable intensity associated with doses. There were differences in the degree of tolerance to 2,4-D,

Table 38. Effects of 2,4-D plus TIBA mixtures on relative ear yields in relation to variety and date of spraying

Entry	<u>Spraying date</u>		Untreated control
	6/15	7/5	
Imbred 577	23.4 ^a	51.4	100 (= 1,264 kg/ha)
Imbred 334	71.4	40.7	100 (= 1,605 kg/ha)
Imbred A632	33.4	88.6	100 (= 3,700 kg/ha)
Hybrid 334 x 695	44.8	105.0	100 (= 6,048 kg/ha)
Hybrid B14 x 577	101.5	83.0	100 (= 6,048 kg/ha)

^a Average of all 2,4-D plus TIBA treatments.

both within imbeds and within hybrids. The hybrids appeared more tolerant to 2,4-D in general. There were interactions of variety by date of spraying. Different varieties could have been at different developmental stages at a given time of spraying. Then the interaction could well be a consequence of actually spraying two varieties on the same date but at different stages of development. Yield depressions of more than 50% were produced in some cases.

Radiotracer Experiments.

Soybeans

The different experiments carried out with C¹⁴-labeled 2,4-D in soybeans can be grouped in three categories according to their major objective and the procedures used. Experiments RS1 and RS2 were

established to study the effect of TIBA on the distribution of ethyl alcohol extractable and residual radioactivity in different parts of the plant. Experiments RS3, RS4, RS5 and RS6 were conducted to study the excretion of radioactivity by the roots to the nutrient solution. Three unnumbered experiments were established for autoradiographic studies.

Experiment RS1 Plants treated with 10 and 50 $\mu\text{g/plant}$ of 2,4-D in all combinations with doses of 0, 50, and 250 ppm of TIBA were harvested 78 hours after treatment and separated in tops above the treated leaf (upper tops), tops below the treated leaf (lower tops), and roots. Alcoholic extraction was performed and the general procedure described was followed for determining radioactivity in extracts and residues. The results are summarized in Table 39. No statistical significance was reached, except for the effect of TIBA on the residual radioactivity below the treated leaf (column 6 in Table 39). The large coefficients of variability obtained, between 25 and 35%, would invalidate any firm statistical inference to a large extent. No trend in the partition or in the total radioactivity translocated was apparently caused by TIBA. Curiously, less radioactivity was detected in fractions of plants treated with the largest doses of 2,4-D. This phenomenon would indicate some detrimental effect of 2,4-D on its own translocation, if not an important systematic error of some sort was introduced. The statistically significant effect of TIBA on residual radioactivity also appeared doses dependent. The low doses of 50 ppm TIBA increased radioactivity remaining in the residue, whereas the highest dose was

Table 39. Experiment RS1. Effect of TIBA on the translocation of radioactivity to different plant parts, 78 hours after application of C¹⁴-labeled 2,4-D (values are given in net CPM obtained in a GM counter; they are averages of two replications)

TIBA ppm	2,4-D μg/pl	Net CPM					
		Upper tops		Lower tops		Roots	
		Extr.	Resid.	Extr.	Resid.	Extr.	Resid.
0	10	408	159	302	127	438	159
0	50	333	115	363	115	288	125
50	10	481	176	454	174	365	139
50	50	394	157	448	153	305	80
250	10	404	131	567	97	293	126
250	50	357	137	581	66	267	164

depressive on the same character. There also was a noticeable amount of radioactivity in the roots.

Experiment RS2 In this experiment, plants were grown in pots. Otherwise, the same procedure as in Experiment RS1 was followed. Table 40 summarizes the radioactivity measured in alcoholic extracts and residues of plants harvested 78 hours after the treatments were applied. The trend of the results, if any, indicates that greater radioactivity was present in all plant parts when TIBA was applied before 2,4-D.

Table 40. Experiment RS2. Effect of TIBA on the amount and distribution of radioactivity in soybean plants, 78 hours after application of C^{14} -labeled 2,4-D (each value is an average of two replications)

TIBA ppm	2,4-D μ g/pl	Net counts per minute			
		Upper top		Lower top	
		extr.	resid.	extr.	resid.
0	5	130	29	34	15
0	50	511	308	64	316
2500 ^a	5	130	32	84	38
2500	50	811	223	--	--
250 ^b	5	---	80	33	22
250	50	1767	308	1153	346

^a Applied as a 0.01 ml drop of 2,500 ppm solution to the top expanded leaf.

^b Applied as 250 ppm solution sprayed over all the plant.

The amounts of C^{14} present in each part also increased with higher doses of 2,4-D approaching linear direct proportionality in some cases. It appears that translocation or at least the amount of C^{14} present was greater in general in the upper part than in the lower part of the plant. A comparison of this sort is acceptable because the total fresh weight harvested was similar in both regions. One replication of this same experiment was harvested six days after the application of the chemicals. The activity detected on these samples was in general very low, barely above background, for the lowest dose of 2,4-D.

Experiment RS3 In Table 41 are summarized the data of excretion of radioactivity by roots of soybean plants treated with three doses of C^{14} labeled 2,4-D and TIBA. Again a tremendous variation from plant to

Table 41. Experiment RS3. Radioactivity excreted by roots of soybean plants treated with C^{14} -2,4-D, as affected by previous application of TIBA

TIBA ppm	2,4-D $\mu\text{g/pl}$	Net CFM ^a		
		R1	R2	Ave
0	0.5	1	4	3
0	5.0	11	19	15
0	50.0	3028	1513	2270
250	0.5	11	159	85
250	5.0	19	9	14
250	50.0	2480	2338	2409

^a Measured eight days after the plants were treated.

plant was present, particularly at the highest levels of 2,4-D applied. The effect of TIBA, if any, might have been toward increasing the exportation of C^{14} out the roots. It was very noticeable the disproportionately large increase in excretion associated with the largest level applied, like suggesting that some mechanism of active control was "over saturated" and became unable to control the rate of excretion beyond certain amount. Actually, most of the amounts of radioactivity were barely above background except for the 50 μ g/plant dose (Table 41).

Experiment RS4 In this experiment the excretion of radioactivity was compared between plants receiving C^{14} -2,4-D alone and plants receiving TIBA before the application of radioactive 2,4-D. Two different TIBA treatments were included: 25 ppm in the nutrient solution and a 100 ppm solution sprayed over the plant tops. Both applications of TIBA were made four hours before 2,4-D. 2,4-D was applied as 0.01 ml drops to the second trifoliated leaf, which was fully expanded. All leaves and cotyledons below the treated leaf had been removed 24 hours before the experiment was started. Six days after treatment the plants were transferred to fresh nutrient solutions and the old ones were extracted with ether. The extracts were transferred quantitatively to planchettes and counted. The radioactivity in the second solutions was assayed six days later by counting a 0.01 aliquot in a scintillation counter. Table 42 summarizes the radioactivities of different treatments at different times. The analysis of variance of the same data is presented in Table 43. The relative effect of TIBA on the excretion of radioactivity under different levels of 2,4-D are

Table 42. Experiment RS4. Radioactivity excreted by roots of soybean plants treated with C¹⁴-labeled 2,4-D

TIBA ppm	2,4-D μg/pl	Net counts per minute								Total
		1st week				2nd week				
		R1	R2	R3	Ave.	R1	R2	R3	Ave.	
0	1	40	94	85	73	337	639	197	391	464
0	10	392	428	216	345	528	522	208	419	764
25	1	197	111	217	175	202	151	192	182	357
25	10	308	469	461	412	160	295	188	214	626
100	1	84	85	114	94	367	365	293	344	438
100	10	149	638	387	391	146	145	140	144	535

Table 43. Experiment RS4. F ratios for the effects of TIBA and doses of 2,4-D on the excretion of radioactivity by soybean roots

Source	d.f.	F ratios	
		1st week	2nd week
TIBA (A)	2	.81	6.1
2,4-D (B)	1	24.2	.83
A × B	2	.1	2.28 ^a
Error	10	---	---
C.V. %	--	46.8	38.3

^a Significant at .25 level.

presented in Table 44. At the end of the first week larger amounts of C¹⁴ had been excreted by the plants receiving the largest dose of 2,4-D. The addition of TIBA slightly increased the amounts of C¹⁴ excreted, but its effect was not significant. At the end of the second week, the trends were to some extent the reversal of the ones shown at the end of the first week. The excretion from plants receiving the low dose of 2,4-D were very similar to those receiving the largest dose. In one case there was even larger excretion from plants receiving the lower dose of 2,4-D. Plants receiving TIBA showed significantly less excretion, only equivalent to 50% to 60% of that in plants treated with 2,4-D alone.

Experiment RS5 In this experiment TIBA was applied in the nutrient solution or as a spray at 200 ppm four hours before applying

Table 44. Experiment RS4. Effect of TIBA on the excretion of radioactivity by soybean plants treated with C^{14} -2,4-D. Figures are relative to the values for plants which received the same amount of C^{14} -2,4-D alone. Each figure is an average of three replications.

TIBA	2,4-D in $\mu\text{g/pl}$			
	first 6 days		second 6 days	
	1	10	1	10
0	100	100	100	100
1 ^a	239	120	46	51
2 ^b	121	113	89	34

^a Applied at 25 ppm in the nutrient solution.

^b Sprayed over the plant as a 100 ppm solution.

The applications of 2,4-D were 0.04 ml/plant of solutions containing 10 and 100 ppm respectively. When TIBA was applied to the roots (level 2) the plants were all transferred to fresh solutions before the application of 2,4-D. Eight days after treatments the nutrient solutions were triple washed with ethyl ether and the ether quantitatively transferred to steel planchettes and counted. The plants were then transferred to fresh solutions which were extracted and the extracts counted a second time four days later. Table 45 includes the net radioactivity detected on both dates. No statistical significance was found for the effects of TIBA on the movement of 2,4-D nor a clear response to the increased doses of 2,4-D. Again extreme variability from plant to plant was found, which, when added to the small number

Table 45. Experiment RS5. Excretion of radioactivity by roots of soybeans in plants treated with C¹⁴-labeled 2,4-D (effect of TIBA applied to the roots or sprayed on tops on the rate of excretion)

TIBA ppm	2,4-D μg/pl	Net CPM						Total
		1st week			2nd week			
		R1	R2	Ave.	R1	R2	Ave.	
0	.4	30	43	36	2	3	3	39
0	4.0	135	214	176	22	4	13	189
200 ^a	.4	58	74	66	1	0	1	67
200 ^a	4.0	535	69	302	7	13	10	312
200 ^b	.4	52	60	56	9	0	5	61
200 ^b	4.0	46	110	78	11	8	10	88

^a TIBA applied to the roots.

^b TIBA sprayed on the leaves.

of degrees of freedom contributed to an "uninformative" statistical analysis. It appeared that root applied TIBA increased the excretion of radioactivity whereas foliar applications tended to decrease the radioactivity excreted. The excretion during the second week was very low, barely above background.

Experiment RS6 Three doses of C^{14} -2,4-D, 0.1, 1.0, and 10 μ g of 2,4-D per plant were applied alone or to plants previously sprayed with a 200 ppm solution of TIBA. The 2,4-D applications were made in 0.01 ml drops to the topmost expanded leaf, which was the first trifoliate leaf. Eight days after treatment, the respective nutrient solutions were extracted with chloroform. The radioactivities produced by the different treatments are summarized in Table 46. The small number of observations and the large variability found make the statistical significance obtained for the effect of TIBA of doubtful value. The effect of TIBA was toward more instead of less mobility of 2,4-D or its radioactive derivatives in the TIBA-treated plants. The response to the doses of 2,4-D applied was far less than proportional. No difference was apparent between 1.0 and 10.0 μ g/plant.

Velvet leaf

Three types of experiments, as described above for soybeans, were carried out using C^{14} -labeled 2,4-D in velvet leaf. The distribution of ethanol-extractable and residual radioactivity in plant parts was studied in Experiment RV1. Experiments RV2 and RV3 dealt with the excretion of radioactivity by roots. The distribution of radioactivity,

as affected by TIBA, was studied by autoradiographic techniques in three undesigned groups of velvet leaf plants.

Table 46. Experiment RS6. Effects of doses of C¹⁴-2,4-D and addition of TIBA on the excretion of radioactivity by roots of soybean (the solutions were counted and extracted eight days after treatment)

TIBA ppm	2,4-D μg/pl	Net CPM			Ave.
		R1	R2	R3	
0	.1	21	20	8	16
0	1.0	11	77	81	56
0	10.0	40	46	49	45
200	.1	59	31	28	39
200	1.0	172	181	66	140
200	10.0	61	65	67	64

Experiment RV1 This experiment had the objective of determining the effect of TIBA on the pattern of distribution of radioactivity in the plant of soybean upon application of C¹⁴-labeled 2,4-D to one leaf. Doses applied and the amounts of radioactivity recovered in the alcoholic extracts and in the residue are summarized in Table 47. All the activities detected were very low. Relatively more radioactivity was translocated to the region above the treated leaf (upper tops) than to the lower tops. The higher doses resulted in higher radioactivity.

TIBA did not induce any appreciable change in the pattern or in the amount of radioactivity translocated.

Table 47. Experiment RV1. Effect of TIBA on the distribution of radioactivity to different plant parts in C^{14} -2,4-D-treated velvet leaf (each figure is an average of two replications)

TIBA	2,4-D μ g/pl	Net counts per minute			
		Upper top		lower top	
		extr. ^a	resid. ^b	extr.	resid.
0	0.2	10	8	3	9
0	2.0	32	21	10	10
1 ^c	0.2	6	10	10	8
1	2.0	49	14	5	8
2 ^d	0.2	5	25	--	8
2	2.0	32	20	5	9

^a 1 : 40 aliquot of the total extract was counted.

^b Counted at infinite thickness.

^c 25 μ g/plant in a 0.01 ml drop.

^d Spray of a 250 ppm solution.

Experiment RV2 In this experiment the effect of TIBA on the excretion of radioactivity by plants of velvet leaf receiving two levels of C^{14} -2,4-D was determined one week after treatment, and another determination was made at the end of a second week in fresh nutrient solution. Results are summarized in Table 48. It shows marked

Table 48. Experiment RV2. Effect of TIBA on the excretion of radioactivity of C^{14} -2,4-D-treated plants in velvet leaf

TIBA ppm	2,4-D μ g/pl	New counts per minute ^a				
		1st week				2nd week
		R1	R2	R3	Ave.	Ave.
0	1.0	244	116	154	171	54
0	10.0	77	93	190	120	46
200	1.0	141	187	182	157	14
200	10.0	99	96	152	116	44

^a Radioactivity in a 0.01 aliquot counted in a scintillation counter.

variability from plant to plant within treatments. TIBA did not show any effect on the amounts of radioactivity excreted. There were indications that more radioactivity was excreted by plants receiving the lower dose of 2,4-D. The amounts excreted during the second week appeared much lower than during the first week.

Experiment RV3 This experiment was conducted under conditions similar to that of Experiment RV2. Mean values of excreted radioactivity at the end of the first and second week are presented in Table 49. The counts were less than twice the background in most cases, denoting very little radioactivity excreted to the solution. No definite trend associated with the addition of TIBA was shown. The indication, if any, was toward a slight inhibition of excretion at the end of the first week. Actually, all the values were very low with

Table 49. Experiment RV3. Effect of TIBA on the excretion of radioactivity by roots of velvet leaf plants treated with C^{14} -labeled 2,4-D

TIBA ppm	2,4-D μ g/pl	Net CPM ^a	
		1st week	2nd week
0	5	4	12
0	10	36	38
200	5	9	14
200	10	12	30

^a Each figure is an average of three replications. A 2,000 counts cycle was taken on each sample.

respect to the total amounts applied. In consequence, even differences relatively large in radioactivity detected in the nutrient solution are of little significance.

Oats

The same three types of experiments, as described for soybeans and velvet leaf, were carried out with oats. The experiments conducted to determine the effects of TIBA on the distribution of radioactivity in plants treated with C^{14} -2,4-D by means of alcoholic extractions and autoradiography failed to detect any significant amount of radioactivity other than in the treated leaf. In cases where some low activity was detectable, no effects of TIBA were apparent. Therefore, only two experiments dealing with excretion of radioactivity via roots are presently reported.

Experiment R01 Plants growing in nutrient solution were treated with two doses of 2,4-D alone or in presence of TIBA at 200 ppm. The amounts of radioactivity detected in the nutrient solution at the of the first and at the end of the second week after treatment are summarized in Table 50. It shows that, as an average, the largest dose of 2,4-D applied resulted in greater radioactivity excreted at the end of the first week after treatment. However, at the end of the second week the lower dose of 2,4-D "caught up" and yielded the largest amount of radioactivity in the solution. The addition of TIBA resulted in a marked inhibition of the excretion of radioactivity at any level of 2,4-D during the whole period of two weeks. No deformations or other symptoms of toxicity were visible in the plants under any of the treatments.

Table 50. Experiment R01. Effect of TIBA on the excretion of radioactivity by oat plants treated with C^{14} -radio labeled 2,4-D (each figure is an average of three replications)

TIBA ppm	2,4-D $\mu\text{g/pl}$	NCPM excreted ^a	
		1st week	2nd week
0	5	12	560
0	50	130	329
200	5	5	6
200	50	3	13

^a Activity in a 0.01 aliquot of the total nutrient solution. Measured in a scintillation counter.

Experiment R02 In this experiment, oat plants were grown in Hoagland solution in the usual manner. The radioactivity excreted was determined at the end of the first and second weeks respectively. Table 51 presents the averages of radioactivity counted for the different treatments in the nutrient solution and present in the treated lamina. It appeared that plants treated with TIBA were able to excrete less radioactivity and to accumulate less radioactivity in the treated leaf. Whether the differences were associated with less absorption, with more 2,4-D being bound in nonether extractable form, or to differential partition to other plant parts was not determined in this experiment. Actually, the differences were caused by the much larger

Table 51. Experiment R02. Effect of TIBA on the excretion of radioactivity by roots of oat plants treated with C¹⁴-2,4-D and on the accumulation of radioactivity in the treated leaf

TIBA ppm	2,4-D ppm	Excreted ^a		Treated leaf ^b	
		week 1	week 2	part 1 ^c	part 2 ^d
0	50	37	265	9175	1090
250	50	21	211	5715	359

^a Activity in a 0.01 aliquot of nutrient solution counted in a scintillation counter.

^b Total extracted radioactivity, counted in a gas-flow counter.

^c Part of the lamina where the C¹⁴-2,4-D was applied.

^d Rest of the lamina in the treated leaf.

values associated with 2,4-D alone in one of the replications, which makes the results less reliable.

Experiments with autoradiography

Plants of soybeans, velvet leaf and oats were treated with radioactive 2,4-D and TIBA, and autoradiographs were taken after different periods of time after the applications, as described in the chapter on methods. Four figures are included, showing results that were consistently found in experiments with two replications each. In a similar number of cases no differences were detected between treatments. In experiments with oats, no differences between treatments were detected in any case in two different experiments. No plants showing effects of TIBA opposite to those in Figures 9, 10, and 11 were found in soybeans or velvet leaf. Figure 9 shows that the translocation of C^{14} -2,4-D or its radioactive derivatives was markedly decreased by the addition of TIBA. When 2,4-D was applied alone, it translocated in larger amounts to both the growing point and down the stem. Another difference shown was that 2,4-D did not move to the lateral leaflets when TIBA was applied. Figures 10 and 11 show the effect of TIBA on the distribution of radioactivity in velvet leaf seedlings of different age. Figure 9 shows that no differences were noticeable three hours after application of the C^{14} -2,4-D. Eighteen hours after the treatment, more translocation was found in plants receiving 2,4-D alone. The distribution remained almost unchanged in the plant receiving a previous application of TIBA. A similar effect was found in older seedlings of velvet leaf, shown in Figure 11. A more intense and generalized translocation

Figure 9. Effect of TIBA on the distribution of radioactivity applied as C^{14} -2,4-D to the first trifoliated leaf in soybeans

Top. C^{14} -2,4-D plus a previous spray
of TIBA at 200 ppm

Bottom. C^{14} -2,4-D applied alone

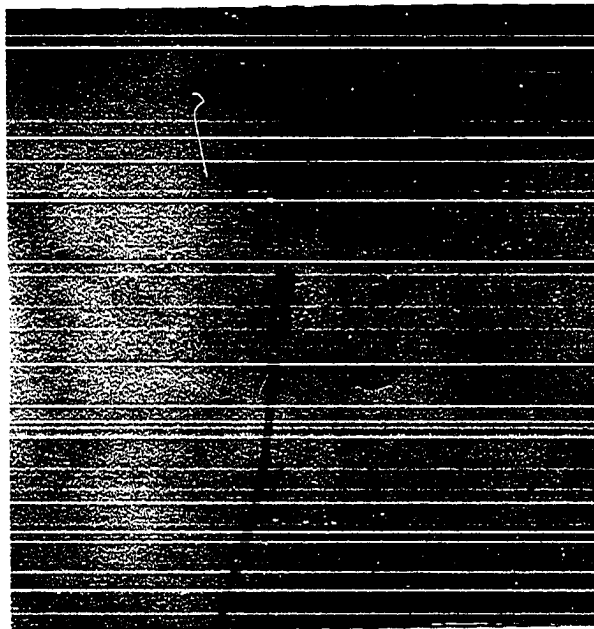
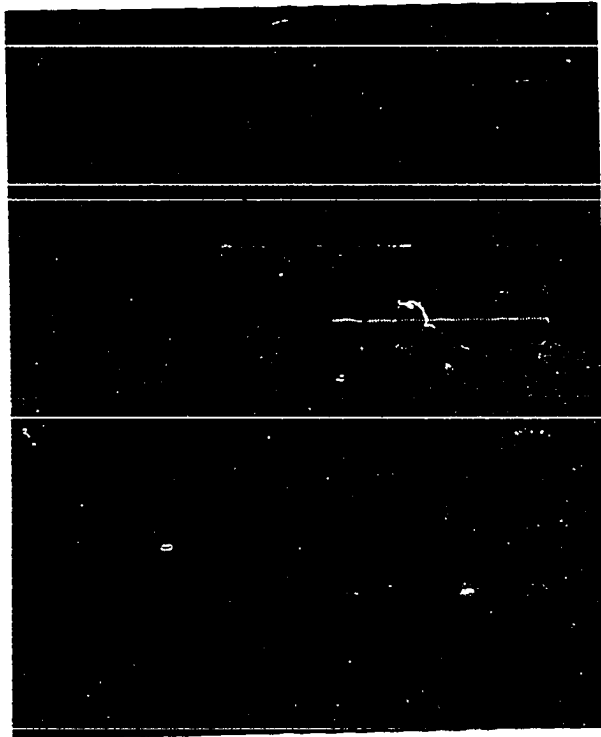
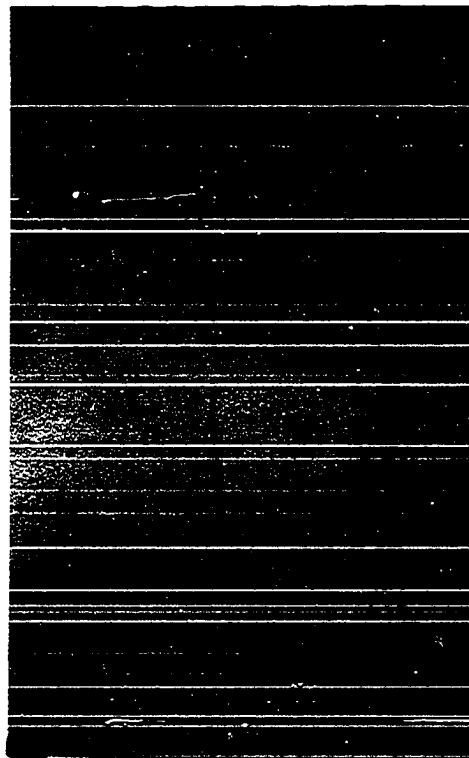
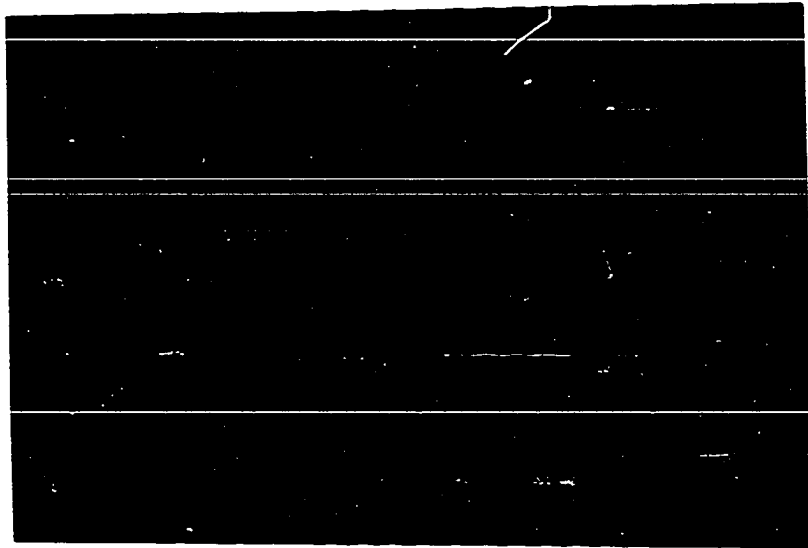


Figure 10. Effects of TIBA on the distribution of radioactivity in velvet leaf seedlings treated with C^{14} -2,4-D on the cotyledonary leaves. From left to right: 2,4-D + TIBA, and 2,4-D alone (18 hours after the treatments). The two impressions at the extreme right were taken three hours after treatment and do not show differences.

Figure 11. Effects of TIBA on the distribution of radioactivity in velvet leaf seedlings treated with C^{14} -2,4-D. Left: 2,4-D + TIBA, right: 2,4-D alone. Distribution 30 hours after treatment.



occurred in plants receiving 2,4-D alone than in those previously treated with TIBA.

Germination Experiments

The objective of these experiments was to determine the combined effects of TIBA and 2,4-D on germinating seeds of oats and velvet leaf. The procedure consisted in placing 10 uniform seeds in a row over a square of blotter paper 15 x 15 cm size. The blotter and seeds were in turn enclosed in a plastic envelope and 20 ml of the corresponding solutions of TIBA, 2,4-D or their combinations were added to moisten the blotter. The "seeded" blotters were stacked in a record holder tilted 45°. The germination period was carried in the dark, at a constant 25°C. Six days after it was observed in oats that two types of roots were characteristically developed and could be easily distinguished within each plant. One type were long roots (longer than 5 cm) which grew down at a narrow angle with respect to the axis of the plant. Others were much shorter roots growing at wide angles with respect to the axis. The number of roots of each type was counted. In some of the envelopes one or two seeds did not germinate, but this lack of germination did not appear associated with any particular treatment. So it was decided to adjust all totals to 10 germinated seeds per envelope, assuming a simple linear relationship. The elongation from the seed to the tip of the leaf was measured to the nearest millimeter and an average length per plant computed for each envelope. The roots were bent and intermingled at the bottom of the envelope. This made impossible any measurement other than counting the roots. Tables 52 and

Table 52. Effects of TIBA and 2,4-D applied to oat germinating seeds on the elongation of the first leaf and the number of roots

TIBA ppm	2,4-D ppm	<u>Root number/blotter^a</u>		<u>Leaf length</u>
		"long"	"short"	mm/plant ^b
0	0	26.5	9.5	60
0	.002	29.5	9.5	70
0	.020	29.5	9.0	57
0	.200	30.0	6.5	66
0	2.00	19.0	31.5	58
2	0	26.0	17.0	60
2	.002	22.5	15.5	58
2	.020	22.5	13.0	62
2	.200	26.0	14.0	50
2	2.00	11.0	30.0	49
20	0	28.0	18.0	59
20	.002	26.5	18.5	53
20	.020	28.0	12.5	59
20	.200	27.5	15.0	51
20	2.00	13.0	30.0	47
200	0	22.5	18.5	42
200	.002	22.5	17.0	38
200	.020	23.0	17.5	49
200	.200	21.0	20.5	40
200	2.00	19.5	22.5	36

^a Averages of two replications.

^b Averages of 18 to 20 plants grouped in two blotters.

53 show the mean yields per treatment and the statistical significance of main effects and interactions respectively. No interactions of TIBA \times 2,4-D were detected as statistically significant. The inhibition of the frequency of long roots by 2 ppm of 2,4-D is well noticeable. Lower doses did not appear to visibly affect the frequency of long roots.

Table 53. Analysis of variance of the effects of TIBA and 2,4-D on the attributes of oat seedlings

Sources	d.f.	Significance of F ratios		
		long roots	short roots	leaf length
TIBA	3	**	*	**
2,4-D	4	**	**	10%
TIBA \times 2,4-D	12	N.S.	N.S.	N.S.
C.V. %	--	14.7	27.6	12.2

Only the level of 200 ppm of TIBA appeared to contribute a slight decrease in long roots. TIBA produced marked increases in the number of short roots when applied alone or combined with lower doses of 2,4-D. When 2 ppm of 2,4-D were added, however, the number of short roots was the highest no matter the addition or absence of TIBA. The interaction of TIBA by 2,4-D was not significant. Both products appeared to increase the proliferation of short roots independently, but when active doses of both products were applied together, the effects were not

Table 54. Effects of TIBA and 2,4-D solutions on the elongation of seedlings and the germination of velvet leaf seeds

TIBA ppm	2,4-D ppm	plant length ^a mm/pl	germinated seeds ^b number/blotter
0	0	152	9.0
0	.002	166	8.0
0	.020	161	8.5
0	.200	73	6.0
0	2.00	27	7.5
2	0	122	7.0
2	.002	127	10.0
2	.020	115	6.5
2	.200	66	8.0
2	2.00	28	8.5
20	0	110	7.5
20	.002	107	7.5
20	.020	83	8.5
20	.200	65	7.5
20	2.00	19	8.5
200	0	78	8.5
200	.002	69	7.5
200	.020	78	6.5
200	.200	54	9.5
200	2.00	25	8.0

^a Averages of 12 to 20 individual plants in two replications.

^b Averages of two replications.

additive but very similar to that of 2,4-D alone. (Compare the number of roots induced by 2 ppm of 2,4-D at any level of TIBA in Table 52.)

Table 55. Analysis of variance of the effects of TIBA and 2,4-D on the seed germination and initial growth of velvet leaf seedlings

Source	d.f.	F ratios	
		Seedling length	% germination
TIBA	3	**	N.S.
2,4-D	4	**	N.S.
TIBA x 2,4-D	12	**	N.S.
C.V. %	--	9.2	11.9
St. error	--	7.9	10.7

A similar procedure and the same doses of TIBA and 2,4-D were applied to seeds of velvet leaf. The seeds had been previously treated in boiling water during one minute which improved germinability up to 95% or more under our conditions. 108 hours after the seeds were put in the dark at 34°C the total length of the seedling from the expanding cotyledons to the root tip and the number of germinated seeds per blotter were determined. Mean values are summarized in Table 54, and the analysis of variance appears in Table 55. The main effects of both products and their interaction were all statistically significant for seedling elongation.

The percent germination was very erratic, showed no statistical significance but great variability. At lowest levels of 2,4-D up to .020 ppm which alone appear inactive the combined effect appeared to be more than additive in inhibiting elongation. The "convergence" of the values at higher levels of 2,4-D indicates effects less than additive. The highest dose of 2,4-D (2 ppm) reduced the elongation down to 15% of the control, but did not arrest the germination as such.

DISCUSSION

The combined effects of TIBA and 2,4-D on various plant attributes were evaluated by diverse type of experiments with a number of cultivated and weed species. The group of experiments conducted with soybeans, velvet leaf and oats in the greenhouse was the one in which more acceptable experimental conditions could be maintained and more consistent results were obtained.

Three successive experiments with soybeans showed statistical significance at 0.01 P level for the main effects of TIBA and 2,4-D, as well as for their interaction on both yield and morphological attributes of the plants, as measured in the portions of the plants developed after the application of the treatments. Fresh and dry matter yields of stems and leaves were all similarly affected. Both TIBA and 2,4-D were progressively inhibitory of yields when increasing doses of either TIBA or 2,4-D were applied alone. The production of lateral branches was a symptom particularly, but not exclusively, associated with applications of TIBA. Gross deformations of leaves and stems were caused only by 2,4-D. The combined effect varied from synergistic to antagonistic, depending on the attribute and the doses considered. In cases where both products were markedly inhibitory individually, the combined effect was almost always less than additive. The toxicity of 2,4-D on leaves and stems appeared differently affected by the addition of TIBA, which was itself inactive on these attributes. When TIBA was added to a given dose of 2,4-D the "2,4-D" symptoms in the leaves were decreased and the symptoms of stem damage were more marked than with the same

amount of 2,4-D applied alone. Also, low levels of 2,4-D, which did not produce any visible damage when applied alone, produced detectable effects when TIBA was added with the low level of 2,4-D. These two apparently opposite effects of TIBA on the activity of 2,4-D supports the hypothesis that TIBA inhibits the free translocation of 2,4-D throughout the plant.

The negative or positive interaction between TIBA and 2,4-D on yield and growth attributes which might be called antagonism or synergism respectively in this thesis in no way implies the existence of a true, more or less direct biochemical interaction of the products at some site in the plant cells. The combined effects I measured in terms of yield and morphological changes could actually be mediated by many other phenomena induced independently by each of the products. Possibly one product modifies the internal conditions of the plant and consequently its ability to respond to the other product applied, but without there being a true interaction between both chemicals. Other factors contributing to the appearance of negative interactions were the methods of measurement themselves, as already was pointed out in the chapter on results. Some amount of plant material was already present in the part of the plant above the treated leaf when the treatments were applied. In addition, the toxic action of TIBA and 2,4-D might not be fully developed instantaneously after the treatment and growth could proceed for a while after the treatments were applied. The final result is that, under the highest levels of TIBA and 2,4-D which might be completely inhibitory, some amount of plant material was harvested and called

"above the treated leaf" or "after treatment" when in fact it was produced "before treatment." These "carry-over" yields were obviously similar in all the pots. When high doses of 2,4-D almost arrested all new growth, the yields obtained were similar with or without addition of TIBA. It is obvious that if one product stops growth and the plant does not "shrink" there is no physical opportunity for a second product to exert any additional effect. The convergence of the plotting, like in Figures 1, 2b, and 3 do not represent real antagonisms but is an artifact of the method and the fact that TIBA and 2,4-D did not induce negative growth. Estimating the carry-over of fresh and dry matter above the treated leaf when the products were applied could result in some degree of improvement of the precision with which the effects of treatments were measured.

Although no systematic observations were made for periods longer than three weeks, it was apparent by then that even the highest levels of 2,4-D and/or TIBA were unable to kill the treated plants. Possibly, 2,4-D at the higher doses damaged the vascular structures which are essential for its transport and systemic toxic action (National Academy of Sciences, 1968). The addition of TIBA to low doses of 2,4-D did not result in any increase of lethal effects on the whole plant, so negating the possibilities of the practical application of TIBA-2,4-D mixtures for weed control. This result is in agreement with the well-known fact that actively growing weeds are necessary for an effective herbicidal action of 2,4-D. The most conspicuous effect of TIBA is precisely the inhibition of growth.

The nitrate content in the leaves was determined in one out the three experiments with soybeans. It gave an interesting example of dose-dependent interaction, which was illustrated in Figure 2a. The main effect of TIBA appeared insignificant (Table 15) but actually TIBA decreased the content of nitrates when applied alone (Table 14). Other authors¹ have found that the addition of TIBA decreased the activity of nitrate reductase which is in a sense opposite to my present results. The application of 2,4-D alone resulted in some degree of nitrate increase at the higher levels, but the effect was relatively small within limits attributable to experimental errors. The combined effect appeared puzzling at first sight in Figure 2a. With up to 50 ppm of 2,4-D, no other than the depressive effect of TIBA is shown. The parallelism of the plots is indicative of no significant effect of 2,4-D at doses of 50 ppm or lower. The increased nitrate content at high levels of 2,4-D with TIBA could possibly be caused by the total disturbance of the plant tissues impeding a normal supply of essential assimilates to the young growing leaves and/or their own photosynthesis. Consequently, some nitrates reaching the leaf via the xylem are not utilized and accumulated. In addition, these leaves formed after high doses of 2,4-D and TIBA were so stunted that any tiny amount of nitrate present could result in high concentrations. No clear possible cause for the synergistic action of TIBA could be found other than TIBA could increase the concentration of 2,4-D in the growing top by impeding its downward translocation.

¹ Anderson, I. C. 1971. Effect of TIBA on nitrate reductase activity. Personal communication. Iowa State University. Ames, Iowa.

The inhibitory effects of 2,4-D and TIBA appeared more marked on the size than on the number of leaves formed after treatment. In preliminary experiments, a close relationship between leaf weight and leaf area measured with a planimeter was found. Leaf weight is the estimator of leaf size used in the present thesis. It was found that 600 ppm of 2,4-D depressed the number of leaves by 25% and 45% in velvet leaf and soybean respectively while the corresponding leaf areas were decreased as much as 65% and 68% respectively. This relatively larger effect on leaf size than on leaf number appears to indicate that the effects of 2,4-D and TIBA are primarily on cell elongation (more than on differentiation).

In the experiments with velvet leaf, the interaction TIBA by 2,4-D reached levels of statistical significance only for a few attributes in a group of three experiments. The coefficients of variability were consistently higher in velvet leaf than in soybeans. Since velvet leaf is a wild species it is possible that a larger genetic variability was present in it, either in terms of growth characteristics and/or in biochemical properties like the type of reaction to active compounds like TIBA and 2,4-D. Possibly, a larger number of plants in a tray would be a better experimental unit than pots with only three plants each. Again the main effects of TIBA and 2,4-D were highly significant on most of the attributes measured. Their interaction was significant in the three experiments only for stem elongation among the quantitative attributes measured. The nature of the significant interaction of TIBA and 2,4-D on lateral branching depended on the doses involved.

Intermediate doses of 80 and 200 ppm of 2,4-D increased the branching induced by TIBA, whereas they alone did not produce any branching. Similar results were obtained in the two experiments where branching was measured. No reasonable explanation for the synergism between TIBA and 2,4-D on this attribute was found. At the higher doses of 2,4-D, branching was strongly inhibited with or without TIBA. Under these conditions all stems were merely a deformed mass of tissue which hardly can be visualized as capable of any growth of buds or leaves. Interaction on the elongation of the stem resulted from the lack of additivity when one or both products were applied at high doses. This aspect was already discussed at the beginning of this chapter. The interaction on branching illustrated in Figure 2a is an example of a combined effect which is both dose-dependent and plant potential limited. For example, TIBA was synergistic to low doses of 2,4-D and apparently antagonistic with respect to the higher levels. We did not apply a dose of TIBA high enough to see up to what extent the action of 2,4-D could be inhibited by TIBA. Considering together Experiments GV1 and GV2, it appeared that the stimulatory effect of 2,4-D on branching occurred at a narrow range of levels, and the levels varied from experiment to experiment. This indicates an extreme sensitivity of the activity of the compound to external and/or internal conditions in the plant. The greater the sensitivity the more problematic its application would be in practical agriculture. The antagonistic action of TIBA on the appearance of 2,4-D-induced leaf deformation was better appreciated at the lower levels of 2,4-D where the total

number of leaves produced was not appreciably decreased by the treatments. Because TIBA was sprayed all over the plant and it is also translocable (Sant' Anna et al., 1970) the question remains about whether TIBA affected the action of 2,4-D by inhibiting its translocation or the biological activity in situ of 2,4-D. A comparison of 2,4-D content between deformed and normal-looking leaves of similar age would be helpful in determining the nature of the action of TIBA in plants. If the normal looking organs contain as much 2,4-D or its derivatives as the deformed ones, it would indicate that the effect of TIBA is at the site of action of 2,4-D. Otherwise it would indicate an inhibition of translocation. A smaller number of deformed leaves was associated with the higher doses of 2,4-D. This apparently resulted from the fact that the actual number of leaves formed after treatment was smaller under the high doses. The first visibly deformed leaf was not the first but the second or third one developed after treatment. Obviously there was not much opportunity for "2,4-D" leaves when only one or two leaves were formed after treatment.

The effects of TIBA and 2,4-D on stem elongation and on the accumulation of fresh and dry matter in the stem and leaves below the treated leaf (lower tops) was determined only in Experiment GV2. These results indicated significant effects of TIBA and 2,4-D on that portion of the plant. Significant increases in dry matter were associated with single applications of TIBA, up to 400 ppm, and with 2,4-D, up to 200 ppm. The effect of those doses combined was less than additive but still yielded more than the untreated control. These results are to a certain

extent comparable to those found by Greer and Anderson (1965), Afolabi (1969), and Johnson (1970), who were able to obtain increased seed yields in soybeans by modifying the pattern of growth and development of the plant with applications of TIBA and/or 2,4-D. The leaves already expanded in the plants of velvet leaf when the treatments were applied did not appear affected. They might sustain a normal rate of assimilation and, because less opportunity for new growth existed in the highly inhibited upper tops, more assimilates were accumulated in the lower tops. This is one of the principles involved in the application of TIBA for increased soybean seed yields. The present results appear to indicate that the partition of assimilates among vegetative organs is possibly manageable too, and some practical application may result for nonseed crops like root and vegetable crops. The lower levels of 2,4-D were able to significantly inhibit the growth in the upper tops and still the total dry matter yield was similar to the untreated control. This indicates that the effects of the compounds, whether direct or indirect, were on the growth processes but apparently not on the assimilative capacity of the mature leaves.

Experiment GV4, where the plants were left to grow for two months after treatment, well illustrates how the combined effects depended on the doses, on the attributes measured and on the lapse of time the products acted on the plant. The low level of 50 ppm of 2,4-D did not induce any visible symptom when applied alone but did show marked effects when TIBA was added to it. Therefore, a sort of synergistic action occurred. The higher levels of 2,4-D stopped apical

growth by killing the growing point with or without addition of TIBA. The regrowth from lower lateral buds was, however, consistently more vigorous and "normal" looking in the plants that received TIBA than in those which received 2,4-D alone, as shown in Figures 5 and 7. If we accept the hypothesis that TIBA inhibits the basipetal movement of 2,4-D, as it does with IAA (Winter, 1967) and perhaps with other growth substances (Libbert, 1959), a possible explanation for the effects observed in Experiment GV4 would be that the lower doses of 2,4-D alone were readily translocated and so "diluted" in the whole mass of plant tissue, without reaching the toxic threshold at any organ of the plant. When TIBA was added, 2,4-D was impeded from moving basipetally and remained relatively more concentrated in the apical part (shorter distance, stronger sink, acropetal movement) where toxic action was then conspicuous. (Pot 12 in Figure 5, center). The larger doses of 2,4-D were toxic enough to kill the apex with or without addition of TIBA, but the action of TIBA could inhibit any significant basipetal movement of 2,4-D. Therefore, the lateral buds remained unharmed and able for the normal regrowth shown in Figure 7. When applied alone, the larger levels of 2,4-D were able to move downward to some extent and inhibited the vigor of the regrowth. Had this experiment been harvested two weeks instead of two months after treatment, most of the effects described above would not have been noticed.

In the experiments carried out in the field it appeared that the whole approach was not adequate to the objectives and to the preliminary or initial characteristics of the study. The natural weed population

resulted in a very complex and variable system (to which I added the further complication of a corn crop, mechanical cultivation and pre-emergence herbicide)! Even the lowest doses of 2,4-D used resulted in highly effective control of broad-leaved weeds and consequently did not give an opportunity for the appreciation of any additional effect of TIBA. After killing the almost pure stand of smartweed, the uncultivated plots at Ames were rapidly repopulated by a similarly vigorous stand of grasses. The critical effect of the experimental site on the type of results obtained is illustrated by the fact that at Ames where the smartweed was dominant, the lowest yields of corn were obtained in the untreated plots, whereas in Madrid, the grassy weeds were predominant and worked together with the toxic effect of 2,4-D to cause the lowest yields in the plots that received the heaviest applications of 2,4-D. In Experiment F2, practically half of the plots were eliminated for the purpose of observing herbicidal effects for none or very few weeds remained alive after the first cultivation. In the others, the results were similar to those in Experiment F1. All technological developments are to be finally tested under field conditions but the development itself should be done under the experimental conditions adequate to the specific problem.

The effects of 2,4-D and TIBA on different corn inbreds and hybrids showed that marked damage can be inflicted to corn growth and yield when doses of 2,4-D high enough are applied. It also was apparent that different degrees of tolerance to 2,4-D exist among varieties. The developmental stage of the different entries was to a certain extent

confounded with the date of spraying which makes the apparent interaction variety by date of spraying of less value. Similar results had been shown with more details in other varieties by Rossman and Stanforth in 1949. No additional effects of TIBA were detectable at any level of 2,4-D.

In the experiments with radio-labeled 2,4-D in soybeans the content of radioactivity in different plant parts extracted with ethyl alcohol appeared relatively uniform 78 hours after the application. No effect of TIBA was noted as modifying the distribution of the radioactivity. The radioactivity translocated out the treated leaf in plants of velvet leaf under similar conditions was very low, insufficient to indicate any effect of TIBA if it existed. Two experiments with oats gave results which were very erratic and highly variable; therefore, they are not further reported. In general it was found that the separation of plant parts only into lower tops and upper tops for determinations of radioactivity was too gross. In each of these fractions, several organs and tissues were present. Even if 2,4-D or its radioactive derivatives had a special affinity for some target tissue, its concentration will be diluted and masked when it was extracted together with the rest of the plant sample. In summary, I believe that large effects of TIBA did not exist in this group of experiments. More detailed separation of plant parts and a more precise assay of the radioactivity possibly could detect effects of TIBA. Chromatographing the radioactive extracts would be instrumental in determining if TIBA affects more or less directly the metabolism of 2,4-D instead of (or in addition to) its

effects on translocation. Repeated washings of the residue with a vacuum filter instead of successive centrifugations and pouring apart the supernatant would improve the separation of extractable and non-extractable radioactivity.

In the studies on excretion of radioactivity, it was oats that surprisingly yielded more interesting results. The experiments with soybean showed large variability among replications and from experiment to experiment. The effect of TIBA was erratic and, if a trend could be indicated, it was in the direction of increasing instead of decreasing the excretion of 2,4-D. The experiments with velvet leaf showed very small amounts of radioactivity excreted into the nutrient solution. The experiments with oats unexpectedly yielded two new results. First, oats excreted significant amounts of radioactivity into the nutrient solution and second, TIBA strongly inhibited the amounts of radioactivity excreted. The problem remains whether the action of TIBA was by affecting the amount of 2,4-D absorbed or by somehow impairing the internal fate of 2,4-D. These results with oats were different from the ones reported by Rakitin et al. (1966). They proposed, based on studies with oats and sunflowers, that the ability to excrete 2,4-D was one major and general characteristic of the susceptible dicot species, as opposed to the ability to bind 2,4-D in inactive forms in the tolerant monocots. In my experiments velvet leaf ("susceptible") excreted consistently lower amounts of radioactivity (possibly as the unaltered 2,4-D molecule or some close derivative). The fact that the oat plants receiving the lower doses showed a relatively large increase in the

amounts excreted during the second week after treatment would indicate that the 2,4-D-binding mechanisms had only a temporary action (Jaworski and Butts, 1952).

The autoradiographs of plants treated with C^{14} -2,4-D alone or in combination with TIBA revealed repeated cases where the basipetal movement of the radioactivity, as C^{14} -2,4-D or its derivatives, was largely suppressed by the addition of TIBA. There also were a similar number of cases which did not show any detectable differences associated with the addition of TIBA. In velvet leaf seedlings, the radioactivity appeared to be arrested "in block." In soybeans it appeared that part of the radioactivity was not controlled by TIBA in its translocation and reached a weak but generalized distribution along the stem and leaflets. This result agrees in parts with the findings of Newman (1970). No differences associated with TIBA were found in oats where the radioactivity detected appeared largely confined within the treated leaf.

SUMMARY

TIBA did interact with the action of 2,4-D on soybeans, velvet leaf, and oats. The signs of these interactions were variable and depended on the doses of 2,4-D, on the simple effects of the doses of TIBA and 2,4-D when applied alone, and on the plant attribute considered.

Synergistic action occurred in cases where both products had produced moderate degrees of inhibition when applied alone. This type of synergism was apparent in the inhibition of fresh and dry matter accumulation in the upper tops of soybeans and velvet leaf, in the induction of branching in velvet leaf, and in the damage of stems in soybeans. In situations where one or both products had been stimulatory of growth or yield, their combined effect was always less than additive. At high doses of 2,4-D, which produced marked toxic effects and growth inhibition in soybeans and velvet leaf, the action of additional TIBA resulted in antagonism, but only to a limited extent. The reason the effects on growth inhibition were less than additive was in part a consequence of the method of measurement.

The studies on the effects of TIBA on extractable and residual 2,4-D radioactivity in different plant parts were imprecise, so inconclusive on the effects of TIBA on the translocation of C^{14} -2,4-D or its radioactive derivatives into different parts of the plant.

No conclusive results were obtained from the studies on the excretion of 2,4-D or derivatives by roots. TIBA did not significantly affect the amounts excreted by soybean roots. The radioactivity excreted by velvet leaf was very low in all cases. Oat plants excreted

significant amounts of radioactivity during the two weeks following their treatment with C^{14} -2,4-D. The addition of TIBA markedly inhibited the amounts of radioactivity excreted by these plants.

The basipetal translocation of C^{14} -2,4-D or its radioactive derivatives was inhibited along the hypocotyls of velvet leaf seedlings, as shown by autoradiography. The movement in soybeans was similarly affected. It appeared that a smaller proportion of radioactive material moved out the treated leaf when TIBA was added but it reached a general distribution in the plant thereafter.

The possible application of synergistic combinations of TIBA and 2,4-D to kill weeds was negated by the results obtained in the field and in the greenhouse. Plants of soybean with the first trifoliated leaf expanded or velvet leaf with the third true leaf expanded were stopped in their growth but rarely killed. The most toxic effects were produced by high doses of 2,4-D and the addition of TIBA did not increase 2,4-D lethal effects.

The method used for evaluating the combined effect of TIBA and 2,4-D on weeds in the uncultivated corn studies was imprecise and unnecessarily complex for the objective under study.

Resistance and susceptibility to 2,4-D are relative concepts. Corn and oats also showed noticeable effects of 2,4-D when they received doses high enough under proper conditions. The effects on corn yields varied among hybrids and inbreds, indicating variable degrees of tolerance. The relative effects on different varieties varied with the dates the chemicals were sprayed. No effect of TIBA on the action of 2,4-D in corn was detected.

2,4-D at 2 ppm or TIBA at 200 ppm did not significantly affect the germination percent in oats and velvet leaf. Both products had markedly inhibited the elongation of velvet leaf hypocotyls and oat leaves at the end of the first week. The proliferation of short roots was increased in oats. No interaction between TIBA and 2,4-D was apparent. The seedlings which were affected by the treatments probably would not have successfully emerged and survived under field conditions.

The autoradiographs of soybean and velvet leaf and other growth responses indicated an inhibition of the translocation of 2,4-D or its derivatives caused by the addition of TIBA. The inhibition of the excretion of radioactivity by roots of C¹⁴-2,4-D treated oats was another indication in the same direction. They do not completely rule out the possibility of an inhibitory effect on the absorption of 2,4-D. The effect mentioned could not be confirmed in the experiments with counting of radioactivity in the various plant parts.

A significant increase in the accumulation of dry matter assimilates in the lower tops of plants of velvet leaf was found associated with the application of doses of 2,4-D and/or TIBA causing moderate but marked inhibition of apical growth. This phenomenon may have some possibility of application in nonseed crops.

LITERATURE CITED

- Aberg, B. 1953. On the interaction of 2,3,5-triiodobenzoic acid and maleic hydrazide with auxins. *Physiologia Plantarum* 6: 277-291.
- Afolabi, N. O. 1969. The interacting affect of 2,3,5-triiodobenzoic acid and 2,4-dichlorophenoxyacetic acid on several physiological processes of soybeans. Unpublished M.S. thesis. Library, Iowa State University, Ames, Iowa.
- Agbakoba, C. H. S. O. and Goodin, J. R. 1970. Pichloram enhances 2,4-D movement in field bindweed. *Weed Science* 18: 19-21.
- Audus, L. J. 1954. Auxin antagonists and synergists. A critical approach. *New Phytologist* 53: 461-469.
- Audus, L. J., ed. 1964. The physiology and biochemistry of herbicides. Academic Press, New York.
- Audus, L. J. and Bakhsh, J. K. 1961. On the adaptation of pea roots to auxin and auxin homologues. In *Plant Growth Regulation*. Pp. 102-126. Iowa State University Press, Ames, Iowa.
- Barrier, G. E. 1956. Absorption and translocation of 2,4-dichlorophenoxyacetic acid and radioactive phosphorous. Unpublished Ph.D. thesis. Library, Iowa State University, Ames, Iowa.
- Beste, G. E. and Schreiber, M. M. 1970. Antagonistic interaction of EPTC and 2,4-D. *Weed Science* 18: 484-487.
- Blacklow, W. M. 1966. The fate of 2,4-D applied to Viking birdsfoot trefoil and a selection being bred for 2,4-D resistance. Unpublished M.S. thesis. Library, Cornell University, Ithaca, New York.
- Blackman, G. E. 1961. A new physiological approach to the selective action of 2,4-dichlorophenoxyacetic acid. In *Plant Growth Regulation*. Pp. 233-246. Iowa State University Press, Ames, Iowa.
- Blackman, G. E. and Sargent, J. A. 1959. The uptake of growth substances II. The absorption and accumulation of 2,3,5-triiodobenzoic acid by the root and frond of Lemna minor. *Journal of Experimental Botany* 10: 480-503.
- Bryan, A. M., Staniforth, D. W. and Loomis, W. H. 1950. Absorption of 2,4-D by leaves. *North Central Weed Control Conference Proceedings* 7: 92-95.
- Chase, G. D. 1959. Principles of radioisotope methodology. Burgess Publishing Company, Minneapolis, Minnesota.

- Chkanikov, D. I. and Pavlovla, N. N. 1966. A study of proteins responsible for 2,4-D detoxification in resistant plants. *Agrokhimiya* 7: 115-119. Abstract in *Weed Abstracts* 17: 1958.
- Chrispeles, M. J. and Hanson, J. B. 1962. The increase in ribonucleic acid content of cytoplasmic particulates of soybean hypocotyls induced by 2,4-dichlorophenoxyacetic acid. *Weeds* 10: 123-125.
- Christie, A. E. and Leopold, A. C. 1965. Entry and exit of indole-acetic acid in corn coleoptiles. *Plant and Cell Physiology* 6: 453-473.
- Colby, S. R. 1965. Greenhouse evaluation of herbicide combinations. *Weed Abstracts* 16: 746.
- Colby, S. R. 1967. Calculating synergistic and antagonistic responses of herbicide combinations. *Weeds* 15: 20-22.
- Colby, S. R., Wojtaszek, T. and Warren, G. F. 1965. Synergistic and antagonistic combinations for broadening herbicidal selectivity. *Weeds* 13: 87-91.
- Colby, S. R. and Feeny, R. W. 1967. Herbicidal interactions of potassium azide with calcium cyanide. *Weeds* 15: 163-167.
- Comar, C. L. 1955. *Radioisotopes in agriculture and biology*. McGraw-Hill Book Co., New York.
- Costa, J. J., Ghelfi, R. A. and Brenzoni, E. O. 1966. Tecnicas utilizadas para la aplicacion de un herbicida marcado con ^{14}C . In *Isotopes in Weed Research*. Pp. 9-16. International Atomic Energy Agency, Vienna. 1966.
- Crafts, A. S. 1961. *The chemistry and mode of action of herbicides*. Interscience Publishers, New York.
- Crafts, A. S. and Yamaguchi, S. 1968. *The autoradiography of plant materials*. University of California (Berkeley) Agricultural Publication Manual 35.
- Crosby, D. G. 1964. Metabolites of 2,4-D in bean plants. *Journal of Agricultural and Food Chemistry* 12: 3-6.
- Danielli, J. R. 1954. Morphological and molecular aspects of active transport. *Society of Experimental Biology Symposium* 8: 502-515.
- Davis, F. S. and Bovey, W. R. 1967. Interactions of paraquat, picloram and 2,4,5-T in plant tissues. *Plant Physiology* 42 (supplement): 49.
- De la Fuente, R. K. and Leopold, A. C. 1970. The transportable auxin pool. *Plant Physiology* 45: 19-25.

- Ennis, W. B. and Boyd, F. T. 1946. The response of kidney bean and soybean plants to aqueous spray applications of 2,4-dichlorophenoxyacetic acid with and without carbowax. *Botanical Gazette* 107: 552-559.
- Fang, S. C. and Butts, J. S. 1954. Studies in plant metabolism III. Absorption, translocation and metabolism of radioactive 2,4-D in corn and wheat plants. *Plant Physiology* 29: 56-60.
- Frans, R. E. 1955. Kinetics of herbicidal action. Unpublished Ph.D. thesis. Library, Iowa State University, Ames, Iowa.
- Freed, W. H. and Montgomery, M. L. 1969. Metabolism of herbicides. *Annals of the New York Academy of Sciences* 160: 133-139.
- Freiberg, S. R. and Clark, H. E. 1952. Effects of 2,4-D upon the nitrogen metabolism and water relations of soybean plants grown at different nitrogen levels. *Botanical Gazette* 113: 322-333.
- Fulton, J. L. and Payne, M. G. 1956. Effects of 2,4-dichlorophenoxyacetic acid and maleic hydrazide on free amino acids, and proteins in potato, sugar beets and bean crops. *Botanical Gazette* 118: 130-133.
- Ghorashi, S. R., Colville, W. L. and Ashworth, D. L. 1969. Effects of 2,3,5-triiodobenzoic acid on the morphology and anatomy of Glycine max (L.) Merrill. *Crop Science* 9: 399-402.
- Goldsmith, Mary Helen M. 1968. The transport of auxin. *Annual Review of Plant Physiology* 19: 347-360.
- Gorter, C. J. 1949. Action of 2,3,5-triiodobenzoic acid on growth of root hairs. *Nature* 164: 800-801.
- Gowing, D. P. 1959. A method of comparing herbicides and assessing herbicide mixtures at the screening level. *Weeds* 7: 66-76.
- Gowing, D. P. 1960. Comments on tests of herbicide mixtures. *Weeds* 8: 379-391.
- Greer, Frances. 1965. 2,3,5-triiodobenzoic acid (TIBA). Unpublished mimeographed review. Library, Research Division International Minerals and Chemical Corporation, Skokie, Illinois.
- Greer, H. A. L. and Anderson, I. C. 1965. Response of soybean to triiodobenzoic acid under field conditions. *Crop Science* 5: 229-232.
- Gregory, F. G. and Veale, J. A. 1957. A reassessment of the problem of apical dominance. *Symposium of the Society of Experimental Biology* 11: 1-20.

- Hallman, N. D. 1970. The effect of 2,4-dichlorophenoxyacetic acid and related compounds on the fine structure of the primary leaves of Phaseolus vulgaris. Journal of Experimental Botany 21: 1031-1038.
- Hardcastle, W. S. and Wilkinson, R. A. 1970. Bioassay of herbicide combinations with rice. Weed Science 18: 336-337.
- Hay, J. R. 1956. The effects of 2,4-dichlorophenoxyacetic acid and 2-3-5 triiodobenzoic acid on the transport of indoleacetic acid. Plant Physiology 31: 118-120.
- Hay, J. R. and Thimann, K. V. 1956. The fate of 2,4-D in bean seedlings I. Recovery of 2,4-dichlorophenoxyacetic acid and its breakdown in the plant. Plant Physiology 31: 382-387.
- Hertel, R. and Leopold, A. C. 1963. Versuche sur analyse des auxin-transports in der koleoptile von Zea mays. Planta 59: 535-562.
- Holm, R. E. and Abeles, F. B. 1967. The role of ethylene in 2,4-D-induced growth inhibition. Planta 78: 293-304.
- Hull, H. M. 1967. Uptake and movement of herbicides in plants. Proceedings on herbicides and Vegetation Management in Forest Ranges and Noncrop Land, Oregon State University, Corvallis, Oregon. September, 1967.
- James, C. S., Prendeville, G. N., Warren, G. F. and Schreiber, M. M. 1970. Interactions between herbicidal carbamates and growth regulators. Weed Science 18: 137-139.
- Jaworski, E. G. and Butts, J. S. 1952. Studies in plant metabolism II. The metabolism of C¹⁴-labeled 2,4-dichlorophenoxyacetic acid in bean plants. Biochemistry and Biophysics Archives 38: 207-218.
- Johnson, C. M. and Ulrich, A. 1954. Analytical methods for use in plant analysis. California Agricultural Experiment Station Bulletin 766.
- Johnson, R. R. 1970. Interaction of TIBA and 2,4-D on growth and yield of soybeans. Unpublished M.S. thesis. Library, Iowa State University, Ames, Iowa.
- Keitt, G. W., Jr. 1968. Is auxin actively transported acropetally? In Vardar Y., ed. The Transport of Plant Hormones. Pp. 130-134. North Holland, Amsterdam.
- Keutzer, T. and Libbert, E. 1961. Blockade of gibberellic acid transport in hypocotyl segments by triiodobenzoic acid, including a new agar block test for gibberellin. Planta 56: 23-27.

- Key, J. L. 1963. Studies on 2,4-D-induced changes in ribonucleic acid metabolism in excised corn mesocotyl tissue. *Weeds* 11: 177-181.
- Key, J. L. 1969. Hormones and nucleic acid metabolism. *Annual Review of Plant Physiology* 20: 449-474.
- Key, J. L., Lin, C. Y., Gifford, E. M. and Dugler, R. 1966. Relation of 2,4-D-induced growth aberrations to changes in nucleic acid metabolism in soybean seedlings. *Botanical Gazette* 127: 87-94.
- Kiermayer, O. 1964. Growth responses to herbicides. In Audus, L. J., ed. *The physiology and biochemistry of herbicides*. Pp. 207-233. Academic Press, New York.
- Korolev, L. I. and Voitekhova, V. A. 1967. Increasing the herbicidal effect of 2,4-D. *Agrokhimiya* 8: 108-111. Abstracted in *Weed Abstracts* 17: 830.
- Krause, B. F. and Boke, N. H. 1968. Effects of 2,3,5-triiodobenzoic acid on the structure of soybean leaves. *American Journal of Botany* 55: 1074-1079.
- Leopold, A. C. and de la Fuente, R. K. 1968. A view of polar auxin transport. In Vardar, Y., ed. *The Transport of Plant Hormones*. Pp. 24-47. North Holland, Amsterdam.
- Levi, E. 1966. Handling plants for macro-autoradiography. In *Isotopes in Weed Research Symposium*. Pp. 189-194. International Atomic Energy Commission, Vienna.
- Libbert, Eike. 1959. Trijodobenzo saure und die stoffleitung Bei Hoheren Pflanzen. *Planta* 53: 612-627.
- Linscott, D. L., Hagin, R. D. and Dawson, J. E. 1968. Conversion of 4-(2,4-dichlorophenoxy) butyric acid to homologues by alfalfa. *Journal of Agricultural and Food Chemistry* 16: 844-848.
- Linser, H. 1964. The design of herbicides. In Audus, L. J. *The Physiology and Biochemistry of Herbicides*. Pp. 483-505. Academic Press, New York.
- Livingston, C., Payne, G. M. and Fults, J. L. 1954. Effects of maleic hydrazide and 2,4-dichlorophenoxyacetic acid on the free amino acids in sugar beets. *Botanical Gazette* 116: 148-156.
- Lucas, E. H. and Hammer, C. L. 1947. Modification of the physiological action of 2,4-dichlorophenoxyacetic acid by simultaneous applications of plant extracts and pH changes. *The Michigan State College Quarterly Bulletin* 29: 256-262.

- Luckwill, L. C. and Lloyd-Jones, C. P. 1960. Metabolism of plant growth regulators I. 2,4-dichlorophenoxyacetic acid in leaves of red and black currant. *Annals of Applied Biology* 48: 613-625.
- McCready, C. C. 1963. Movement of growth regulators in plants I. Polar transport of 2,4-dichlorophenoxyacetic acid in segments from petioles of Phaseolus vulgaris. *New Phytologist* 62: 3-18.
- McRae, D. H., Foster, R. J. and Bonner, J. 1953. Kinetics of auxin interaction. *Plant Physiology* 28: 343-355.
- Miller, M. D., Mikkelsen, D. S. and Huffaker, R. C. 1962. Effects of stimulatory and inhibitory levels of 2,4-D, iron and chelate supplements on yield and growth of soybean. *Crop Science* 2: 114-116.
- Mitchell, J. W. and Livingston, G. A. 1968. Methods of studying plant hormones and growth-regulating substances. U.S.D.A. Agricultural Research Service Agricultural Handbook No. 336.
- Moody, K. 1969. Uptake and release of herbicides by soybean roots in culture solution. Unpublished Ph.D. thesis. Library, The University of Wisconsin, Madison, Wisconsin. [University Microfilm Order 70-3635, Ann Arbor, Michigan]
- Moreland, D. E. 1967. Mechanisms of action of herbicides. *Annual Review of Plant Physiology* 18: 365-386.
- Morgan, P. W. and Hall, W. C. 1962. Effect of 2,4-dichlorophenoxyacetic acid on the production of ethylene by cotton and grain sorghum. *Physiologia Plantarum* 15: 420-427.
- Morgan, P. W. and Hall, W. C. 1963. Metabolism of 2,4-D by cotton and grain sorghum. *Weeds* 11: 130-135.
- National Academy of Sciences--National Research Council. 1968. Principles of plant and animal pest control. Vol. 2. Weed Control. National Academy of Sciences--National Research Council Publication 1597.
- Newman, I. A. 1970. Auxin transport in Avena. *Plant Physiology* 46: 263-272.
- Niedergang-Kamien, E. and Leopold, A. C. 1957. Inhibitors of polar auxin transport. *Physiologia Plantarum* 10: 29-38.
- Niedergang-Kamien, E. and Skoog, F. 1956. Studies on polarity of auxin transport in plants I. Modification of polarity and auxin transport by triiodobenzoic acid. *Physiologia Plantarum* 9: 60-73.

Nitsch, J. P. and Mme Colette Nitsch. 1961. Synergistes naturels des auxines et des gibberellines. Bulletin de la Societe Botanique de France 108: 349-362.

Nolla, J. A. B. 1948. Control of grass weeds in sugar cane fields in Puerto Rico. Science 108: 112-113.

O'Brien, T. P. 1968. The approach of a plant physiologist to the selective toxicity of 2,4-D. Proceedings First Victorian Weed Conference 1968. Victoria, Australia. Reprint from Victoria, Australia.

Ohki, K. 1968. Effects of root-absorbed 2,3,5-triiodobenzoic acid on nutrient absorption and growth of soybean. Plant Physiology 43 (supplement): 48.

Parr, J. F. and Norman, A. G. 1965. Considerations in the use of surfactants in plant systems: a review. Botanical Gazette 126: 86-96.

Parry, D. W. 1956. The effects of 2,4-dichlorophenoxyacetic acid, 2,3,5-triiodobenzoic acid and thiourea on the vegetative and reproductive growth of Avena sativa. Journal of Experimental Botany 7: 381-386.

Phillips, I. D. J. 1969. Apical dominance. In Wilkins, M. B., ed. The Physiology of Plant Growth and Development. Pp. 163-202. McGraw-Hill Book Co., New York.

Rakitin, Y. V., Zemskaya, W. A., Voronina, E. I. and Chernikova, L. M. 1966. Peculiarities of the detoxification of 2,4-D in plants sensitive and resistant to this herbicide. Soviet Plant Physiology 13: 30-38.

Rossman, A. C. and Staniforth, D. W. 1949. Effect of 2,4-D on inbred lines and single crosses of maize. Plant Physiology 24: 60-74.

Sant' Anna, R., Ohlrogge, A. J., Christian, J. E. and Breckinridge, G. E., Jr. 1970. Foliar absorption and distribution of 2,3,5-triiodobenzoic acid (TIBA) in soybeans (Glycine max). Crop Science 10: 731-735.

Sargent, J. A. 1968. The role of growth regulators in determining the penetration of 2,4-D into primary leaves of Phaseolus vulgaris. In Vardar, Y., ed. 1968. The Transport of Plant Hormones. Pp. 365-379. North Holland, Amsterdam.

Sargent, J. A. and Blackman, G. E. 1962. Studies on foliar penetration I. Factors controlling the entry of 2,4-dichlorophenoxyacetic acid. Journal of Experimental Botany 13: 348-368.

- Slife, F. W., Key, J. L., Yamaguchi, S. and Crafts, A. S. 1958. Penetration, translocation, and metabolism of 2,4-D and 2,4,5-T in wild and cultivated cucumber plants. *Weed Science* 10: 29-31.
- Snow, R. 1925. The correlative inhibition of the growth of auxillary buds. *Annals of Botany London* 39: 841-859.
- Snyder, W. O. 1949. Some responses to 2,3,5-triiodobenzoic acid. *Plant Physiology* 24: 195-206.
- Staniforth, D. W., ed. 1970. North Central Weed Control Conference. 27th Annual Research Report. Department of Botany, Iowa State University, Ames, Iowa.
- Staniforth, D. W. and Loomis, W. E. 1949. Surface action in 2,4-D sprays. *Science* 109: 628-629.
- Still, G. G., Davis, D. G., and Zander, G. L. 1970. Plant epicuticular lipids: alteration by herbicidal carbamates. *Plant Physiology* 46: 307-314.
- Stoller, E. W. 1969. The kinetics of Amiben absorption and metabolism as related to species sensitivity. *Plant Physiology* 44: 854-860.
- Summerford, C. R. 1954. Synergism and synergists. A review. *Journal of Agricultural and Food Chemistry* 2: 310-327.
- Sweet, R. D. and Lynch, M. R. 1971. A little can go a long way when you combine weed killers. *Crops and Soils* 23: 7-8.
- Taylor, T. D. and Warren, G. F. 1970. The effect of metabolic inhibitors on herbicides movement in plants. *Weed Science* 18: 68-73.
- Thimann, K. V. and Bonner, W. D. 1948. The action of triiodobenzoic acid on growth. *Plant Physiology* 23: 158-161.
- Thompson, A. K. 1970. The effect of sub-toxic concentrations of 2,4-D in combination with mineral nutrients on the potato (Solanum tuberosum). *Journal of Agricultural Science* 74: 505-509.
- Tutass, H. O. 1967. Relationships among molecular structure, metabolism, and biological activity of halogen-substituted phenoxyacetic acids. *Dissertation Abstracts* 29: 877-B.
- Van Overbeek, J. 1959. Theory on the mode of action of herbicides. *Botanical Review* 25: 269.
- Van Overbeek, J. 1966. Plant hormones and regulators. *Science* 152: 721-731.

- Vardar, Y. 1967. Auxin factor in apical dominance. (Reprint). Botany Department, Ege University, Izmir, Turkey.
- Vardar, Y. 1968. Agents modifying the longitudinal transport of auxin. In Vardar, Y., ed. The Transport of Plant Hormones. Pp. 156-188. North Holland, Amsterdam.
- West, S. H., Hanson, J. B. and Key, J. L. 1960. Effect of 2,4-dichlorophenoxyacetic acid on the nucleic acid and protein content of seedling tissue. Weeds 8: 333-348.
- Whiting, Geraldine A. and Munay, Mary A. 1948. Abscission and other responses induced by 2,3,5-triiodobenzoic acid in bean plants. Botanical Gazette 109: 447-473.
- Wickson and Thimann, K. V. 1960. The antagonism of auxin and kinetin in apical dominance. Vol. II. The transport of IAA in pea stem in relation to apical dominance. Physiologia Plantarum 13: 539-554.
- Widholm, J. M. and Shaffer, R. 1969. The effect of TIBA and 2,4-D on growth and metabolic processes in soybean hypocotyl sections. Unpublished mimeographed report, International Mineral and Chemical Corporation Growth Sciences Center, Libertyville, Illinois.
- Winter, A. 1967. The promotion of the immovilization of auxin in Avena coleptiles by triiodobenzoic acid. Physiologia Plantarum 20: 330-336.
- Wort, D. J. 1962. The application of sublethal concentrations of 2,4-D alone and in combination with mineral nutrients. World Review of Pest Control 1: 6-19.
- Wort, D. J. 1966. Effects of 2,4-D nutrient dusts on growth and yield of beans and sugar beets. Agronomy Journal 58: 27-29.

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