SOME ASPECTS OF GROWTH REGULATOR APPLICATION TO PLANTS OF _ZINNIA ELEGANS_ AND _VIGNA SINENSIS_ INOCULATED WITH TOBACCO RINGSPOT VIRUS

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Donald J. de Zeeuw
Charles L. Hamner
Major professor

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By

JAMES GLYNN KARAS

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ABSTRACT

SOME ASPECTS OF GROWTH REGULATOR APPLICATION TO PLANTS OF *ZINNIA ELEGANS* AND *VIGNA SINENSIS* INOCULATED WITH TOBACCO RINGSPOT VIRUS

by James Glynn Karas

Qualitative differences and similarities in plants of the Blaze cultivar of *Zinnia elegans* variously treated with 2,4-dichlorophenoxyacetic acid and inoculated with tobacco ringspot virus were evaluated for several characteristics. Growth suppressant effects of the virus and the growth regulator appeared additive. Darkening of root color was induced by the growth regulator treatment more than by inoculation. Root quantity for either virus or growth regulator treatment did not differ significantly from the control.

Plants variously treated with growth regulator, inoculated with virus or both were evaluated later for elongation of lateral shoots. Either the virus or the growth regulator reduced elongation; but the combination treatment induced normal elongation at the cotyledonary node.

Nitrate content, which is assumed to be important in virus multiplication, appeared to increase in growth

regulator treated plants. In tobacco ringspot virus inoculated plants of *Zinnia elegans* nitrate was significantly correlated with the amount of virus.

Application of the compound, 2,4-dichlorophenoxyacetic acid, at the two to four leaf pair stage caused a reduction of recoverable virus from plants of the Blaze cultivar of *Zinnia elegans* at six, fourteen, and at twenty-one days from inoculation. Plants in the first flower bud stage inoculated only on the third pair of leaves and treated with growth regulator were assayed at three and at ten days from inoculation for the presence of tobacco ringspot virus in nodes one through six. At three days no virus was found in the noninoculated leaves. At ten days virus could be recovered from leaf pairs three, five, and six, suggesting that a relationship existed between virus movement and the phyllotactic arrangement of the leaves.

Plants of the Blaze cultivar of *Zinnia elegans* variously treated with 2,4-dichlorophenoxyacetic acid and inoculated with virus were assayed for virus presence at six and twenty-one days. More virus was recovered from inoculated plants at six than at twenty-one days. Less virus was present in combination inoculated and growth regulator treated plants

at six days than at twenty-one days and more virus was present in the combination treatment at twenty-one days than in the inoculated plants at the same stage. There was a significant drop in virus between six and twenty-one days in plants not receiving the growth regulator.

The growth regulator analogs 2-chloro, 4-chloro, and 2,4-dichlorophenoxyacetic acids were applied at forty-four, twenty-four, or one and one-half hours before inoculating plants of the "S" cultivar of _Vigna sinensis_ with tobacco ringspot virus. The longer exposure periods reduced infection quotients significantly. When the 3,4-dichloro analog was applied after inoculation, on the other hand, the reduction effect gradually diminished with increasing time.

The 2-chloro and 3,4-dichloro analogs proved nearly as effective in suppressing lesion formation in inoculated plants of the "S" cultivar of _Vigna sinensis_ as the 2,4-dichloro analog, but had fewer phytotoxic effects.

Evaluation of the effect of concentration of 2,4-dichlorophenoxybutyric, 2-chlorophenoxyacetic, phenoxyacetic, and 3,4-dichlorophenoxyacetic acids on tobacco ringspot virus lesion production in _Vigna sinensis_ indicated that all compounds had inhibitory ranges, while the 2-chloro analog had a stimulatory range as well. Certain major variations encountered in the control and treatment curves were consistent and appear to relate to total incident solar radiation. Reduction in virus lesions paralleled major drops in radiation intensity.

An investigation was made on the effect of a mixture of kinetin, gibberellic acid, and indoleacetic acid on lesion production. When the solution was applied in physiologic concentration with or after inoculation, infection was reduced. If applied before inoculation, infection was increased.

Evaluations of other compounds were made. One such compound was given further trial. A choline derivative (2-bromoethyl)trimethylammonium bromide, was applied at three concentrations. When applied before inoculation, increasing concentrations of the compound appeared to increase lesion number. When applied after inoculation, however, increasing concentrations decreased lesion number.

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TABLE OF CONTENTS

LIST OF TABLES

Table		Page

LIST OF FIGURES

Figure | Page

INTRODUCTION

Many viruses are important hindrances in the production of man's food supply and are for this and other reasons of considerable interest to the biologist. One such important virus is tobacco ringspot virus. It has been transmitted to over 178 species and in approximately forty-eight different families of dicotyledenous plants. Having such an extensive host range the virus is additionally useful in studies on host metabolism, morphology, physical characteristics, and interactions.

Tobacco ringspot virus is a very high molecular weight nucleoprotein, about twenty-six millimicrons in diameter, and polyhedral in shape. It contains about three per cent phosphorus and thirty-four and one-half per cent nucleic acid. The nucleic acid contains about nine per cent phosphorus, an unusually large amount (88).

Various research workers have demonstrated the biological activity of naturally occurring and synthetic growth regulators possessing the ability to exert gross as well as subtle alterations in the metabolism, morphology, and physical characteristics of the plant (4, 98). One of the most extensively investigated synthetic growth regulators is 2,4-dichlorophenoxyacetic acid. Extensive investigations have demonstrated that 2,4-dichlorophenoxyacetic acid causes yellowing of the terminal portions of the plant, terminal leaf clasping, leaf epinasty, and extreme overgrowth of internodal areas as well as other prominent host morphological alterations (4, 38, 87).

Research by Karas et al. (40), deZeeuw et al. (16) and Karas (39) have demonstrated qualitatively somewhat similar gross effects in Zinnia elegans inoculated with tobacco ringspot virus or treated with 2,4-dichlorophenoxyacetic acid. These similarities appear to offer an excellent opportunity to determine the more basic relationships present in the single treatments and in the combination treatment with the growth regulator and virus. Among these basic relationships of importance are virus multiplication, virus movement, local lesion development, and changes in biological trends as affected by concentration.

REVIEW OF LITERATURE

Since the initial discovery that certain compounds influence the growth, form, and physiology of treated plants, several thousand research reports have been published on various aspects of the problem. Numerous fine texts, summaries, and reviews are available. Among these are Audus (4), Leopold (48), Mitchell (67), Strong (89), Tukey (94), Went and Thimann (98), and the Annual Reviews of Plant Physiology. Research pertinent to the present topic is reported.

Growth Regulator Effects

Movement of the growth regulator. The movement of auxin has been shown to be influenced by other growth regulating chemical structures. Leopold and Lam (49) and Niedergang-Kamien and Leopold (70) showed inhibitions of zero to fifty per cent of the uptake and movement of indoleacetic acid in sunflower hypocotyl sections by ring chlorinated phenoxyacetic acid analogs. Indoleacetic acid also competitively inhibited the uptake and/or translocation of these materials depending on the ratio of the two compounds.

Conditions favoring rapid movement of food from the leaves during and after a period of rapid synthesis in the light also favor 2,4-dichlorophenoxyacetic acid transport and, when such rapid food movement from darkened leaves stops, so also does the 2,4-dichlorophenoxyacetic acid movement. The sieve tubes apparently play a vital role. Xylary transport occurs only if the growth regulator is applied to the roots or when applied in high, local applications to the bark and as a result of leakage into the xylem (4).

Morphological and physiological responses to growth regulators. The morphological alterations produced by auxins or auxin-like growth regulators are varied. In foliar treated plants of *Zinnia elegans* and other species depending on the compound, its concentration, formulation, season, adjuvants, etc., there may be growth stimulation, growth suppression, or death of the plant (16,37,39,40). More specifically, there may be suppression or elongation of the main axis and laterals, decrease or increase in cumulative lateral growth, infloresence malformation, green weight and dry weight variation, and increase or decrease in internode length (37,39,40).

Other responses include epinasty, yellowing of the green areas, leaf reflexing, leaf strapping, rosetting, pigmentation, bud suppression, and toothed and fused leaflets. Johnson (38) also indicated that Zinnia elegans responded to 2,4-dichlorophenoxyacetic acid sprayer residues by leaf dropping, leaf rolling, very narrow leaves, and yellow apical leaves which soon die.

There was stimulation of vascular tissue differentiation with all concentrations of substituted benzoic acids applied to Zinnia elegans (3). In addition plants sprayed with low concentrations of these same compounds maintained their parenchyma cells in meristematic condition, while plants treated with high concentrations produced undifferentiated or unexpanded cells. Jacobs (36) showed that xylem differentiation around a wound coincided with auxin movement. Removal of leaves above a wound resulted in slow healing, whereas, removal of leaves below had no effect.

Indoleacetic acid and 2,4-dichlorophenoxyacetic acid are equally effective in decreasing the heat coagulability of protein. Weak auxins such as 2,3,5-trichlorobenzoic acid are slightly active and the antiauxin 4-chlorophenoxyisobutyric acid is not effective as an antidenaturant (26).

The growth regulators 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid at concentrations as low as 10^{-4} $\underline{M}$ inhibited oxidation of glycolic acid and ascorbic acid in cell free enzyme systems (65). They felt that this was compatible with the view that herbicidal activity is dependent on alteration of enzyme systems of the treated plant.

Tonzig and Marré (93) consider ascorbic acid as a growth regulator. The rise of ascorbic acid in a tissue or of its synthesis resulted in reduced growth rate. It has been noted that an increase in ascorbic acid in pea internode segments limits growth rate, apparently through the increase of dehydroascorbic acid which is the actual inhibitor. There is an associated reduction in respiration and an increase in the glutathione oxidation-reduction ratio. It was shown by their work that indoleacetic acid increased the ascorbic acid level and decreased the dehydroascorbic acid. Marré and Arrigoni (61) have demonstrated that pea internodes treated with solutions of ascorbic acid containing 10^{-3} or more $\underline{M}$ per liter had increased oxidized to reduced glutathione ratio. It is apparent that an application to pea internode segments of

concentrations of indoleacetic acid less than 10 parts per million increased the ascorbic acid and decreased the dehydroascorbic acid; whereas, segments to which concentrations greater than ten parts per million have been applied exhibit the opposite trend. Other workers indicate that plants to which 2,4-dichlorophenoxyacetic acid has been applied had less oxidation of ascorbic acid (65).

A reduction in photosynthesis in beans as a result of 2,4-dichlorophenoxyacetic acid application has been frequently reported (2,55). Using labeled carbon, Akers and Fang (2) found that carbon incorporation into aspartic and glutamic acids was increased to three to four times the control as a result of an application of 2,4-dichlorophenoxyacetic acid. The peak occurred the third day after treatment (2).

Luecke et al. (57) demonstrated that red kidney bean leaves treated with 2,4-dichlorophenoxyacetic acid have less thiamin, riboflavin, nicotinic acid, and carotene and more pantothenic acid. In the stems all these materials except carotene increased.

Rebstock et al. (75) demonstrated that plants to which 2,4-dichlorophenoxyacetic acid had been applied as a foliar spray contained less total phosphorus, acid and alcohol soluble phosphorus and nucleic acid phosphorus in the leaf. In stems of treated plants total phosphorus and the acid soluble phosphorus was higher, alcohol soluble phosphorus was the same, and the nucleic acid phosphorus was twice the check. As indicated in Satarova (79), there was an increase in ribonucleic acid in meristematic areas of 2,4-dichlorophenoxyacetic acid treated tissues. Loustalot et al. (56) in investigations with Commelina and Xanthosoma found more water soluble phosphorus in treated plants. One thousand parts per million of 2,4-dichlorophenoxyacetic acid applied as a foliar spray to Blanca Bonita white bean showed an increase in inorganic phosphorus in the leaves, stems, and roots two days after treatment. Seven days following treatment the inorganic phosphorus had increased greatly in roots, was the same status in stems, and had decreased in the leaves of treated plants. Fang and Butts (21) indicated that application of 2,4-dichlorophenoxyacetic acid to leaves of plants greatly reduced the upward movement of radioactive phosphorus to the leaves. The degree is inversely related to 2,4-dichlorophenoxyacetic acid concentration. Indoleacetic acid and other compounds also

modify the distribution and accumulation pattern of radioactive phosphorus.

Pectase and phosphorylase decreased in the leaves and stems of cranberry bean plants as a consequence of 2,4-dichlorophenoxyacetic acid foliar treatment (68).

In bean stem tissue to which 2,4-dichlorophenoxyacetic acid had been applied there was a movement of carbohydrate reserves and nitrogen to the meristematic areas (82).

Two year old leaves of Euonymus to which applications of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid have been applied had more total nitrogen in treated parts, while nontreated parts of 2,4-dichlorophenoxyacetic treated leaves had less nitrogen than the controls. Carbon containing materials as well may move to the treated areas thereby serving to delay aging (72).

Faludi and Daniel (20) have investigated the effect of various concentrations of 2,4-dichlorophenoxyacetic acid in potato tissue cultures. Generally, there was a reduction in amino acid content. Arginine, asparagine, aspartic acid, alanine, proline, methionine, and valine were reduced slightly. Glutamine, glutamic acid, glycine, threonine, and tyrosine showed a marked reduction. Plants to which 2,4-dichlorophenoxyacetic acid had been applied contained no leucine. As the concentration of 2,4-dichlorophenoxyacetic acid increased the total alpha keto acid section increased. The decrease in amino acids was probably not due to transaminase inhibition, but due to an increase in deamination.

Sell et al. (80) demonstrated in red kidney bean stems increase in total protein, aspartic acid, lysine, valine, methionine, and phenylalanine, decrease in reducing and non-reducing sugars, carbohydrate reserves, and acid hydrolyzable polysaccharides as a consequence of 2,4-dichlorophenoxyacetic acid treatment. They concluded that much of the carbohydrate loss was a result of its utilization in protein synthesis.

Davis and Dimond (13) showed that plants to which 2,4-dichlorophenoxyacetic acid and alpha-naphthaleneacetic acid was applied had less reducing sugars. In other instances bean plants variously treated with 2,4-dichlorophenoxyacetic acid had less leaf protein and a concomitant doubling of stem protein (11). Catalase activity and respiration in stems was increased (11,87). Its effect on

mitochondria was one of mere adsorption on the mitochondrial surface (23).

In confirmation of the work of Sell et al. (80), Hoffman and Schmeling (34) indicated an increase in simple sugars at the expense of high molecular weight carbohydrates.

Tomato plants responded to foliar applications of low concentrations of 2,4-dichlorophenoxyacetic acid by an increase in the polyphenoloxidase, peroxidase, and catalase enzymes in the flowers, fruits, leaves, and tip leaves. Other increases were apparent in the diurnal assimilation capacity of the leaves in dry weight, reduction of water loss, and mobilization of nutritional reserves to the ovaries (87). Ravazzoni (73) found that 2,4-dichlorophenoxyacetic acid activates horseradish peroxidase.

Bean stem tissue culture grown on one per cent starch agar medium and treated with 2,4-dichlorophenoxyacetic acid had a higher redox capacity. Extensive areas of the medium showed starch decomposition (25). Kidney bean plants to which 2,4-dichlorophenoxyacetic acid had been applied had less pectase and phosphorylase in the stems

and leaf tissues. No apparent indication of a phosphatase acting on D-glucose-1-phosphate was noted.

Lustinec (58) demonstrated increased phosphate and decreased catalase activity in hardwood grape cuttings, to which beta-indolebutyric acid had been applied. Bean stems treated with 2,4-dichlorophenoxyacetic acid had less alpha and beta-amylase activity. The compound is ineffective on the beta-amylase of leaves (69). Ravazzoni (73) found that apple leaf tissue had less amylase activity, while Ricinus seed amylase remained unchanged, and potato amylase was the same. Plants of Ricinus to which one thousand parts per million of 2,4-dichlorophenoxyacetic acid had been applied to the leaves had less lipase (73).

In the review of Audus (4) it was noted that the growth of stems treated with ten parts per million indoleacetic acid was stimulated.

The application of a solution containing ten parts per million of indoleacetic acid to sunflower hypocotyls greatly increased the uptake of amino acids (76). Plants treated with growth regulator applications seemed to have more protoplasmic streaming either as a consequence of lowered viscosity or of the injection of energy into an energy requiring system, or by an energy regulating property of the auxin (4).

Relationship of auxin-like growth regulators to other systems. The general opinion is that each organ requires different ratios of the various components of the growth regulation system for normal growth correlation (4). In support of this view Kefford and Goldacre (41) incorporated the experimental evidence derived from several investigations on auxins, gibberellins, and kinins. If kinetin only is present in the cell, then we might expect only the first of the various steps in cell division to occur, namely deoxyribonucleic acid synthesis. If auxin only is present then we might expect just cell enlargement. Tissues to which kinetin has been applied undergo cell division if auxin is supplied. Gibberellic acid and indoleacetic acid interact in cell enlargement processes. It is apparent that a spectrum of tissues exists, from those that require both auxin and kinin to those requiring one or the other, to those that require neither for normal growth correlation.

It is believed that auxin may be more basic, but less specific than either kinetin or gibberellin and is a predisposing agent in that it results in the production of something else in limiting amounts which is required in both cell division and cell enlargement. In the presence of kinetin, cell division proceeds, while in the presence of gibberellic acid, only elongation occurs. If auxin is limiting, then there is competition between cell division and cell enlargement, the net result depending on the kinetin-gibberellic acid ratio (41).

Other Biologically Active Compounds

Purine group. Dedolph et al. (15) demonstrated that freshly harvested asparagus spears treated with the purine analog, 6-benzylamino purine, had decreased respiration, reduced spear elongation, and less weight for five and one-half days. Reduced water loss, less chlorophyll loss, and inhibition of senescence were also apparent. Unpublished research indicates that broccoli treated with this compound had reduced respiration (14). This compound differs from kinetin in that the five membered furan ring on the six position of purine has been replaced with a six membered benzene group.

Strong (89) showed that carrot and tobacco callus tissue responded to one part per billion of kinetin. Kinetin (6-furfurylamino purine) treated cells have increased cell division. Many derivatives such as benzyl and beta-phenoxyacetate possessed equally high activity. Application of both indoleacetic acid and kinetin to excised tobacco pith tissue cultures resulted in continuous cell division and cell enlargement; whereas applications of indoleacetic acid alone resulted only in enlargement; and kinetin, cell division only. The ratio of kinetin to indoleacetic acid (2 parts per million) appeared to be critical. At one two-hundredths of one part per million of kinetin rooting resulted; at one-half to one, budding; whereas, intermediate concentrations resulted in no differentiation in new tissue. Concentrations above one part per million of kinetin inhibited the growth of the tissue. Cultures treated with indoleacetic acid alone produced roots; with kinetin alone, produced buds; while the combination reduced root formation. Kinetin applications reproduced the red light effect in lettuce seed germination, bean leaf disc expansion, and _Avena_ section stimulation. Both indoleacetic acid and kinetin are apparently required for deoxyribonucleic acid synthesis (64,89).

Lettre and Endo (50) showed that normal and malignant animal tissue cultures were not affected by kinetin, nor was growth rate or mitotic rate in growing animal tumor cells altered. Kinetin did not antagonize colchicine activity. Applications of the 6-beta-indolethylamino purine analog at one-ten thousandth of one part per million to fibroblasts and tumor cells inhibited division of fibroblasts and tumor cells and produced cells with two nuclei. The compound had an activity one hundred times that of kinetin. The specifity of the indole derivative was apparent, since it was not antagonized by kinetin, while the benzyl or phenylethyl derivatives were inactive.

Choline group. Acetyl choline, an analog of choline, is well known among animal physiologists and biochemists. The biological significance of choline in plants was not generally known until Tolbert (92) discovered that as much as thirty percent of the total soluble phosphorus in a plant root and sometimes in the leaves was present as phosphorylcholine.

An exceedingly wide range of chlorinated and non-chlorinated derivatives of choline possessed plant growth regulator activity (90,91). The most characteristic

growth responses to analogs of (2-chloroethyl)trimethyl-ammonium chloride are thicker stems, shorter internodes, intensely dark green leaves, and shorter plants (90,91 . Wittwer and Tolbert (102) demonstrated a slightly greater, but significant increase in dry weight as a result of a 10^{-7} M (2-chloroethyl)trimethylammonium chloride application to the roots of tomato plants. Growth reduction occurred above this concentration. Soil applications accelerated flowering in tomato plants over a wide range of internode shortening concentrations.

Wittwer and Tolbert (102) suggested that (2-chloroethyl)trimethylammonium chloride, gibberellic acid, and indoleacetic acid activated mechanisms are closely related. The first two are mutually antagonistic. The first compound suppressed light-induced lettuce seed germination, vegetative extension of genetically dwarf and normal plants, elongation of plants in light and in dark, and lettuce flowering. Avena coleoptiles, in the presence of or in the absence of indoleacetic acid, failed to elongate when solutions of allyltrimethylammonium chloride higher than 2×10^{-2} M were applied. Tomato ovary growth was not affected by (2-chloroethyl)trimethylammonium chloride;

however, in combination with 10^{-3} $\underline{M}$ indoleacetic acid and 10^{-5} $\underline{M}$ gibberellic acid, ovaries showed growth synergism beyond that noted for the indoleacetic acid-gibberellin combination.

Soil application of (2-chloroethyl) trimethylammonium chloride to bean seedlings grown in the light was considerably more effective in height suppression than applications made to similar plants grown in the dark (103).

Rhythmic Variations in the Plant

Biddulph (7) indicated a maximum in downward translocation of foliar applied radioactive phosphorus from the leaf about 10:00 A.M. and a minimum about 10:00 P.M. and a maximum in the slight upward movement about noon. Light was associated with phosphorus movement, but the maxima did not coincide.

Hastings (32) showed a peak in phosphorus incorporation into nucleic acid at six, fourteen, and twenty-two hours with minima at two, ten, and eighteen hours on twelve hours of light and twelve hours of darkness sequence in *Gonyaulax* at 25° C. In *G. polyedra* there was no diurnal rhythm in nucleic acid synthesis. Kinetin at 3 x 10^{-4} M and gibberellic acid at 10^{-4} $\underline{M}$ had relatively little effect upon the phase of the rhythm, while cyanide and dinitrophenol shifted the phase.

Halberg (29) in work with mouse liver demonstrated a maximum in ribonucleic acid synthesis at 6:30 P.M. (just after dark initiation) and a minimum at 6:00 A.M. (just after light started). In liver, deoxyribonucleic acid synthesis peaks at 4:00 A.M. (eight hours of darkness elapsed), with a minimum at 6:30 P.M.

Van Die (96) demonstrated a diurnal rhythm in three month old tomato plants grown under controlled conditions insofar as total amino acids present in root exudate fractions collected over a sixty hour period were concerned. A maximum occurred about 11:00 A.M. to 1:00 P.M. with a minimum at night.

Hamner (30) in investigating the total number of nodes flowering on ten plant plots of Biloxi soybean pretreated with six hours of initial light and altering the total exposure to darkness in the subsequent seventy-two hours cycle found that maxima occurred at twenty-four, forty-eight, and seventy-two hours of darkness and minima at eighteen, thirty-six, and sixty hours of darkness. A

diurnal leaf movement was indicated in *Phaseolus multi-floris* (2).

Host Virus Relationships

Selman and Milne (81) working with tomato spotted wilt virus in tomato demonstrated a maximum virus concentration in leaves, stems, and terminals eleven days after inoculation. Roots were barely above a zero virus index reading for the entire nineteen day period.

Virus lesion formation. Farkas et al. (22) and Solymosy et al. (83) mention that many workers have suggested that local lesions were thought to be a protective mechanism as a result of the disruption of the organization of a small group of host cells highly sensitive to the pathogen. Oxidation-reduction systems acting on polyphenols were upset in the area to allow an accumulation of toxic polyphenol oxidation products with the eventual damage and death of the host cells in the area. They found that local lesions had high polyphenol oxidase activity: whereas, systemically invaded hosts had essentially normal levels. If this was the cause of lesion formation then ascorbic acid or other compounds which either reduce quinones or inhibit their formation should eliminate lesions. Ascorbic acid, glutathione, and cysteine applications reduced lesion numbers by five, fourteen, and seventeen percent, respectively, when vacuum infiltrated into the leaf. If their concentration is allowed to decrease, lesions appear. Cell disorganization did not produce the unusually high oxidase activity noted, since leaves damaged with a needle had considerably less oxidase activity than the virus inoculated plots. Polyphenol oxidase disruption therefore, was the cause and not the result of the cell breakdown.

Farkas et al. (22) in confirmation demonstrated that the polyphenoloxidase and the peroxidase activity increased very rapidly in infected plants. Lesion development was accompanied by a decrease of o-dihydric phenols essentially chlorogenic acid in the host tissues. Histochemical analysis suggested that toxic quinones accumulate in the necrotic areas and produce rapid breakdown of added ascorbic acid by maintaining phenol reduction level, and not through any effect in inhibiting the polyphenol oxidase system. Yamaguchi and Hirai (105) demonstrated a respiratory increase when lesions appeared.

<u>Host tissue</u> - <u>virus interactions</u>. Wu, Jia-Hsi et al. (104) investigated tumors produced by the cross <u>N</u>. <u>tabacum</u>

x N. glutinosa and found that young tissue cultures (mostly meristematic) were more resistant to tobacco mosaic virus infection and/or to subsequent multiplication than were older cultures composed largely of enlarging or senescent cells. With clone M222 of crown gall origin, active cell division accounted for low infectivity per unit fresh weight of tissue and the authors implied that nucleoprotein synthesis required for cell division is competitive with tobacco mosaic virus synthesis.

Spencer (84, 85) investigated the effect of host nutrition and other factors on tobacco mosaic virus in bean and tobacco. He found a definite relation between host nutrition and host susceptibility to virus infection. Host susceptibility is not governed just by host vigor as indicated by growth rate, but by some other as yet unknown factor(s). Areas of greater meristematic activity and less differentiation such as the terminal leaves versus the lower leaves appeared more susceptible.

Movement of virus within the plant. Virus movement is considered to be of two types (19, 78).

Movement in the vascular system is by means of the phloem or the xylem. Curly top virus moves up to fifteen centimeters in six minutes after inoculation and can move out of beet cotyledons and produce symptoms within thirty hours. Dodder transmission of virus is apparent in a vector three hours after inoculation and at a distance of thirty centimeters from the area of inoculation.

The second method of virus transport by means of the parenchyma or epidermis is rather slow, possibly two millimeters per day, until a vascular element is reached at which time a rate of several centimeters per day is observed. This slow intercellular movement is probably via the plasmodesmata. The twenty to two hundred millimicron size would allow passage of an oriented rod of tobacco mosaic virus two hundred and eighty by fifteen millimicrons or tobacco ring-spot virus (twenty-six millimicrons). The physical form of virus transport is not known. Movement of maize streak virus through the phloem cells approached twenty centimeters per hour. Movement is probably by mass flow (19).

It was noted that in a leaf inoculated in the middle and wounded at the base, that virus was recovered from the wounded and inoculated areas. The areas between which served as channels of movement for the virus often remained virus free.

The previously reported so-called directing action of meristems on virus movement was not due to the primary meristems themselves in all likelihood, but due to the active young tissues such as the leaf primordia, which acted as a directing factor for the virus. In addition mature or differentiated cells reversed to the juvenile condition through wounding influence virus movement for about seventy-two hours after such wounding (8) .

Samuel (78) in his investigations with tobacco mosaic virus in tomato showed that the virus did not move from the inoculated leaf for three to four days. Once it first appeared in the petioles of the inoculated leaf, however, the virus was detected in the roots within twelve hours and in the apex in twenty-four hours. This movement did not depend on the multiplication of the virus and often intermediate areas between these extremes were virus free. The virus left the inoculated leaflet, bypassed leaflets on the same leaf, and appeared in the root in four days. From the root it went to the apex within five days and then throughout the entire stem. At ten days it was in the leaflets on the inoculated leaf and in the lowest true leaf. From there it entered the lowest two full size leaves and all other leaves in eighteen days. By the end of twenty-five days full systemic invasion was apparent.

In an older fruiting plant the virus entered the root in four days and was entering all fruits between, but not the leaves. In five days it was in the tip and a few subterminal leaves and all intermediate fruits. In thirty days it was in several of the upper leaves and was progressing down the stem (78).

Kunkel (43) demonstrated that tobacco mosaic virus frequently moved out of the inoculated tomato leaves in less than two days. The speed may approach many inches per hour as indicated previously. The virus moved to the stem, then to the roots and up to the apex in that order, but at times to either the apex or to the roots only. The roots may not always be the intermediate stop in the route to the terminal meristem. Long sections of stem may act merely as a tube. The movement is probably via the phloem with different strands accounting for basipetal and acropetal movement. Stem sections often appeared to be virus free when rooted later. Plants produced from these sections could show the presence of tobacco mosaic virus as a result of the entrapment of a virus particle in one of the vascular elements which later for some reason or another broke loose or finds itself in a region possessing suitable conditions for reduplication.

Beemster (6) demonstrated with *Physalis floridana* that the presence of viruses X and Y in the same plant did not alter the movement rate in plants in the light. When the question of darkness was considered it was found that plants kept in the dark exhibited a retarded movement of virus X from the leaves in many cases.

Plant Growth Regulator-Virus Interaction

Effects of growth regulators on virus movement. Limberk (51) studied the influence of heteroauxin applications on tobacco mosaic virus inoculated tobacco plants. He found that inoculated plants produced symptoms only on those leaves above the inoculated area and then primarily on the apical leaves. If uninoculated plants were decapitated and then rubbed with a lanolin infusion of virus and auxin, the symptoms appeared on the basal shoots first. In the absence of auxin the decapitation procedure resulted mainly in the involvement of the upper leaves. The auxin apparently speeded virus movement and changed its direction, the particles being passively swept along with the heteroauxin, according to the author.

Corbett and Peterson (10) in their investigations with potato plants inoculated with potato Y virus, mentioned that the application of indoleacetic acid to the petioles of inoculated leaves did not appear to alter the plant's production of local lesions, but delayed leaf abscission. Systemic infection occurred only in growth regulator treated plants. Mechanical inoculation resulted in the production of local lesions only; whereas, vector and graft transmission of the virus resulted in systemic invasion. It seems fairly certain that virus movement was influenced by the indoleacetic acid.

Millikan and Guengerich (66) investigated systemic invasion of plum trees inoculated with necrotic ringspot virus and found that repeated applications of one hundred parts per million of 8-azaguanine or one hundred and forty parts per million of thiouracil to branches destined for use as budwood resulted in about fifteen to twenty per cent virus free trees from the treated buds, while those trees derived from buds obtained from branches treated with both compounds were not virus free.

Lindner et al. (53) investigated the effect of 8-azaguanine on tobacco mosaic virus replication. They concluded that the compound altered the plant's metabolism in such a way that 1) viral replication sites were altered, 2) virus particles with a low order of infectivity and virus particles with a high order of infectivity were produced, and 3) rates of systemic virus movement were reduced. The opinion is that the compound may do more than act as a replacement for guanine in the ribonucleic acid molecule. It may even have altered the replication site.

Effects of growth regulators on virus multiplication. Fulton (24) demonstrated that materials such as trypsin, milk, an extract of *Phytolacca decandra*, bovine serum, and an extract of *Aspergillus niger* influenced tobacco, cucumber, and bean mosaic viruses and potato and tobacco ringspot viruses. The results in general indicated a direct inhibitory effect on the virus and not an indirect effect through the host.

Van Kammen et al. (97) suggested that carnation sap inhibitors of tobacco mosaic virus in *Nicotiana glutinosa* blocked the virus receptor sites through a competitive inhibition mechanism. This was substantiated through a close fit of the observed and theoretical curves.

An inhibitory effect of a solution containing fifty parts per million of alpha-naphthalene acetic acid on tobacco mosaic virus lesions in Nicotiana glutinosa is demonstrated (28). Leaves so treated also had increased catalase activity.

Gondo (27) demonstrated that the number of tobacco mosaic virus lesions was reduced in plants to which a solution containing forty parts per million of 2,4-dichlorophenoxyacetic acid was applied. One hundred parts per million solution of the compound in the inoculum, however, did not reduce the number of local lesions. The growth regulator probably affected only the host.

Davis (12) demonstrated that plants inoculated with tobacco mosaic virus to which 4-chloro, 3,5-dimethyl phenoxyethanol was applied as ten daily soil applications of fifty milliliters each had as much as thirty percent fewer local lesions. A mixture of the virus and the compound did not appear to reduce infectivity.

Kutsky (46) in his investigations with tobacco mosaic virus in tobacco stem tissue culture determined that only indolebutyric acid at one hundred parts per million was useful in reducing the virus concentration. Purine bases and nucleic acids were generally without effect.

Kurtzman (44) and Kurtzman et al. (45) investigated extensively the inhibitory effects of purine derivatives on the production of tobacco mosaic virus by tissue cultures of *N. tabacum*. They found that 6-chloropurine, purine, and 8-chloroxanthine were essentially equivalent to 8-azaguanine. Reduplication of the virus as well as the host was inhibited at levels between ten and one hundred parts per million of the purines.

Ulrychova-Zelinkova (95) in their investigations with tobacco mosaic virus infections of the Samsun cultivar of *N. tabacum* indicated that soil applications of cadmium ions before inoculation, after inoculation, and three days after inoculation often reduced the quantity of virus by seventy percent. The inhibitory activity of the compound was less if it was applied twenty-four hours before inoculation. Application of large doses to certain young tobacco plants produced spots resembling the necrotic lesions on *N. glutinosa* leaves. The cadmium may disturb phosphorus metabolism. There may have been an interaction between zinc and cadmium in nucleoprotein metabolism such as is found in animal metabolism. There is an important role of zinc in the conversion of tryptophane to indoleacetic acid.

Bawden and Pirie (5) reported the isolation from tobacco leaves of a tobacco mosaic virus inactivating system possessing the properties of aldehydes and derivatives of ascorbic acid. The role of ascorbic acid will be considered.

Lindner et al. (53) felt that 8-azaguanine affected the amount of tobacco mosaic virus in a plant by (1) inhibiting virus replication sites and (2) by producing a non-infective virus.

Lindner et al. (52) applied the purine analog kinetin (6-furfurylamino purine) to cucumber cotyledons and found that is was ineffective against tobacco mosaic virus; however, it was not tried on plants inoculated with tobacco ringspot virus.

Brants (8) found that tobacco plants in which the stem tips and axillary buds had been treated with tetrachloronitrobenzene and inoculated with tobacco mosaic virus contained less virus, had less virus transport, and had a lower growth rate. As the plant inhibition subsided, plant growth resumed, and virus multiplication and transport increased.

Rawlins (74) demonstrated that floating tobacco leaf discs receiving applications of one-half and one-tenth percent solutions of the compound (2-chloroethyl)trimethylammonium chloride had ninety-two percent and eighty-one percent less virus, respectively, than the controls.

Williams and Howles (101) demonstrated the effectiveness of a six day soaking in 2,4-dichlorophenoxyacetic acid in the inactivation of virus in tomato seeds. The compound is also fairly effective on the flower distortion virus of *Chrysanthemum*. The compound 2,4-dichlorophenoxyacetic acid and its 3-chloro analog when applied to leaves of certain plants resulted in the production of twenty-two and three percent respectively, of apparently virus free plants six months after treatment.

Howles (35) demonstrated that 2,4-dichlorophenoxyacetic acid and other phenoxyacetic acid analogs can control tomato aspermy virus in *Chrysanthemum morifolium*.

Hartman and Price (31) demonstrated a synergism between virus and the growth regulator with Southern bean mosaic virus in bean and aster. The virus caused production of greater axillary branching on infected plants. Plants treated with growth substances, such as 2,4-dichlorophenoxyacetic acid and alpha-naphthalene acetic acid, did not reduce branching as freely as the controls. In bean plants treated with alpha-naphthaleneacetic acid an hour after inoculation there was a reduction in virus activity. A fifteen day delay in application of alpha-naphthaleneacetic acid led to increased virus activity. The investigators stated that growth substances appear to delay host tissue maturation so that the multiplication of the virus continued for a longer period of time. There is scattered evidence to indicate that auxin levels within the plant drop as a plant ages or approaches maturity. Critical levels may be the important factor. Hitier and Izard (33) mentioned that 2,4-dichlorophenoxyacetic acid and alpha-naphthaleneacetic acid at this stage apparently possessed very little utility in the field control of tobacco mosaic virus infection of tobacco.

Locke (54) investigated the effect of a two thousand parts per million foliar spray on leaf roll virus infected potato hills. The compound induced complete masking of

the virus symptoms in subsequent new growth and in shoots from tubers produced in the hill. He felt that the virus was reduced quantitatively rather than the symptoms being merely masked. The first generation plants resulting from the tubers produced in the 2,4-dichlorophenoxyacetic acid treated hill were also resistant to virus introduced by graft transmission.

Rich (77) demonstrated that lettuce plants inoculated with lettuce big vein virus were more vigorous if they had a pre-planting watering with a growth regulator such as fifty parts per million of alpha-naphthaleneacetic acid or indoleacetic acid or one hundred parts per million 2,4,6-trichlorophenoxyacetic acid. The trichloro derivative has been reported to possess anti-auxin characteristics.

Matthews (63) found that foliar applications of 8-azaguanine to a plant infected with turnip yellow mosaic virus resulted in the production of a virus form with a lower order of infectivity; however, no apparent chemical differences existed between the two virus forms. The general opinion was that the guanine analog was probably incorporated into the ribonucleic acid and the reduced activity resulted from the production of non-functional nucleic acid rather than enzyme blockage. Plants treated with the compound had less total virus nucleic acid in the plant sap and in the nucleoprotein. The percentage of phosphorus and of nucleic acid in the two viruses was identical.

Matthews (62) found that plants to which the guanine analog guanazolo was foliar applied had no lesions in certain cases, and systemic invasion was reduced for periods as long as twenty-three days. Soil applications had equivalent lesion suppressant activity, but were not as effective in suppression of systemic invasion. In treated plants inoculated with cucumber and pea mosaic viruses the soil applications delayed systemic development of mechanically introduced virus, but not virus introduced by an aphid vector.

Kirkpatrick and Lindner (42) investigated the response of cucumber to stone fruit ringspot virus. They found that with application of 8-azaguanine to plants before inoculation, the protection against the virus varied directly with the concentration of the compound and inversely with the titer of the inoculum. Plants treated both before and after inoculation displayed the greatest reduction of

systemic symptoms and severity.

The triazolo analog of adenine damaged plants and possessed very little virus antagonism. The hypoxanthine analog did not have any toxic affects and was more effective in tobacco than any of the other analogs in reducing virus activity. Beans inoculated with one of several viruses were not altered in their response to the virus by the addition of a purine derivative. Plants to which foliar applications of 10^{-2} $\underline{M}$ guanazolo were made had delayed or suppressed systemic transfer of cucumber mosaic virus. Its effectiveness as a soil application depended upon the method of transfer of the virus.

<u>Amelioration of virus symptoms with gibberellin</u>. Gibberellic acid has been tried as a virus suppressant in certain virus investigations. In an early paper on the subject Maramorosch (59, 60) indicated that plants foliar treated with ten and one hundred parts per million of the compound at the time of inoculation and with two additional applications at three day intervals showed regrowth; whereas the plants not so treated did not evidence any regrowth. The nodes of stunted corn and aster plants elongated to about twice the length of the non-treated

inoculated plants after three applications of one hundred parts per million each. The effect was apparent in corn inoculated with corn stunt, in aster with aster yellows, and in clover with wound tumor. Results were apparent in forty-eight to one hundred and twenty hours. The application of the compound in no case resulted in suppressing the ability to recover the virus, since leafhoppers could still recover the virus. Diseased plants showed other symptoms of virus invasion such as leaf symptoms and slight residual dwarfing. The elongation phenomenon may indicate that viral, physiological, and genetical dwarfing agents may have a common, basic mechanism.

Orlob and Arny (71) investigated the effect of foliar applications of gibberellic acid to barley plants inoculated with barley yellow dwarf virus. The usual stunting was partly reduced, but the results never equalled the performance of the resistant variety suggesting that the stunting principle was still in operation.

Ela et al. (18) demonstrated that foliar applications of one hundred parts per million of gibberellic acid to young abaca plants resulted in the best restoration of growth rate. Plants treated with higher concentrations had a greater

incidence of, and mortality due to, stem rot.

Yerkes (106) applied a solution containing fifty parts per million of potassium gibberellate to the leaves of vining type bean plants inoculated with a Mexican bean mosaic stunting virus. Virus-free plants showed internode elongation almost at once. Growth of inoculated plants was suppressed after several days and completely ceased within two weeks after inoculation. The inoculated plants treated with potassium gibberellate did not respond to the compound. These results are in contrast to other reported effects of gibberellic acid on virus inoculated plants.

Karas (39) investigated the effect of foliar applications of potassium gibberellate to tobacco ringspot virus inoculated plants of _Zinnia elegans_. Treated plants exhibited a partial restoration of growth rate; however, other virus symptoms were still apparent.

METHODS AND MATERIALS

Virus assay with *Vigna sinensis*. The general procedure for assay work follows. The plant employed in this investigation was the "S" selection from the commercial cultivar Black of the cowpea, *Vigna sinensis*. Seed of massed populations derived from single plant selections was used for almost all of the work. This stock possesses greater uniformity of reaction to experimental manipulation than commercial stocks.

Seeds were sown singly about three-quarters inch deep in steam pasteurized three inch clay pots filled with a steamed medium composed of two parts Conover loam, two parts peat moss, and one part sand by volume. The pots were placed in flats on a greenhouse bench in which thermostats were adjusted to provide a 70 degree night temperature. Germination usually required three to five days. In about nine to eleven days the primary leaves were usually fully expanded.

Plots of equal size in a given experiment containing between ten and twenty leaves (five to ten plants) were set up. Aberrant and atypical plants were discarded. Appropriate checks were evaluated when necessary.

Recognized statistical procedures were applied to data where appropriate.

A standard culture of the common strain of tobacco ring spot virus derived from muskmelon was maintained in Nicotiana tabacum var. xanthii n.c. Periodically it was reisolated from virus lesion on cowpea and reinoculated into new tobacco stock plants.

The inoculum was prepared from the expressed sap, silicon carbide, and pH 7.0, 0.01 M phosphate buffer in proportions previously determined to be adequate for the environmental conditions. The inoculum was prepared the day it was to be used and filtered before the addition of abrasive to remove as much plant debris as possible. The degree of dilution depended on the environment, virus concentration in the stock, and the purpose of the experiment. In general suitable dilutions were between 1:50 and 1:100.

The leaves of plants to be used in an experiment were lightly atomized with water, dusted very lightly with 400 mesh silicon carbide, and permitted to dry. Application of the prepared inoculum was made with the ground glass surface of a specially constructed glass spatula. In a given experiment, and depending on the experimental objectives, one or two strokes were made with the spatula dipped in the prepared inoculum for each primary leaf. Care was exercised to reduce excessive injury due to mechanical abrasions and maintain a uniform pressure during inoculation throughout an experiment.

Lesions appeared on the primary leaves in two to three days as small discrete brown areas, which enlarged and often coalesced in a short time when crowded. Systemic invasion occurred about five to seven days after inoculation. Lesions were counted three to six days after inoculation and recorded as the number of characteristic lesions per primary. Death of the assay plant generally occurred about ten days after inoculation. The observational period included the first eight days after inoculation.

The following represents the general procedures for *Zinnia elegans* experiments (Table 1):

Plant production and assay of Zinnia elegans. Many investigators have theorized about the utilization of simple and complex nitrogenous compounds in virus multiplication. Such compounds as nitrate supplied in the medium, amino acids, and nitrogenous bases have produced alterations

Table 1.

Critical data for typical investigations with the Blaze cultivar of *Zinnia elegans* conducted under Sylvania Daylight VHO fluorescent tubes, 16 hour photoperiod, 6,400 foot candle hours supplementing daylight.

Experiment	Experiment Series No.		
	FL 1	FL 2	FL 3
Sown	March 8	April 12	May 16
Potted	March 13	April 17	May 20
Treated	March 30	May 2	June 7
Virus assays	April 13	May 8	------
		May 16	
		May 23	
Plants harvested	------	------	June 12

in virus quantity. It appears that treatments which alter the nitrogen level or balance in a plant may also affect the virus quantity of an inoculated plant.

An effort was made to determine if a simple relation exists between virus recovery and inorganic nitrate nitrogen in the leaf. Measurements of nitrate evaluations were conducted on leaf samples identical to the samples used in the virus assay and concurrently with the assay.

Seed of the Blaze variety of Zinnia elegans, a variety previously determined to be of value in virus research (40) was sown and germinated in flats of compost and maintained under daylight supplemented by fluorescent light on a sixteen hour photoperiod until transplanting. Thermostats in the greenhouse were adjusted to provide a seventy degree night temperature.

Four seedlings were transplanted into each four inch clay pot filled with the compost. The potted plants were held in a greenhouse at seventy degree night temperature and illuminated by Sylvania Daylight T 12 VHO fluorescent lamps for sixteen hours per day. Light intensity as measured with a Weston Illumination Meter was between thirteen hundred and fifteen hundred foot candles. A

complete commercial fertilizer solution was applied about twice a week or as needed.

When three pairs of true leaves had developed the pots were assorted into four, twenty pot replicates. The seedlings in each pot were thinned to three per pot. The replicates were then divided into four treatments each. Plants in appropriate treatments were inoculated in each of the four replicates with 1:25 inoculum derived from tobacco as previously described.

Filtered inoculum containing one percent abrasive was applied to the plants with a DeVilbiss pressure atomizer at a pressure of thirty pounds per square inch. The inoculum usually dried within an hour. The appropriate plot was then sprayed with a solution containing 4.12×10^{-5} $\underline{M}$ of the sodium salt of 2,4-dichlorophenoxyacetic acid to the point of runoff by means of a small fly sprayer. The plots were allowed to dry and then returned to the experimental area in the greenhouse.

At periodic intervals assays were conducted with the "S" cultivar of Vigna sinensis. An evaluation of the virus present in plants of the Blaze cultivar of Zinnia elegans was made by grinding a small piece of tissue in buffer,

adding additional buffer, and rubbing out the resultant slurry in a uniform manner over the surface of the predusted cowpea leaves. Typical local lesions of the virus appearing on the assay leaves were positive evidence of establishment of the virus in leaves of the *Zinnia elegans* plant being assayed.

A quantitative assay involved grinding of a known quantity of tissue from a leaf of *Zinnia elegans* with a known volume of buffer, and carefully rubbing it onto a series of half or whole primary leaves of the "S" cultivar of *Vigna sinensis*. The average number of lesions produced for each primary leaf was counted and recorded as a representation of the virus concentration.

EXPERIMENTAL RESULTS--PLANT GROWTH REGULATOR--TOBACCO RINGSPOT VIRUS INTERACTION

Variation in physical characteristics of Zinnia elegans treated with growth regulator and inoculated with virus. In an investigation of virus growth regulator interactions a knowledge of the morphological and physical differences manifested in variously treated plants is helpful, if not mandatory, in providing an index to aberrations and other effects of the various treatments. In an attempt to determine these relationships, fresh weight, root color and quantity, and other characteristics were measured.

The Blaze cultivar of Zinnia elegans was used to determine the various aspects of the virus-growth regulator interaction. These experiments are referred to as FL 1 and FL 2 (Table 1).

Total vigor was recorded at the end of about three and one-half weeks from the date of treatment and inoculation as fresh weight in grams per plot. Plant values were computed.

The plants were at the two to four leaf pair stage on the day of inoculation and treatment. Inoculum consisting of plant sap from tobacco ringspot virus infected tobacco plants, buffer, and abrasive was prepared and used as described previously. Care was taken to direct the sprayed inoculum onto leaves in the area of the terminal meristem.

After the inoculum had dried a solution containing 4.12×10^{-5} $\underline{M}$ per liter of the sodium salt of 2,4-dichlorophenoxyacetic acid was applied as previously described.

In experiment 1 it is apparent that replicate 1, especially the 2,4-dichlorophenoxyacetic acid treatment, is aberrant (Table 1). The control in this replicate appears lower than others (Table 2). The statistical evaluation is presented in the last column. Through the use of Duncan's range test it is apparent that of all treatments only the virus, 2,4-dichlorophenoxyacetic acid combination is significantly different than the others.

In experiment 2 all means differ significantly at the five percent level (Table 2). Plots receiving the single treatments had lower fresh weight. Difference due to the combination treatment appeared to be the cumulative effects of the single treatments. It is apparent that the growth regulator application had a greater effect than in experiment 1.

Table 2

Fresh weight of plants of the Blaze cultivar of *Zinnia elegans* treated as indicated with a 4.12×10^{-5} $\underline{M}$ solution of sodium 2,4-dichlorophenoxyacetate and inoculated with tobacco ringspot virus.[a]

Experiment 1

Treatment	Mean	Significance
	g	
Virus, 2,4-D	13.9	
Virus	15.1	
2,4-D	15.8	
Control	16.1	

Experiment 2

Treatment	Mean	Significance
	g	
Virus, 2,4-D	13.0	*
2,4-D	15.5	
Virus	19.2	
Control	21.7	

[a]Means connected by a line are not significantly different (17).

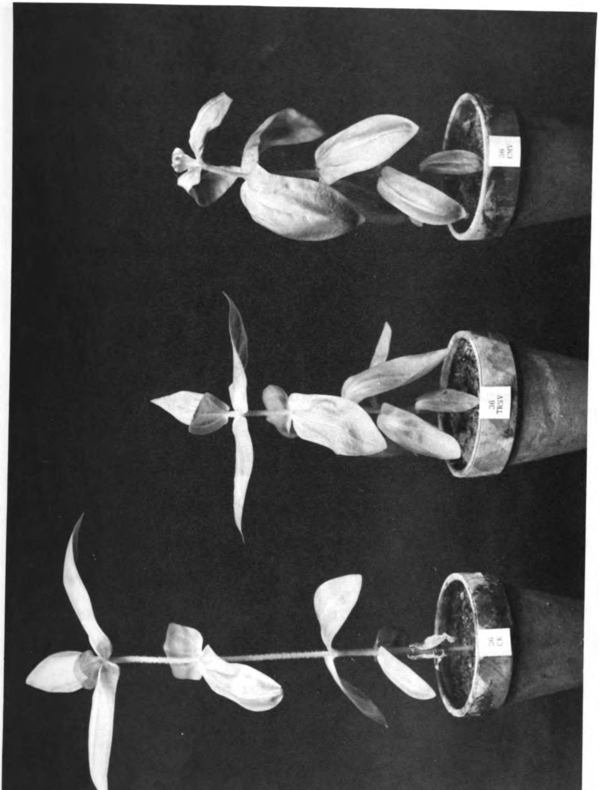
Aberrant values for the control and for the 2,4-dichlorophenoxyacetic acid treatments are apparent in replicate 1. The one factor of significance is that this replicate was near the end of the light fixture where edge drop off in light intensity could have been a factor. In subsequent experiments plants were arranged to avoid this problem.

Results were more consistent in experiment 2. The indication that the combined effect is the sum of the reductions attributed to the single effects suggests that, at least for this experiment, the agents were working on different growth processes in the plant. If they had been acting on the same group of growth mechanisms, such close agreement would not have been expected.

It is apparent that tobacco ringspot virus is not as severe as certain other viruses, even on _Zinnia elegans_, but the inoculated plants are about eleven percent lighter. Comparative symptoms produced by tobacco ringspot virus and cucumber mosaic virus are illustrated in Figure 1. Agronomically, this reduction in yield can be of significance. The differences in intensity of 2,4-dichlorophenoxyacetic acid, symptoms at various concentrations, and times after

Figure 1

Symptoms of tobacco ringspot virus and cucumber mosaic virus on the Blaze Cultivar of _Zinnia elegans_ twenty-three days after inoculation. Average gain for, left to right: control--20.3 cm.; tobacco ringspot inoculation--7.8 cm.; cucumber mosaic virus--9.7 cm. Distortion and mottle no longer apparent in tobacco ringspot inoculated plants but increasing in severity in cucumber mosaic inoculated plants. Comparative inoculation with cucumber mosaic by D. J. deZeeuw.


36
CMV
36
TRSV
36
CK

applications on the Blaze cultivar of *Zinnia elegans* are illustrated in Figures 2 and 3. Concentrations of eleven parts per million (5×10^{-5} *M*) or stronger approached the range of severe formative effects on *Zinnia elegans*.

In discarding the plants of the first experiment it was noted that there were somewhat consistent differences in certain treatments especially with respect to darker color of the roots and less so with respect to root color.

Plants of the Blaze cultivar of *Zinnia elegans* used in experiment 1 grown as indicated in general procedure were used for evaluation of root quantity and root color. The conditions of inoculation and growth regulator application are given in the general procedure for *Zinnia elegans*. The plants in each plot were harvested about six to seven weeks after sowing. The soil balls were removed from the pots and coded to avoid bias of judgment during recording. The condition of the root ball was evaluated for root quantity and root color (Table 3).

Figure 2

Symptoms produced by applications of (L-R) 0, 1.1, 110, and 1105 p.p.m. (0, 5×10^{-6}, 5×10^{-4}, 5×10^{-3} M) 2,4-dichlorophenoxyacetic acid in the Blaze Cultivar of *Zinnia elegans*.

A. Approximately 48 hours after treatment.

B. One month after treatment.


0
1.1
110
1105

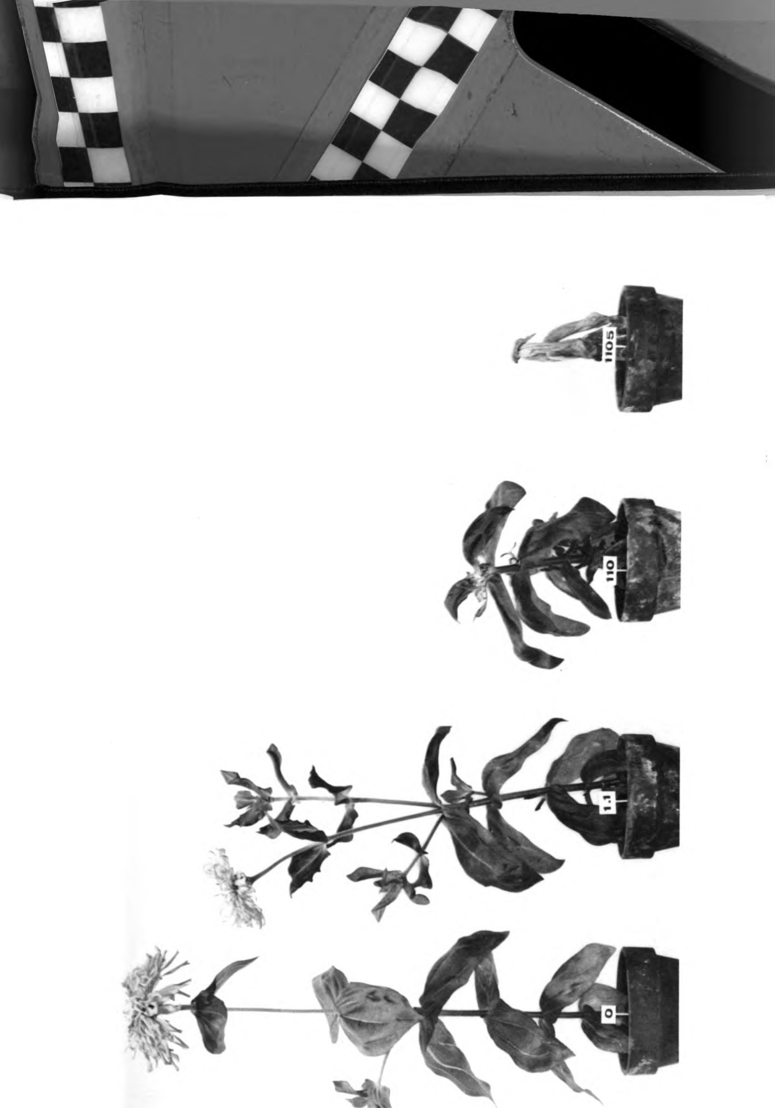
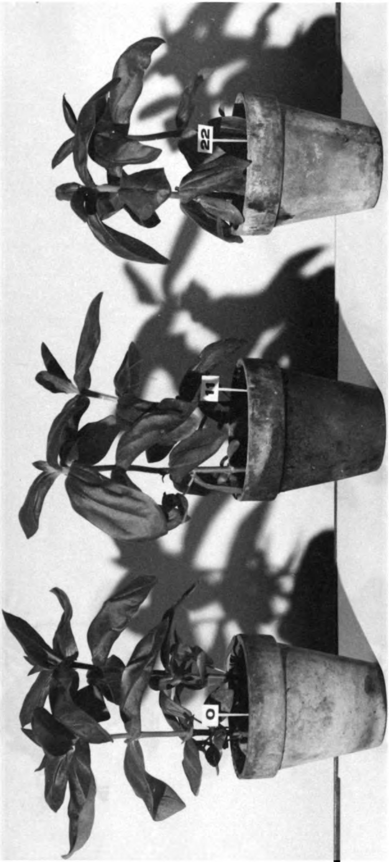

0
1.1
110
1105

Figure 3

Symptoms produced by applications of (L-R) 0, 11, and 22 p.p.m. (0, 5 x 10^{-5}, 10^{-4} M) 2,4-dichlorophenoxyacetic acid on the Blaze Cultivar of *Zinnia elegans*.

A. Approximately 48 hours after treatment.

B. One month after treatment.


0
11
22


0
11
22

Table 3.

Variations in root quantity and root color in plants of the Blaze cultivar of *Zinnia elegans* treated with a 4.12 x 10^{-5} M solution of sodium 2,4-dichlorophenoxyacetate and inoculated with tobacco ringspot virus as indicated.

Treatment	Root quantity[b] Mean	Significance[a]
Virus, 2,4-D	1.9	
Virus	2.2	
2,4-D	2.6	
Control	2.8	

Treatment	Root color[c] Mean	Significance
Virus, 2-4D	1.0	
Virus	1.2	
Control	1.8	
2,4-D	2.0	

[a]Means connected by a line are not significantly different at the five percent level.

[b]Quantity: 1, sparse; 2, medium; and 3, many roots.

[c]Color; 1, light tan and 2, white.

It is apparent that essentially no variation with respect to root quantity occurs. There was quite consistent variation with respect to differences in root color. Plants inoculated with tobacco ring spot virus have a consistently and significantly darker root color than uninoculated treatments. The application of 2,4-dichlorophenoxyacetic acid did not seem to alter root color.

Variation in root color can be indicative of root growth differences in various treatments. A relatively dark color may suggest that the roots are growing less actively than the situations where the soil ball is whiter. Presumably, this is because of the preponderance of older roots. Other physical alterations such as alteration in phenoloxidase systems may cause darkening of certain tissues. However, data of the present investigation are not indicative of such a conclusion.

Inoculated plots had a lower value suggesting that the cause of darkening may rest with some aspect of virus invasion. Definitive evidence would permit more extensive conclusions.

The role and importance of certain plant growth regulators in altering the growth of such plant parts as lateral buds has been demonstrated.

The development of lateral buds appears to be closely regulated by the apical bud and the auxin diffusing from its general area. The concept of apical dominance relies upon the assumption that auxin diffusing out of the actively growing apical bud diffuses down the stem and inhibits the lateral bud development through its supra-optimal amounts. It had been demonstrated that removal of the apical bud allowed development of the lateral bud. Replacement of the bud or application of a growth substance in a lanolin paste will restore the original inhibition.

Observation of the responses initiated by tobacco ringspot virus in various cultivars of Zinnia elegans suggests a possible alteration of the native growth regulators within the plant. An approach to this problem would be to measure lateral bud development at a node where the physiological effect of the native growth regulators might be most pronounced. The buds at the cotyledonary node seem to possess the desired qualifications.

General description of the procedure is the same as recorded previously (Table 1). Plants of the Blaze cultivar of Zinnia elegans in experiment 2 served as the experimental material. Virus application and growth regulator treatment are as described. About ten days after treatment lateral development at the cotyledonary node was determined for each of the two hundred and forty plants in the experiment. Elongation of laterals was measured as indicated (Table 4).

The plants treated with 2,4-dichlorophenoxyacetic acid and those inoculated with tobacco ringspot virus demonstrated inhibited lateral development at the cotyledonary node (Table 4). It is further apparent that the control and the plants treated with both the growth regulator and with the virus gave equivalent responses.

According to the limited data the lateral suppressing capability of the compound and of the virus were mutually antagonistic.

From the literature it appears that exogenous applications of auxin suppress lateral bud development. The data on Zinnia elegans presented here lend support to this theory, and there also appears to be lateral suppressant activity by the virus as well. This would suggest that the physiologically active quantities of auxin are increased or the efficiency of the endogenous auxin is increased.

Table 4

The development of lateral shoots at the cotyledonary node in plants of the Blaze cultivar of _Zinnia elegans_ inoculated with tobacco ringspot virus and sprayed with a 4.12 x 10^{-5} _M_ solution of sodium 2,4-dichlorophenoxyacetate as indicated.[a]

Treatment	Mean	Significance[b]
2,4-D	1.65	│
Virus	1.70	│
Virus, 2,4-D	1.90	│
Control	1.95	│

[a]Mean shoot elongation at the axis for each of sixty plants in a treatment: 1, elongation 1/4 centimeter or less and 2, 1/4 - 1 centimeter elongation.

[b]Means connected by a line are not significantly different at the 5% level.

The puzzling aspect is why should the combination treated plants have a lateral development equivalent to the control. It seems logical that lateral development should be no greater than the effects of the least active treatment.

Leopold and Lam (49) have shown that 2,4-dichlorophenoxyacetic acid competitively inhibits the uptake and/or transport of indoleacetic acid. Audus (4) suggested that exogenous applications of a growth regulator such as 2,4-dichlorophenoxyacetic acid did not actually destroy endogenous auxin where there was an interaction between the two, but might partly or fully block an enzyme site and render the site unavailable for further use. Some investigators have intimated that exogenous auxin may be partly or fully effective in certain physiological processes, and be ineffective in certain other auxin triggered processes.

The effects observed on lateral development may be explained on this basis. Exogenous auxin applications suppress lateral development due to the supraoptimal amounts of auxin. The presence of the virus suppresses lateral development by altering the balance between auxins and anti-auxins or by releasing additional auxin through the cleavage of auxin from host protein-auxin complexes being utilized for virus synthesis.

In a plant inoculated and treated with 2,4-dichlorophenoxyacetic acid it is possible that the exogenous auxin partially blocks enzyme sites. Additional supplies of physiologically active auxin released as a consequence of virus invasion would be in competition with the 2,4-dichlorophenoxyacetic acid.

The 2,4-dichlorophenoxyacetic acid may also competitively interfere with the uptake and/or transfer of endogenous auxins released as a result of protein complex degradation. Accordingly, it might be expected that the plants of *Zinnia elegans* inoculated with virus and treated with growth regulator would exhibit normal lateral development. Without more definitive evidence on auxin concentrations in the various treatments, this explanation should be regarded merely as an attempt to explain the observed data and subject to change as additional information is accumulated.

The effect of growth regulators on virus recovery, multiplication, and movement and the relation between nitrate content and the ability to recover tobacco ringspot virus. Plants of the Blaze cultivar of *Zinnia elegans* as previously described were the source of plant material used to assay for the presence of virus and for nitrate analysis. A leaf sample was used for virus assay and an adjacent one was used for nitrate analysis (experiment series FL 3).

A modified tissue testing procedure utilizing 0.04 percent of diphenylamine in concentrated sulfuric acid to develop the blue color was used (86). A quantity of nitrate standard equivalent to the fluid content of each tissue sample was added with a micropipette to a depression in a small porcelain plate. One-half milliliter of the reagent was used. Standards were run with each group of unknowns. Readings of the standards and unknowns were compared at a uniform time after addition of the reagent and consistent with ability to discern differences in the standards.

Comparison of the color developed in the plant material was made to a graded series of nitrate standards (KNO_3) and recorded directly as parts per million.

The results can be ranked in decreasing nitrate content as follows: virus and 2,4-dichlorophenoxyacetic acid; 2,4-dichlorophenoxyacetic; virus; and control (Table 4). Comparison of the nitrate content in parts per million and the amount of virus in a plant of the Blaze cultivar of Zinnia elegans expressed as the number of lesions for each leaf of Vigna sinensis revealed a significant linear correlation coefficient of +0.809.

Comparison of the results obtained in lesion assays and nitrate at time of assay reveals a direct relationship (Tables 5 and 6). The apparent accumulation of nitrate in plants containing more virus may be merely a result of reduced growth rate brought about by the presence of the virus and not directly related to the quantity of recoverable virus.

Considerable research has been conducted on the effect of certain chemicals in reducing virus multiplication. Many compounds have been of value in reducing the ability to recover virus from inoculated plants (16,53).

Work by deZeeuw et al. (16) showed that plants of Zinnia elegans treated with 2,4-dichlorophenoxyacetic acid at several concentrations have less recoverable tobacco

Table 5.

Nitrate content in plants of the Blaze cultivar of _Zinnia elegans_ twenty-one days after inoculation with tobacco ringspot virus and treated with a 4.12×10^{-5} _M_ solution of sodium 2,4-dichlorophenoxyacetate as indicated. Experiment Series (FL 2).

Treatment	Mean[a]	Significance[b]
	p.p.m.	
Control	15.1	│
Virus	28.3	│
Virus, 2,4-D	98.9	│
2,4-D	105.9	│

[a]Adjusted weight samples: control 1.000 (.00936 grams per disc), control/virus inoculated, 0.979; control/2,4-D, 0.791, and control/combination, 0.642.

[b]Means connected by the same line are not significantly different.

Table 6.

Relation of nitrate content at time of virus assay to the amount of virus. Experiment Series (FL 2).

Nitrate	Local lesions[a]		
p.p.m.	Mean		
22.8	21.1		
19.5	21.9		
31.0	7.5	r + 0.809*	
110.8	19.5	r for 5%	0.707
172.7	35.5	1%	0.834
162.9	44.0		
146.6	35.5		
162.9	54.0		

[a]Average lesions per primary of *Vigna sinensis* used to assay each plant of *Zinnia elegans*.

ringspot virus than untreated plants. Hartman and Price (31) have shown that a growth regulator application seems to reduce the quantity of virus when applied just after inoculation, but has a reverse effect when applied fifteen to sixteen days after inoculation. They attributed the effects to maintenance of the juvenile condition by the growth regulator.

In an effect to further substantiate these results a series of investigations was initiated. The general procedure is the same as discussed under general procedure.

Plants of the Blaze cultivar of Zinnia elegans contained in experiment 2 served as the experimental materials. Plants of the "S" cultivar of Vigna sinensis were used for virus assay at six, fourteen, and twenty-one days after treatment using the techniques described previously.

The assays at six and at fourteen days consistently reveal that 2,4-dichlorophenoxyacetic acid reduced the quantity and/or recoverability of tobacco ringspot virus from plants of the Blaze cultivar of Zinnia elegans (Tables 7 and 8). At twenty-one days the trend was reversed; that is, the inoculated plots treated with 2,4-dichlorophenoxyacetic acid had more virus than those with only the virus treatment (Table 9).

Percentage of plants of Blaze cultivar of *Zinnia elegans* indicating absences of tobacco ringspot virus on assay. Plants variously treated with 4.12 x 10^{-5} $\underline{M}$ solution of sodium 2,4-dichlorophenoxyacetic acid and inoculated with the virus.

Treatment	Experiment number			
	FL 2 assay at 6 days	FL 1 assay at 14 days	FL 2 assay at 14 days	FL 2 assay at 21 days
	(pct)	(pct)	(pct)	(pct)
Control	-----	100	100	-----
Virus	6.7 *	1.7 **	5.0 **	100
2,4-D	-----	100 **	100 **	-----
Virus, 2,4-D	21.7 *	13.3 **	28.3 **	1.7

* Difference between treatments within each column significant at 5%.

** Significant at 1%.

Table 8.

Semi-quantitative tobacco ringspot virus assay of the Blaze cultivar of _Zinnia elegans_ fourteen days after inoculation with tobacco ringspot virus and treatment with 4.12 x 10^{-5} _M_ solution of sodium 2,4-dichlorophenoxyacetate. Experiment Series (FL 1).

Treatment	Local lesions Mean	Significance[b]
Control	0	
2,4-D	0	
Virus, 2,4-D	1.3[a]	
Virus	1.9	

[a]Average rating: 0, no lesions; 1, a few scattered lesions; and 2, many lesions per assay leaf of _Vigna sinensis_.

[b]Means connected by the same line are not significantly different.

It is indicated that the recoverable virus in inoculated plots is less at twenty-one than at six days (Table 9). The major drop apparently occurs after fourteen days. It is shown that the recoverable virus from the combination virus and growth regulator plots was greater at twenty-one days than at six days. This increase apparently also occurs sometime after fourteen days (Table 7). Although the virus content in the combination treatment had not yet reached its peak at twenty-one days, it is doubtful that the peak would ever attain that of the inoculated plots which were at a maximum between six and twenty-one days (Table 9).

Sequential pairing of plants in the virus inoculated plots with those in the combination plots and pairing of plants for twenty-one versus six days points up other relationships.

Considering the two assay periods it is apparent that far more plants treated with the growth regulator showed an increase in virus content than plants in the untreated plot (Table 10). Inoculated plants had more virus than the combination at six days; whereas, at twenty-one days the combination treatment had more virus than those only

Table 10.

Comparison of relative virus concentration in the Blaze cultivar of *Zinnia elegans* at six and at twenty-one days after inoculation with tobacco ringspot virus or inoculation and treated with a 4.12×10^{-5} $\underline{M}$ solution of sodium 2,4-dichlorophenoxyacetate.

Treatment	Assay higher at six days	Assay higher at twenty-one days
Virus only[b]	53[a]	7
Virus, 2,4-D	28	32

Assay period	Assay of virus treated plants higher than in combination treatment	Assay of virus treated plants lower than in combination treatment
6[b]	54[a]	6
21	10	50

[a]Each value represents data on sixty plants.

[b]Rows significantly different at 5%.

inoculated with virus. This resulted from a reduction in virus in the inoculated plots and an increase in virus by the end of twenty-one days in plots receiving both growth regulator and virus (Table 10).

The data provide indirect evidence for a 2,4-dichlorophenoxyacetic acid interference with virus multiplication. If the observed growth regulator effects were on virus movement it is doubtful whether the presence or absence assays or the six and twenty-one days quantitative assays would result in as high a virus recovery in the combination treated plants.

Investigations on virus movement in plants made by Samuels (78), Kunkel (43), Brant (8) and others reveal that there is a greater quantity of virus in certain meristematic centers or in regions near these centers in other areas of the plant. There is very little published information on the role of growth regulators in virus movement.

Seedlings of the Blaze cultivar were grown two in a pot as described previously. When the fourth pair of leaves had expanded fully on all plants, the number of plants was thinned to one per pot; the pots were sorted into ten two-pot replicates; and the third leaf pair spray

inoculated with a 1:10 dilution of filtered ground tobacco ringspot virus inoculum. Care was exercised to avoid inoculating other plant parts. To assure adequate inoculation two passes were made on each of the two leaves at the third node.

A solution of 4.12×10^{-5} M of 2,4-dichlorophenoxyacetic acid was applied about one hour later, when the inoculum had dried completely. All plants were sprayed until runoff was about to occur.

The plants were allowed to remain in the short night conditions of late summer. Flower buds were visible in all inoculated plants the day of inoculation.

Three to four days after inoculation assay discs eight millimeters in diameter were removed from leaf pairs one through five with a cork borer. The discs were weighed and sufficient buffer added to give an inoculum concentration of 1:15. The preparation was then applied with a glass spatula to one-half of each primary on each of three plants of the "S" cultivar of Vigna sinensis. The other half of each primary was used for the plants inoculated and treated with 2,4-dichlorophenoxyacetic acid.

A repeat assay was made on the same leaf pairs of *Zinnia elegans* ten days after inoculation. General procedures were the same except that the sample was diluted 1:25 with buffer.

The assay made at three days from the time of inoculation and treatment, was essentially negative except in the inoculated leaf. The virus had not moved out of the inoculated leaf to any great degree, or if it had then it had not multiplied sufficiently to be recoverable by the techniques herein employed (Table 11).

The Wilcoxon paired replicates test (99, 100) indicates that leaf pairs one, two, four, and five had significantly less virus than leaf pair three which was inoculated; but did not differ significantly among themselves at the five percent level. Recovery from inoculated growth regulator treated plants was not significantly different from the inoculated plants. At this stage the direction of virus movement in the plant did not appear to be influenced by growth regulator application (Table 11).

The assay at ten days showed considerable movement from the inoculated third pair of leaves into the sixth pair of leaves and some of the fifth pair (Table 11).

Table 11.

Recovery of tobacco ringspot virus three and ten days after inoculation from plants of the Blaze cultivar of *Zinnia elegans* variously inoculated and/or treated with 4.12×10^{-5} *M* solution of sodium 2, 4-dichlorophenoxyacetate.

Node number	Three day		Ten day	
	Virus[a] lesions	Virus, 2,4-D lesions	Virus lesions	Virus, 2,4-D lesions
1	0	0	-----	-----
2	.02	0	0	0
3 (inoculated)	19.3[b]	26.0[b]	33.7[b]	48.5[b]
4	0	.04	0	0
5	.02	0	5.2	5.9
6	-----	-----	62.9[b]	70.5[b]
Mean	3.87	5.21[c]	20.4	25.0[c]

[a]Average number of local lesions per half primary leaf of assay plants of *Vigna sinensis*.

[b]Significantly different from other nodes.

[c]Means connected by the same underline not significantly different.

The usual analysis of variance is applicable only to the main effects. The generally consistent, but small increase of recoverable virus from inoculated growth regulator treated plants for nodes three, five, and six was not significant at the five percent level (Table 11).

Analysis by the Wilcoxon test indicated that nodes two, four, and five were not significantly different in virus content from one another, but were significantly different from nodes three and six. Nodes three and six differed significantly from one another.

The data indicated that there is an association of virus movement and the location of meristematic areas (Table 11). Application of 2,4-dichlorophenoxyacetic acid did not alter the direction of movement of the virus at three days nor its speed at either three days or ten days from inoculation (Table 11).

It is apparent that 2,4-dichlorophenoxyacetic acid did not greatly affect either the amount of virus in an older plant of *Zinnia elegans*, or its relative direction of movement.

The marked inability to recover virus at three and at ten days from the second and fourth leaf pairs which are oriented ninety degrees to the inoculated pair of leaves suggested that virus movement is associated with phyllotactic arrangement of the leaves and with its stage of development the day that translocation of virus from the inoculated leaves occurred. A secondary site of virus multiplication may exist in the region of the terminal meristem and the virus produced there moves via the vascular strands into areas below it. The observed data on basic movement and sites of virus multiplication are in fairly close agreement with work of others (8,43,78).

The influence of interval between inoculation and growth regulator application and lesion production in Vigna sinensis. The effectiveness of growth regulators in the protection of plants against viruses is dependent on a number of factors. A series of investigations were initiated to determine one of these, the effect of time of application in relation to inoculation, on lesion number in *Vigna sinensis*.

Five plant plots of the "S" cultivar of *Vigna sinensis* were treated with a solution containing 5×10^{-3} *M* of either 2-chlorophenoxyacetic acid, 4-chlorophenoxyacetic acid, or 2,4-dichlorophenoxyacetic acid forty-

four, twenty-four, and one and one-half hours before inoculation. Infection quotients were derived from the average number of lesions produced on the inoculated control.

Time elapsed between growth regulator application and subsequent inoculation of plants of "S" cultivar of Vigna sinensis is important (Table 12). The twenty-four hour and forty-eight hour intervals were not significantly different from one another; however, they are significantly different from the one and one-half hour time interval. No differences exist in the performances of the three compounds.

A somewhat general increase in the infection quotient occurred between twenty-four and one and one-half hours before inoculation (Table 12). Variation existed between time replications 1 and 2.

The data as presented suggest that time elapsed between growth regulator application and inoculation may be crici-cal. Inconsistency between replicates in time is noted. The reason for the observed variations is not known at this time.

At this point it seemed desirable to investigate the effect of various time intervals following inoculation before application of the growth regulator in order to determine

Table 12.

The effect of time of application of 5 x 10^{-3} M solutions of each of three phenoxyacetic acid analogs on lesion production in the "S" cultivar of *Vigna sinensis*.

Growth regulator	Infection quotients[a] at:			
	44 hrs.[b]	24 hrs.	$1\frac{1}{2}$ hrs.	Mean[c]
2-chlorophenoxy-acetic acid	78.8	83.2	115.7	92.5
4-chlorophenoxy-acetic acid	84.0	54.1	96.0	78.0
2,4-dichlorophen-oxyacetic acid	53.5	58.8	91.0	67.8
Mean[c]	72.1	65.3	100.9	

[a]Infection quotients = percentage of inoculated control.

[b]Hours between growth regulator application and inoculation.

[c]Means connected by the same line are not significantly different.

physiological trends associated with the virus-host interaction. General procedures as outlined previously were employed for the production of assay plants with these exceptions; seven plants were used for each plot in the initial experiment and eight in the confirmatory experiment.

Plants were inoculated as described previously. Plants were sprayed to the point of runoff with 5×10^{-3} $\underline{M}$ solution of 3,4-dichlorophenoxyacetic acid at successively greater intervals after inoculation with a DeVilbiss atomizer as indicated previously.

In the first experiment the plots consisted each of seven cowpea plants in optimum condition for production of local lesions with the virus. They were inoculated with the glass spatula method and then sprayed with 5×10^{-3} $\underline{M}$ solutions of 3,4-dichlorophenoxyacetic acid per liter at the predetermined time intervals. The second trial was made in the same manner.

Two time interval studies are summarized in Table 13. In the first experiment there appears to be a slight positive relationship between lesion number and the length of the interval between inoculation and application of 3,4-dichlorophenoxyacetic acid. The coefficient of linear

Table 13.

Effect of time of application of a solution of 5×10^{-3} $\underline{M}$ 3,4-dichlorophenoxyacetic acid following tobacco ringspot inoculation on local lesions in the "S" cultivar of *Vigna sinensis*.

Experiment 1		Experiment 2	
Inoculation to treatment	Lesions[a]	Inoculation to treatment	Lesions[a]
Minutes		Minutes	
0	14.9	0	26.3
28	26.8	48	23.4
58	21.7	103	26.9
90	23.9	151	48.8
120	29.1	205	39.1
148	17.8	248	51.9
178	40.0	335	73.1
238	23.9	Control	
358	39.1	(Virus only)	124.4
r^b + 0.647 N.S.		r + 0.918 **	
r 5% 0.666		r 1% 0.874	

[a]Average number of virus lesions on each of the leaves of the "S" cultivar of *Vigna sinensis*.

[b]Coefficient of linear correlation and test for significance.

correlation is +0.647. A value of +0.666 is necessary for significance at the five percent level with seven degrees of freedom.

The relatively high r value suggested that a further investigation might be of some advantage. The correlation coefficient for the second experiment was +0.918. A value of +0.874 is required for significance at the one percent level with five degrees of freedom.

The number of lesions in the control indicates that reduction in lesion number in growth regulator treated plants may occur for several hours. The relationship held in this case for the five or six hour period before sunset, during which both experiments were performed.

The evidence leads to the conclusion that the influence of the growth regulators may depend on the host physiology. It is reasonable to presume that the virus nucleoprotein has entered the cell after two or three hours have elapsed.

The positive relation between the length of the interval separating inoculation and growth regulator application and lesion number implies that the physiological processes leading to lesion production become less responsive to growth regulator application as the interval increases.

<u>The influence of growth regulator application on virus lesion development</u>. Although the effects of auxins and auxin-like compounds on virus lesion production have been reported, little has been reported on the effect of growth regulator dilution beyond the point of apparent morphological effect and/or physiological alterations. Kurtzman et al. (45) reported one of the few evaluations of plants treated with extended dilution series using various purine analogs.

An earlier investigation (40) in the present series demonstrated that under certain conditions, an inverse relationship between lesion number and concentration of the growth regulator may not hold. The growth regulator need not be inhibitory throughout the dilution range.

The experiments to determine dilution effects were conducted from early summer through early winter. Most of the materials were dissolved in the concentrated form with the addition of a few drops of alcohol. The exception was 2,4-dichlorophenoxybutyric acid which required slight increase in temperature, alcohol, and a small quantity of Triton X-100 surfactant in most cases to render it suitable for application. Appropriate checks were maintained.

The compounds were applied to primary leaves of the "S" cultivar one hour after inoculation with a DeVilbiss sprayer. Seven concentrations ranging from 5×10^{-3} to 5×10^{-9} M per liter were used of 2-chlorophenoxyacetic acid; 3,4-dichlorophenoxyacetic acid; and 2,4-dichlorophenoxybutyric acid. These appeared to possess the most desirable characteristics of all the 2,4-dichlorophenoxyacetic acid analogs evaluated.

In fifteen of sixteen experiments at least one concentration inhibited lesion development when the compound was applied one hour before inoculation. In twelve of twelve comparisons at least one concentration clearly inhibited lesion development when the compound was applied one hour after inoculation (Table 14).

In thirteen of sixteen experiments one concentration of the compound stimulated lesion development when the compound was applied one hour before inoculation; while, in only four of twelve experiments was there an increase in lesion number demonstrated when the compound was applied one hour after inoculation.

Plants treated with 2-chlorophenoxyacetic acid before inoculation have fewer lesions in nineteen of twenty-one

Table 14

The effects of concentration and time of application of analogs of phenoxyacetic acid on tobacco ringspot virus local lesions on the "S" cultivar of <u>Vigna sinensis</u>.

Exp. 1	POA[a]		2-ClPOA		3,4-ClPOA		2,4-ClPOB	
Conc.[b]	DV[c] lesions[d]	VD lesions	DV lesions	VD lesions	DV lesions	VD lesions	DV lesions	VD lesions
5×10^{-3}	17.4	13.9	27.9	11.9	10.3	1.3	18.3	14.7
5×10^{-4}	38.1	14.1	54.3	37.4	28.4	8.3	49.1	12.2
5×10^{-5}	28.1	13.5	49.8	21.0	42.6	20.8	64.4	15.1
5×10^{-6}	31.8	10.8	44.9	12.3	42.6	19.7	38.1	24.0
5×10^{-7}	27.5	22.0	33.4	13.2	42.5	21.4	39.7	21.3
5×10^{-8}	39.3	15.4	46.6	13.9	40.9	21.1	33.6	13.9
5×10^{-9}	25.1	11.8	45.3	15.7	47.6	21.3	43.4	7.6
Control	25.6	31.0	25.6	31.0	25.6	31.0	25.6	31.0
Control	34.4	19.5	34.4	19.5	34.4	19.5	34.4	19.5
Exp. 2								
5×10^{-3}	180.0	106.5	135.5	163.5	93.5	75.0	107.0	162.5
5×10^{-4}	189.5	178.0	189.5	157.5	174.5	165.5	156.6	191.0
5×10^{-5}	169.0	155.0	195.0	189.0	185.0	180.0	193.0	184.5
5×10^{-6}	203.0	146.5	181.0	199.0	196.0	180.0	177.0	198.0
5×10^{-7}	172.5	170.5	177.0	197.5	186.5	198.0	183.5	194.5
5×10^{-8}	176.5	194.0	154.0	182.5	197.0	183.0	201.0	181.5
5×10^{-9}	201.0	174.0	186.0	194.0	165.0	178.5	197.5	186.0
Control	184.5	188.5	177.5	178.5	177.5	178.5	157.5	178.6
Control	177.5	194.0	157.5	194.0	157.5	194.0	199.5	194.0

[a] POA = phenoxyacetic acid; 2-ClPOA = 2-chlorophenoxyacetic acid; 3,4-ClPOA = 3,4-dichlorophenoxyacetic acid; 2,4-ClPOB = 2,4-dichlorophenoxybutyric acid.

[c] DV = plants treated one hour before inoculation, VD = one hour after inoculation.

Table 14. Continued.

Conc.	POA		2-ClPOA		3,4-ClPOA		2,4-ClPOB	
	DV lesions	VD lesions	DV lesions	VD lesions	DV lesions	VD lesions	DV lesions	VD lesions
Exp. 3								
5 x 10^{-3}	120.5	72.9	95.0	76.5	34.5	98.5	85.5	49.8
5 x 10^{-4}	157.0	150.0	131.0	131.5	93.5	116.5	121.0	123.5
5 x 10^{-5}	155.5	135.0	190.0	96.5	111.5	158.5	112.5	129.5
5 x 10^{-6}	159.5	143.5	137.0	155.0	121.0	128.5	149.5	130.5
5 x 10^{-7}	155.0	89.0	142.5	192.0	105.0	119.5	141.5	156.5
5 x 10^{-8}	173.0	134.0	158.0	113.0	119.5	130.0	137.0	139.5
5 x 10^{-9}	158.0	171.5	151.5	104.5	150.5	143.5	128.5	147.5
Control	180.5	177.0	180.5	177.0	156.0	191.5	180.5	191.5
Control	156.0	191.5	156.0	191.5	109.5	180.5	156.0	180.5
Exp. 4								
5 x 10^{-3}	104.0		124.3		26.1		37.3	
5 x 10^{-4}	109.8		129.0		115.8		67.4	
5 x 10^{-5}	139.0		143.3		173.1		109.2	
5 x 10^{-6}	136.5		127.8		191.8		105.5	
5 x 10^{-7}	150.5		150.8		187.7		99.2	
5 x 10^{-8}	128.3		147.0		174.3		80.6	
5 x 10^{-9}	155.2		150.7		212.5		87.2	
Control	131.5		127.8		151.1		62.0	
Control	107.5		131.5		127.8			

[b]Fraction of a Mole per liter of application solution. The surfactant Triton X-100, a non-ionic type was used to achieve solution of 2,4-ClPOB. Control plants were sprayed with a solution of this surfactant.

[d]Average number of lesions per leaf of <u>Vigna sinensis</u>.

comparisons. Other compounds evaluated displayed less consistent results. Plants treated before inoculation with 2-chlorophenoxyacetic acid had more lesions than those treated with 3,4-dichlorophenoxyacetic acid in eighteen of thirty-one comparisons and plants treated with the four strongest concentrations had more lesions in eleven of twelve comparisons. Plants receiving 2-chlorophenoxyacetic acid had more lesions in twenty-three of thirty-one comparisons than those plants receiving 2,4-dichlorophenoxybutyric acid; whereas, in the stronger concentrations there were more lesions in eleven of twelve comparisons.

Variations in the controls and in the treatments do not permit extensive interpretation of the data. Plants treated with 2-chlorophenoxyacetic acid, however, appear to have responded in the concentration range between 5×10^{-4} and 5×10^{-5} $\underline{M}$ per liter by displaying consistent increases in lesion number.

Differences in method of application and differences between analogs may account for observed variation in the effects obtained. The host plant variability (even though it is an improved type), temperature, and certain suspected variations in the control series limit the confidence one can place in some of the small differences obtained.

The effect of 2-chlorophenoxyacetic acid as a preinoculation treatment has been presented (Table 14, Figure 4). This serves to demonstrate differences between 2-chlorophenoxyacetic acid and 3,4-dichlorophenoxyacetic acid (Figures 4 and 5).

Lesion suppressant ranges appeared in plants treated with either analog. In contrast to 2-chlorophenoxyacetic acid, no obvious lesion stimulation range appeared to exist for 3,4-dichlorophenoxyacetic acid.

The apparent lesion stimulation range in plants treated with 2-chlorophenoxyacetic acid and the absence of a stimulation range in plants treated with 3,4-dichlorophenoxyacetic acid suggests the existence of subtle metabolic differences in the response to various analogs. Such differences may suggest that structures possessing virus antagonism, but without apparent host disruption may exist. These may assist in the understanding of the role of exogenous growth regulator application in the virus-host relationship.

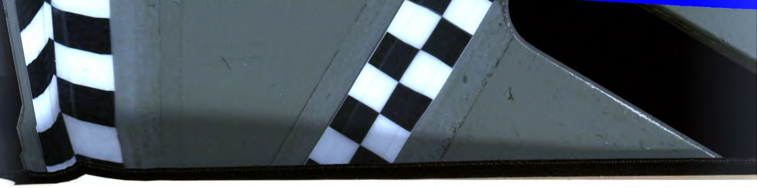
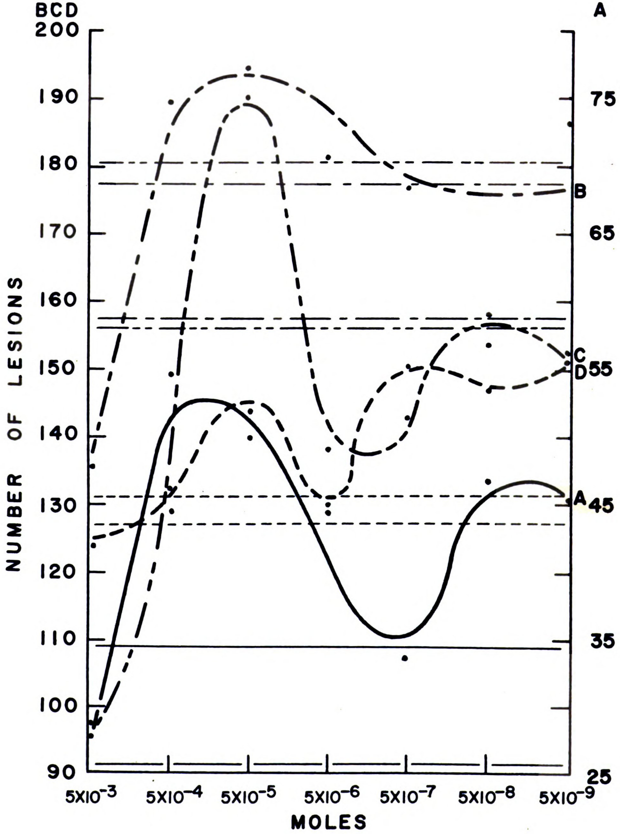


Figure 4.

The effect of various concentrations of 2-chlorophenoxy-acetic acid applied before tobacco ringspot virus inoculation on lesion number in plants of the "S" cultivar of Vigna sinensis. Control limits indicated by lighter weight horizontal lines of the same type as experimental curve. Letters denote duplicate experiments.


BCD
A
200
190
180
170
160
150
140
130
120
110
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90
75
65
55
45
35
25
B
C
D
A
NUMBER OF LESIONS
5X10^{-3}
5X10^{-4}
5X10^{-5}
5X10^{-6}
5X10^{-7}
5X10^{-8}
5X10^{-9}
MOLES

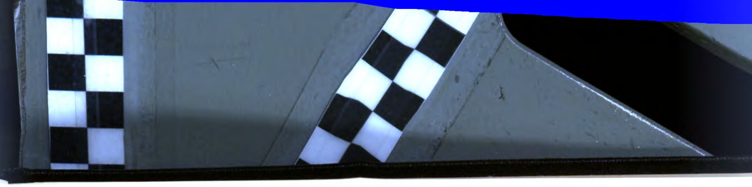
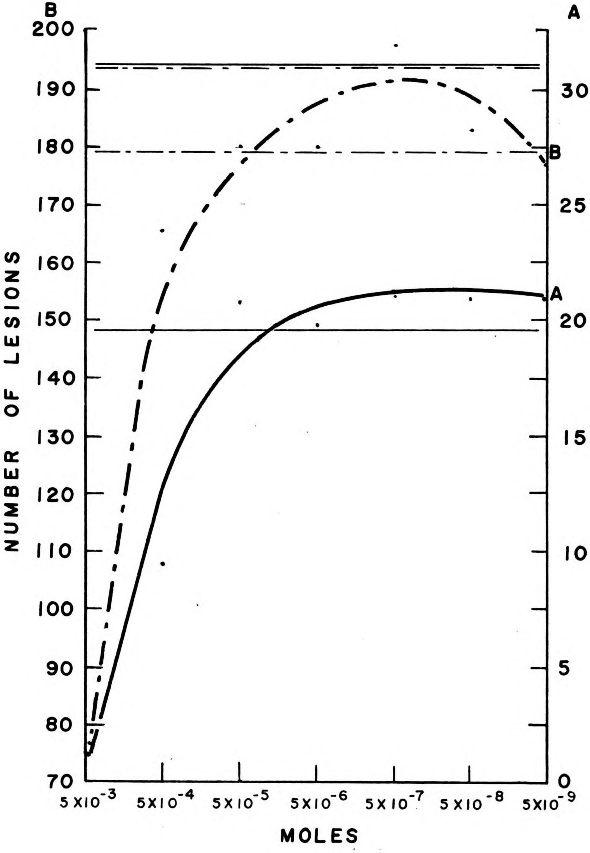


Figure 5.

The effect of concentration of 3,4-dichlorophenoxyacetic acid, applied after inoculation, on tobacco ringspot virus lesion number in plants of the "S" cultivar of Vigna sinensis. Control limits indicated by lighter weight horizontal lines of the same type as experimental curve. Curve A, Experiment 1; Curve B, Experiment 2.



B
A
200
190
180
170
160
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140
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120
110
100
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80
70
30
25
20
15
10
5
0
NUMBER OF LESIONS
B
A
5X10^{-3}
5X10^{-4}
5X10^{-5}
5X10^{-6}
5X10^{-7}
5X10^{-8}
5X10^{-9}
MOLES

<u>The effect of different phenoxy acid analogs on lesion production in the "S" cultivar of Vigna sinensis inoculated with tobacco ringspot virus.</u> Initial investigations into the effect of growth regulators on virus recovery revealed that 2,4-dichlorophenoxyacetic acid materially reduced the recoverable virus (16). The extreme formative effects of this compound strongly indicated the need for a more desirable analog. An investigation of other analogs of the phenoxy series was conducted. Since time of application may be important, the effects of applications of growth regulator prior to, simultaneous with, and after inoculation were also evaluated.

Some modifications of the general methods were employed. Growth regulator solutions containing 2×10^{-4} *M* were applied to plants of the "S" cultivar of *Vigna sinensis* to the point of runoff with a small flysprayer. Tobacco ringspot virus inoculum was applied one hour before the growth regulator; as a 1:9 mixture of growth regulator and inoculum simultaneously; or one hour after treatment. Repetitions of the experiments were conducted at different times.

The results are summarized in Table 15. Considerable variation existed in the performance of the various analogs.

Table 15.

Relative effectiveness of some halogen derivatives of phenoxy acids in altering local lesion production in plants of the "S" cultivar of Vigna sinensis inoculated with tobacco ringspot virus. Growth regulators applied before, simultaneous with, and after inoculation.

Growth regulator, virus			Simultaneous		Virus growth regulator	
Analog	Local lesion mean[a]	Range test	Analog	Local lesion mean[b]	Analog	Local lesion mean[b]
4(2,4-Cl)POB[c]	1.55		2-Me,4-ClPOA	9.64	2(4-Cl)POP	9.18
2,4-BrPOA	1.88		2(4-Cl)POP	10.73	2,4,5-ClPOA	9.64
3,4-ClPOA	3.34		2(2,4-Cl)POP	11.32	POA	9.99
2,3,6-ClPOA	3.43		2,6-ClPOA	11.95	2,4-BrPOA	10.23
2,4-ClPOA	3.43		4(2,4-Cl)POB	12.77	3,4-ClPOA	10.41
4-ClPOA	3.43		2(2,4,5-Cl)POB	13.50	4(2,4-Cl)POB	10.41
2-ClPOA	3.43		2,4,5-ClPOA	13.73	2,4,6-ClPOA	11.91
2(2,4,5-Cl)POP	3.73		2(2-Me,4-Cl)POP	13.82	2,4-ClPOA	12.23
2,3-ClPOA	3.75		2,4-ClPOA	14.41	2,5-ClPOA	12.27
2,3,4,6-ClPOA	3.75		4(2,4,5-Cl)POB	14.59	2(2-Me,4-Cl)POB	12.36
2,4,6-ClPOA	3.82		2,3,6-ClPOA	14.82	2(2,4,5-Cl)POP	12.64
POP	4.30		2,4-BrPOA	14.86	4(2,4,5-Cl)POB	12.68
POA	4.64		4(2-Me,4-Cl)POB	15.09	2,3,6-ClPOA	12.86
2,4,5-ClPOA	4.88		2,5-ClPOA	15.23	2(2,4-Cl)POP	13.14
2,5-ClPOA	5.15		POP	16.09	2,6-ClPOA	13.18
2-Me,4-ClPOA	6.00		3,4-ClPOA	16.36	2,3-ClPOA	13.77
2,3,4,6-ClPOA	6.15		2,3,4,5,6-ClPOA	17.00	4(2-Me,4-Cl)POB	14.77
2,3,4,5-ClPOA	6.45		3-ClPOA	17.00	2-Me,4-ClPOA	15.09
2,6-ClPOA	6.54		2,4,6-ClPOA	17.36	POP	15.18
2(2-Me,4-Cl)POP	6.75		4-ClPOA	17.95	2,3,4,6-ClPOA	15.54
4(2,4,5-Cl)POB	6.88		2,3,4,5-ClPOA	19.41	4-ClPOA	17.18
4(2-Me,4-Cl)POB	7.94		NONE	19.59	2,3,4,5-ClPOA	17.23
2(4-Cl)POP	10.66		2,3,4,6-ClPOA	19.68	2,4,6-ClPOA	17.36
2(2,4-Cl)POP	10.73		2,3-ClPOA	25.09	2,3,4,5,6-ClPOA	21.95
NONE	13.09		POA	28.50	3-ClPOA	22.59
2,3,4,5,6-ClPOA	13.30		2-ClPOA	31.72	NONE	25.91

[a]Means (three replicates) not significantly different are connected by a line.

[b]Means (two replicates) do not differ significantly.

[c]All growth regulators used at 2 x 10^{-4} M concentration.

POA (1)	Phenoxyacetic acid
2-ClPOA	2-chlorophenoxyacetic acid
3-ClPOA	3-chlorophenoxyacetic acid
4-ClPOA	4-chlorophenoxyacetic acid
2,3-ClPOA	2,3-dichlorophenoxyacetic acid
2,4-ClPOA	2,4-dichlorophenoxyacetic acid
2,5-ClPOA	2,5-dichlorophenoxyacetic acid
2,6-ClPOA	2,6-dichlorophenoxyacetic acid
3,4-ClPOA	3,4-dichlorophenoxyacetic acid
2,3,6-ClPOA	2,3,6-trichlorophenoxyacetic acid
2,4,5-ClPOA	2,4,5-trichlorophenoxyacetic acid
2,4,6-ClPOA	2,4,6-trichlorophenoxyacetic acid
2,3,4,6-ClPOA	2,3,4,6-tetrachlorophenoxyacetic acid
2,3,4,5-ClPOA	2,3,4,5-tetrachlorophenoxyacetic acid
2,3,4,5,6-ClPOA	2,3,4,5,6-pentachlorophenoxyacetic acid
POP	phenoxypropionic acid
2(4-Cl)POP	2(4-chlorophenoxy)propionic acid
2(2,4-Cl)POP	2(2,4-dichlorophenoxy)propionic acid
2(2,4,5-Cl)POP	2(2,4,5-trichlorophenoxy)propionic acid
4(2,4-Cl)POB	4(2,4-dichlorophenoxy)butyric acid
4(2,4,5-Cl)POB	4(2,4,5-trichlorophenoxy)butyric acid
2,4-BrPOA	2,4-dibromophenoxyacetic acid
2-Me,4-ClPOA	2-methyl, 4-chlorophenoxyacetic acid
2(2-Me,4-Cl)POP	2(2-methyl, 4-chlorophenoxy)propionic acid
4(2-Me,4-Cl)POB	4(2-methyl,4-chlorophenoxy)butyric acid

Differences between time of application were apparent. Means of plants treated with growth regulators before inoculation differed significantly. Analog performance groups are indicated by vertical lines. Means in columns two and three are not significantly different.

Significant differences between means in the prior application series probably were, in part, the result of one additional time replication which increased the statistical precision of the evaluation. Freed (23) and Leaper and Bishop (47) demonstrated differences in growth regulator activity in the phenoxy series with 2-chlorophenoxyacetic acid and 3,4-dichlorophenoxyacetic acid being milder in formative effects than 2,4-dichlorophenoxyacetic acid. In this work (Table 15, column 1) they were nearly the same as to lesion suppression. More extensive conclusions concerning the role of substituents on the ring and side chain await further investigation.

<u>The effect of various growth regulator concentrations on tobacco ringspot virus lesion production in *Vigna sinensis*</u>. In the previous experiments it was mentioned that certain unaccounted variations of the inoculated plots were observed. In an effort to determine the

significance of these variations on the apparent inhibitory and stimulatory ranges of the compound, it was considered necessary to make an additional test with modification of the procedure. The number of growth regulator concentrations was increased and the dilution extended. Additional virus treatments were included to assist in the delineation of a control trend that would follow changes in the environment more closely.

An improved selection of the "S" cultivar of *Vigna sinensis* was used as an assay plant. The seed used was a bulk population derived from the second selection out of the original specially selected line. The improved type gave more uniform tobacco ringspot virus local lesions and was also of more uniform leaf size and character.

Growth regulator solutions and water sprays were applied sequentially beginning with the 1×10^{-12} concentration through the 5×10^{-3} *M* concentration. Inoculated plots (sprayed with water only) were placed in the series after each two growth regulator treated inoculated plots and at the beginning and end of the experiment. All plants were inoculated as nearly as possible to one hour after they had received the growth regulator spray. Care was taken to reduce inactivation of the growth regulator solutions and the virus inoculum by using low temperature ice baths for the solutions. Inoculum of 1:200 dilution was used in this experiment to assure readily counted numbers of lesions on the leaves.

Experimental data are presented in Table 16 and graphically in Figure 6. The vertical axis (Figure 6) represents the average number of lesions for each primary, while the horizontal axis represents decreasing growth regulator concentration as well as increasing time from about 11:55 A.M. through 1:15 P.M.

Plots inoculated with virus but not treated with a growth regulator are indicated as V, while the growth regulator treated, inoculated plots are indicated by a negative exponent, e.g. 5×10^{-3}.

At first glance the control and treatment lines appear inconsistent. Further examination reveals that the variations in control values are generally closely matched with variations in the treatment curve for growth regulator treatments containing less than 7.5×10^{-5} M of 2-chlorophenoxyacetic acid. Over seventeen values on one

Table 16.

The influence of various concentrations of 2-chlorophenoxy-acetic acid on lesion production in an improved selection of the "S" cultivar of _Vigna sinensis_.

Treatment	Time[b]	Lesions[c]	I.Q.[d]
Concentration[a]			
V	2.6	55	
1×10^{-12}	3.9	67	1.22
2.5x	5.2	66	1.06
V	6.5	62	
5.0x	7.8	68	1.10
7.5x	9.1	93	1.25
V	10.4	74	
1×10^{-11}	11.7	56	.76
2.5x	13.0	79	1.13
V	14.3	70	
5.0x	15.6	76	1.09
7.5x	16.9	80	.96
V	18.2	83	
1×10^{-10}	19.5	74	.89
2.5x	20.8	83	1.54
V	22.1	54	
5.0x	23.4	50	.93
7.5x	24.7	82	1.22
V	26.0	67	
1×10^{-9}	27.3	57	.85
2.5x	28.6	72	1.47
V	29.9	49	
5.0x	31.2	45	.92
7.5x	32.5	42	.60
V	33.8	70	
1×10^{-8}	35.1	94	1.34
2.5x	36.4	62	1.09
V	37.7	57	
5.0x	39	56	.98
7.5x	40.3	62	.97

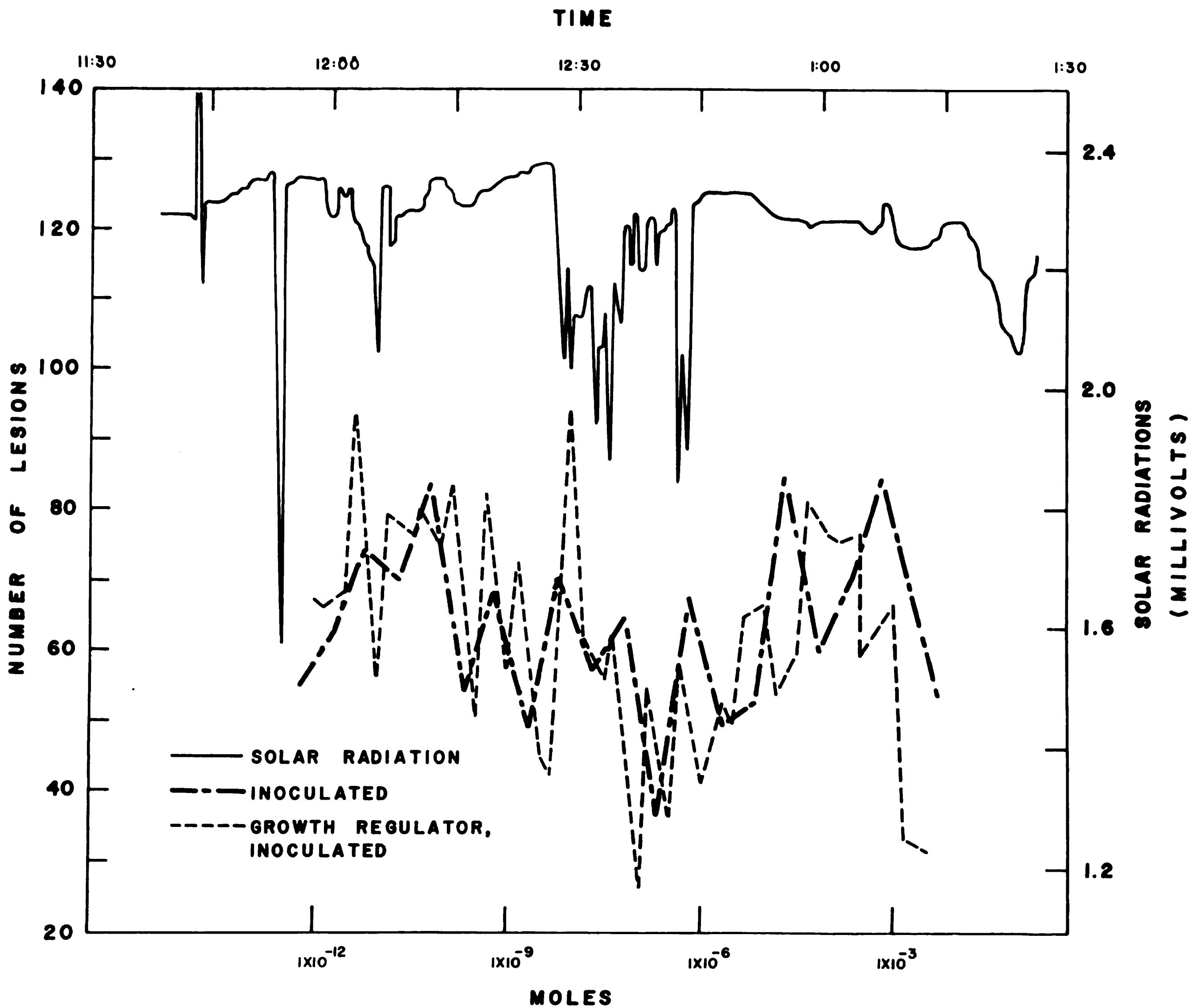
Table 16. Continued

Treatment	Time[b]	Lesions[c]	I.Q.[d]
Concentration[a]			
V	41.6	64	
1×10^{-7}	42.9	26	.41
2.5x	44.2	54	1.50
V	45.5	36	
5.0x	46.8	36	1.00
7.5x	48.1	57	.85
V	49.4	67	
1×10^{-6}	50.7	41	.60
2.5x	52.0	52	1.06
V	53.3	49	
5.0x	54.6	49	1.00
7.5x	55.9	60	1.15
V	57.2	52	
1×10^{-5}	58.5	66	1.27
2.5x	59.8	53	.63
V	61.1	84	
5.0x	62.4	59	.70
7.5x	63.7	81	1.37
V	65.0	59	
1×10^{-4}	66.3	76	1.29
2.5x	67.6	75	1.09
V	68.9	69	
5.0x	70.2	76	1.10
7.5x	71.5	59	.71
V	72.8	83	
1×10^{-3}	74.1	66	.80
2.5x	75.4	33	.51
V	76.7	65	
5.0x	78.0	32	.49
V	79.3	53	

[a]Concentration expressed as Moles per liter of the free acid.
[b]Time of treatment in minutes after initiation of experiment.
[c]Average lesion number per leaf for a plot of ten leaves.
[d]Infection quotient expressed as the computed ratio of a treatment and its nearest virus control (V).

Figure 6.

Relationship between variations in numbers of tobacco ringspot virus lesions on the "S" cultivar of <u>Vigna sinensis</u> treated variously with 2-chlorophenoxyacetic acid and subsequently inoculated with the virus, and variations in total solar energy measured on a horizontal surface one-half mile away. Curve A, inoculated with tobacco ringspot virus; Curve B, treated with 2-chlorophenoxyacetic acid one hour before inoculation with tobacco ringspot virus; and Curve C, total incident solar radiation recorded in millivolts on March 26, 1962.

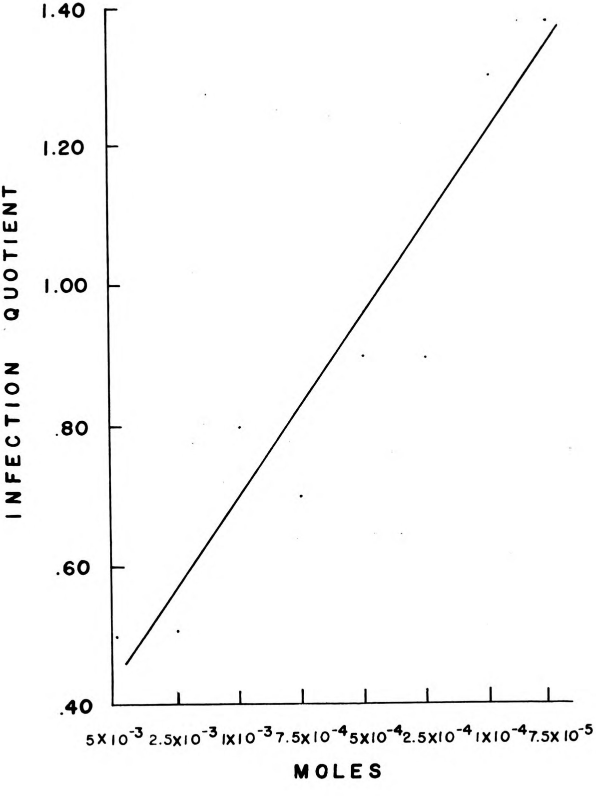

TIME
11:30
12:00
12:30
1:00
1:30
NUMBER OF LESIONS
140
120
100
80
60
40
20
SOLAR RADIATIONS (MILLIVOLTS)
2.4
2.0
1.6
1.2
SOLAR RADIATION
INOCULATED
GROWTH REGULATOR, INOCULATED
1×10^{-12}
1×10^{-9}
1×10^{-6}
1×10^{-3}
MOLES

curve fell very close to the extended lines of the other curve. The portion of the curve from 1 x 10^{-12} to 5 x 10^{-5} M appears to follow the control curve particularly well. The part of the curve from 7.5 x 10^{-5} to 5 x 10^{-4} M is above the control; while the part of the curve from 7.5 x 10^{-4} to 5 x 10^{-3} M is below the curve. The last two ranges represent what are assumed to be stimulatory and inhibitory concentrations. Plants treated with this group of concentrations are also those that show effects usually attributed to growth regulator application. All plants in the experiment, regardless of growth regulator treatment, were systemically invaded by the virus within eight days after inoculation.

The data derived from a conversion of these described values to an infection quotient utilizing the adjacent virus check are plotted (Figure 7). The eight points lie very close to an estimated straight line of best fit. Four of the points lie above and four points below the control line. The coefficient of linear correlation for these eight values, -.802, is significant at the five percent level and almost significant at the one percent level.

Figure 7.

The influence of various concentrations of 2-chlorophenoxyacetic acid on the infection quotient of plants of the "S" cultivar of *Vigna sinensis* inoculated with tobacco ringspot virus.


INFECTION QUOTIENT
1.40
1.20
1.00
.80
.60
.40
5X10^-3
2.5X10^-3
1X10^-3
7.5X10^-4
5X10^-4
2.5X10^-4
1X10^-4
7.5X10^-5
MOLES

When each virus control and its subsequent growth regulator treatment was paired the correlation was +0.501 which is significant at the five percent level (Table 16, Figure 7).

In investigating the effect of wide differences in concentration of phenoxyacetic acid analogs it was noted that in certain cases there appeared to be concentrations which at times resulted in more lesions per plant. The compound 2-chlorophenoxyacetic acid was most consistent and lesion increase appeared at certain concentrations in four experiments.

The data from this experiment give further evidence for an increase in lesion production in plants treated with the concentrations from 5×10^{-4} to 7.5×10^{-5} M. This range coincides with that observed in four of the previous experiments (Table 14, Figure 4) which were made at different times and under other conditions.

The significant inverse relation for the infection quotients lends additional support to the view that there is, in fact, an inhibitory as well as a stimulatory range.

The significant slope of the infection quotients (Figure 7) also means that the smaller values of X falling

falling above the line are different from the larger values falling below the line. If they were not, the slope would not differ significantly from zero.

It is reported that growth regulators of the phenoxy-acetic acid series have inhibitory as well as stimulatory properties in the growth and physiology of roots, leaves, and buds. It is reasonable to believe that such effects might also occur in virus inoculated plants treated with certain growth regulators.

A remarkable coincidence between treatment and control trends was noted. A comparison of this trend with that for total incident solar radiation as measured only one-half mile from the site of the experiment indicates a further coincidence. Major depressions in both occur at nearly the same time.

Variations in the treatment curve containing twice as many points appear to occur at approximately the same time as variations in the control curve. Graphical presentation and correlation analysis suggest, but by no means prove conclusively, a further effect of the environment or of an environmentally triggered endogenous rhythm.

<u>The influence of growth regulator mixtures on virus lesion production</u>. In an effort to determine the value of more definitive work on the effect of mixtures of certain naturally occurring compounds on plant growth response and plant systems, a trial was initiated with approximately physiological strengths of three compounds. These indicated as molar equivalents per liter were: potassium salt of gibberellin A_3 at 1.87×10^{-5}; 6-furfurylaminopurine (kinetin) at 4.65×10^{-5}; and indoleacetic acid at 5.71×10^{-5}.

Uniform plots containing four to five plants of the "S" cultivar of <u>Vigna sinensis</u> (eight of ten primaries) were prepared for treatment. The compounds were applied as a single mixture either before, with, or after the virus inoculation. The time interval was either one or three hours in each case. The simultaneous application consisted of a mixture of the inoculum and three growth regulators. Inoculum and growth regulator mixtures were stored in the freezer or in the refrigerator where necessary to preserve their activity during the time of the experiment.

The results of a prior, subsequent, and simultaneous application of kinetin-gibberellin-indoleacetic acid in relation to inoculation are presented in Table 17. Statistical comparisons and Wilcoxon test results are also listed. The large number of zeros precludes the use of the usual analysis of variance methods since the normalcy of the data is questionable. The Friedman two-way test indicates significance between treatment means at the one percent level. The Wilcoxon matched pairs signed ranks test demonstrates significant differences between all treatment means at least at the five percent level (Table 17).

Plants receiving an application of the growth regulator mixture before inoculation had more lesions than the control, plants receiving a subsequent application had a lower lesion number, while plants receiving a mixture of growth regulator and virus preparation had no lesions (Table 17).

Since the components of the growth regulator mixture are at approximately physiological strength, it is possible that the general physiology of the plant has been stimulated by prior application of the growth regulator mixture with the result that more substrate, more energy, etc. are available for virus utilization. It is further possible that the kinetin is utilized as an additional source of purine base considered necessary in virus reduplication.

Table 17.

Effect of various methods of application of a mixture of kinetin (4.65×10^{-5} $\underline{M}$), indoleacetic acid (5.71×10^{-5} $\underline{M}$), and gibberellin A_3 (1.87×10^{-5} $\underline{M}$) on the number of tobacco ringspot virus lesions in the "S" cultivar of *Vigna sinensis*.

	Treatment[a]			
	M lesion	VK lesion	V lesion	KV lesion
Mean*	0	21.9	31.4	42.1

[a]M = mixture of inoculum and growth regulator solution

VK = inoculated one to three hours before treatment

V = inoculated only

KV = growth regulator applied one to three hours before inoculations

*All means differ significantly

The role of auxin in speeding protoplasmic streaming in the cell at this concentration should not be neglected, since lesions normally remaining small in size might become more pronounced as a result of the movement of the virus to an area of the cell containing entry sites to an adjacent cell. Auxin effect on permeability of cells and levels of ascorbic acid may enter the picture since lesions are believed to be the consequences of alterations in certain polyphenoloxidase systems in the plant.

The subsequent application of the compound mixture may have reduced entry or the subsequent multiplication of the virus. Certain components of the compound mixture may have substituted for native substrates in the oxidase systems of the plant and thus interfered with the usual sequences leading to lesion formation.

The nature of the inhibition involved in the combined mixture of kinetin, indoleacetic acid, and gibberellic acid and virus preparation remains to be demonstrated. It is reasonable to believe that the time the inoculum was held in the experimental period did not influence the results nor did the dilution, since comparable inoculated controls behaved normally.

<u>The effect of (2-bromoethyl) trimethylammonium bromide on tobacco ringspot virus lesion production in *Vigna sinensis*</u>. To broaden the scope of the investigation and to determine if other growth regulating structures might have a more subtle effect on the host-virus interaction sixteen additional compounds with representative structures were given preliminary evaluation. One of these, (2-bromoethyl) trimethylammonium bromide, was selected for further evaluation.

Plots consisting of six or seven plants of uniform size of the "S" cultivar of *Vigna sinensis* were employed. All inoculations were made in mid-morning and chemical treatments applied at intervals before, simultaneously with, or after inoculation. Treatment solutions contained either 10^{-2}, 10^{-3} or 10^{-4} *M* of (2-bromoethyl) trimethylammonium bromide.

It was observed that inoculated plants treated with this compound had fewer lesions in some cases than the inoculated controls (Table 18). Results of the applications of the growth regulator as a mixture with the inoculum were not consistent. When applied before inoculation and in daylight, lesion production was increased with increasing concentration of the chemical; whereas, when applied

Table 18.

Influence of (2-bromoethyl)trimethylammonium bromide on development of tobacco ringspot virus lesions in inoculated plants of the "S" cultivar of *Vigna sinensis*.

Time of treatment - Hours before/after inoculation	Treatment concentration M	Local lesions experiment 1	2	3
		Mean	Mean	Mean
12 before (dark)	5×10^{-2}	7.7		
	5×10^{-3}	8.2		
	5×10^{-4}	7.8		
5 1/2 before	5×10^{-2}		84.3	
	5×10^{-3}		72.0	
	5×10^{-4}		56.2	
1 before	5×10^{-2}	9.2	148.3	
	5×10^{-3}	5.6	100.7	
	5×10^{-4}	1.9	87.5	
Virus only	None	8.6[a]	107.0[a]	10.2[a]
Simultaneous	5×10^{-2}	4.2	98.6	
	5×10^{-3}	2.2	121.3	
	5×10^{-4}	2.9	104.2	
1 after	5×10^{-3}			3.7
	5×10^{-4}			10.2
12 after (dark)	5×10^{-2}	4.1		
	5×10^{-3}	4.1		
	5×10^{-4}	4.9		
25 after	5×10^{-2}		108.8	
	5×10^{-3}		115.8	
	5×10^{-4}		134.4	

[a]Single control value in Experiment 1; mean of two values in Experiments 2 and 3.

after inoculation, lesion numbers increased with the decreasing of concentration (Figure 8). These relationships were most striking in experiment 1 (Table 18) one hour before inoculation; experiment 2 at five and one-half hours before, one hour before and 25 hours after inoculation; and in experiment 3 where treatment was only applied one hour after inoculation.

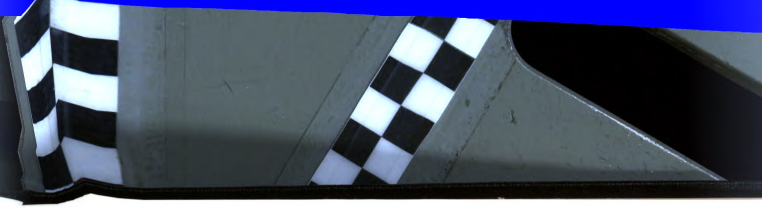
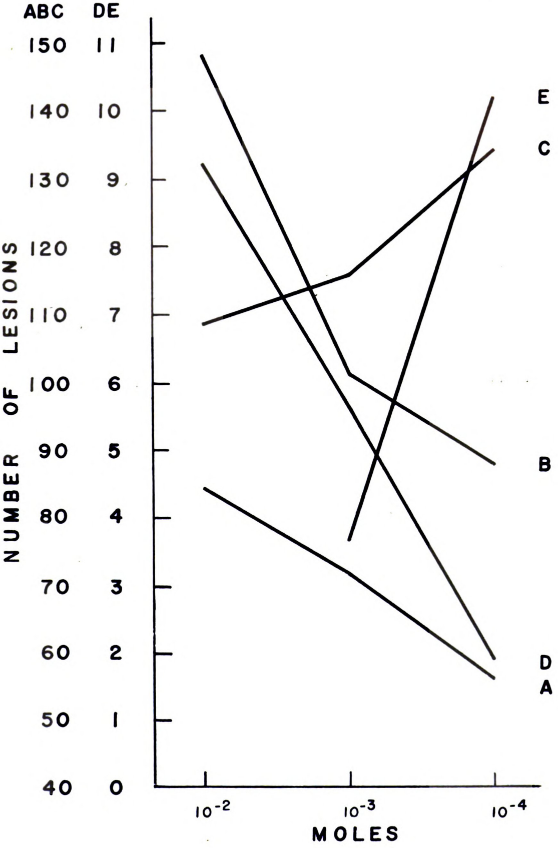


Figure 8.

The influence of (2-bromoethyl)trimethylammonium bromide on development of tobacco ringspot virus lesions in inoculated plants of the "S" cultivar of _Vigna sinensis_. Curve A, treated five and one-half hours before inoculation--experiment 2; Curve B, treated one hour before inoculation--experiment 2; Curve C, treated twenty-five hours after inoculation--experiment 2; Curve D, treated one hour before inoculation--experiment 1; and Curve E, treated one hour after inoculation--experiment 3.



ABC DE
150 11
140 10
130 9
120 8
110 7
100 6
90 5
80 4
70 3
60 2
50 1
40 0
NUMBER OF LESIONS
E
C
B
D
A
10^{-2}
10^{-3}
10^{-4}
MOLES

GENERAL DISCUSSION

The effects of moderately high concentrations of 2,4-dichlorophenoxyacetic acid in reducing the ability to recover virus or in reducing the amount of virus in plant tissue may have been the result of interference in virus coordinated processes or to certain other physical or physiological imbalances. The purpose of this discussion is to emphasize certain of these features which may be responsible. These possibilities await confirmation from more truly definitive work.

It has been noted that plants treated with 2,4-dichlorophenoxyacetic acid have inhibition of respiration or a reduction in the amount of energy available for physiological processes (93). Treated plants in many cases have reduced photosynthesis (55). Treated plants have lower rates of glycollic acid and of ascorbic acid oxidation resulting in an increase in these compounds (65) at regulator application levels approximating those in the present study.

The compound 2,4-dichlorophenoxyacetic acid may also interfere in energy and its distribution through a reduction

of phosphorylation (68). It has been noted by other investigators that catalase levels (87) and peroxidases are increased (73). Bean stems (69) and apple leaf tissue (73) treated with 2,4-dichlorophenoxyacetic acid have decreased amylase activity. It is possible that these changes may have a pronounced effect on virus multiplication.

Freed et al. (23) reports that the 2,4-dichlorophenoxyacetic acid effect on the energy associated mitochondrial system is simply a result of its being adsorbed on the surface of the mitochondrial particle. If the compound is, in fact, adsorbed on the particle surface, then these sites may no longer be available for energy transfer functions.

It was mentioned earlier that ascorbic acid increases are apparent in 2,4-dichlorophenoxyacetic acid treated tissues. Tonzig and Marré (93) indicate that its increase inhibits growth rate through the increase of dehydroascorbic acid which is the actual growth inhibitor. There is an associated reduction in respiration and an increase in the glutathione oxidation-reduction ratio. Ascorbic acid has been reported to be involved with auxin metabolism and may also cause significant variation in this way as well.

A further way in which 2,4-dichlorophenoxyacetic acid may interfere with virus reduplication is by its effect on enzyme systems. The compound reduces important co-factors such as thiamin, riboflavin, nicotinic acid, and the pigment carotene (57).

Some investigators have suggested a relationship between virus multiplication and maturity of the plant (104). Osborne and Hollaway (72) claimed that accumulation of carbon containing materials by the plant in the growth regulator treated areas serves to delay aging. High molecular weight polysaccharides are reduced in amount with a concomitant increase in simple sugars such as monoses (34). Audus (4) reported that plants treated with growth regulators appear to have accelerated protoplasmic streaming either as a result of lowered viscosity or of the injection of energy into an energy requiring system or by an energy regulating property of the auxin. These alterations may influence virus multiplication and movement.

There is some evidence to show that a reduction of auxin occurs in plants as the flowering stage as approached. This seems logical since at this stage compared with earlier one we would expect to find a smaller percentage of meristematic areas which generally seem to be associated with sites of high auxin activity. This may explain the increase in virus in older plants treated with growth regulator (31).

The virus nucleoprotein requires certain essential building blocks such as the elements nitrogen, phosphorus, and sulfur, the amino acids, and more complex units such as guanine and other nucleic acid bases. It is possible that a disruption in these systems or the substitution of an analog via growth regulator treatment will affect virus reduplication at some critical stage.

Rebstock et al. (75) demonstrated that 2,4-dichlorophenoxyacetic acid treated plants had less total phosphorus, acid and alcohol soluble phosphorus, and nucleic acid phosphorus in the leaf. Reduced movement of phosphorus to the leaves of treated plants has been reported (21). Satarova (79), on the other hand, reported that 2,4-dichlorophenoxyacetic acid treated plants had more ribonucleic acid in the meristems. Smith (82) reported that nitrogen is mobilized to the meristematic areas. If the net effect of the ribonucleic acid increase and the nitrogen translocation would be to siphon off substrate from areas of the plant where virus has been introduced, such as the leaf, then we might expect that virus reduplication would be decreased. If the virus were present in the areas receiving the additional substrates, however, the reverse might well be true.

Faludi and Daniel (20) showed that plants treated with 2,4-dichlorophenoxyacetic acid had a general decrease in amino acids. Glutamine, glutamic acid, glycine, threonine, and tryptophane were drastically reduced. Leucine completely disappeared. Arginine, asparagine, aspartic acid, alanine, proline, methionine, and valine were reduced slightly. These authors concluded that the decrease in amino acids was probably due to an increase in de-amination rather than the inhibition of transamination. We may conclude that for normal virus multiplication rates to persist, a variety of essential substrates such as amino acids is necessary. Reduction in the quantity of certain essential ones would reduce virus multiplication.

The data for the experiment with 2-chlorophenoxyacetic acid indicate (Table 16) that a third factor, presumably environmental in nature, may well be involved. An endogenous factor triggered by environmental variations such as light may operate. There was some evidence of the influence of total incident solar radiation in this experiment.

At this point it is apparent that the growth regulator effects on the host parasite relationships are very complex. The relationship with the environment may be far more critical than previously anticipated.

The present investigations (Table 18, Figure 8) confirm, in part, the results obtained by Rawlins (74) in that the quantity of a virus may be reduced in plants treated with one of the halogenated choline compounds. The results also indicate that time of application may be critical in lesion suppression.

Tobacco ringspot virus contains an abnormally high phosphorus content (88). Treatments creating disturbances in phosphorus metabolism could alter the activity of the virus. Tolbert (92) reported that as much as thirty percent of phosphorus in roots occurred as phosphoryl choline. It is interesting to speculate that (2-bromoethyl)trimethylammonium bromide may alter lesion production through disruption of phosphorus metabolism. Reasons for the apparent reversal of the concentration curves are not well understood and more extensive investigations on the phenomenon should be made.

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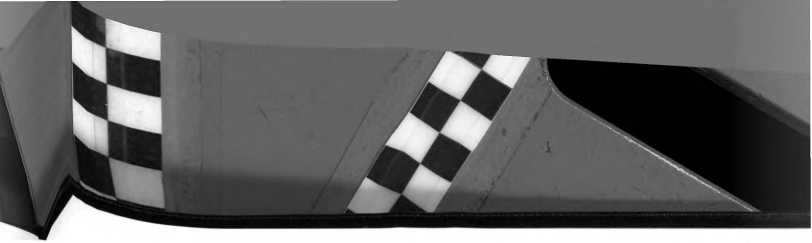

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