

Utah State University

DigitalCommons@USU

All Graduate Theses and Dissertations

Graduate Studies

5-1974

Anatomical Effects of Dicamba on Pea Root Tissues

Brent George Ovard
Utah State University

Follow this and additional works at: <https://digitalcommons.usu.edu/etd>

 Part of the [Plant Sciences Commons](#)

Recommended Citation

Ovard, Brent George, "Anatomical Effects of Dicamba on Pea Root Tissues" (1974). *All Graduate Theses and Dissertations*. 3514.

<https://digitalcommons.usu.edu/etd/3514>

This Thesis is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.



ANATOMICAL EFFECTS OF DICAMBA

ON PEA ROOT TISSUES

by

Brent George Ovard

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1974

ACKNOWLEDGMENTS

I am grateful for the opportunity to have attended Utah State University.

I especially appreciated Dr. J. LaMar Anderson, under whose direction this study was conducted.

Special thanks is extended to Dr. William Campbell for his advice and words of wisdom and to Dr. Colburn Williams for his helpful suggestions.

I would also like to thank my father-in-law, Paul C. Glissmeyer, for his help in duplication of the photos.

Grateful appreciation is extended also to my dad, Joseph W. Ovard, (now deceased), and to my mom, Retta F. Ovard, for their encouragement to attend college.

Finally, to my wife, Cheryl, for her support and patience, I extend my gratitude.

Brent George Ovard

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENT	ii
LIST OF FIGURES	iv
LIST OF APPENDIXES	v
ABSTRACT	vi
INTRODUCTION	1
REVIEW OF LITERATURE	2
MATERIALS AND METHODS	5
RESULTS AND DISCUSSION	7
SUMMARY AND CONCLUSION	44
LITERATURE CITED	46
APPENDIXES	49
VITA	51

LIST OF FIGURES

FIGURE	Page
1. Pea root elongation following dicamba treatment	8
2. Nuclear volume of untreated pea root meristem cells	10
3. Nuclear volume of pea root meristem cells	11
4. Nuclear volume of pea root meristem cells	14
5. Cross-section of pea root 1 mm from tip 0.5 ppm dicamba - 24 hrs. after treatment	16
6. Cross-section of untreated pea root 1 mm from tip 24 hrs. after time of treatment	18
7. Diameter of root tip 1 mm from root end	20
8. Diameter of pea root tip as measured by number of cells 1 mm above root end	21
9. Cross-section of pea root 1 mm from tip 0.5 ppm dicamba - 48 hrs after treatment	22
10. Cross-section of untreated pea root 1 mm from tip 48 hrs. after time of treatment	24
11. Cross-section of pea root 1 mm from tip 0.5 ppm dicamba - 72 hrs. after treatment	27
12. Cross-section of untreated pea root 1 mm from tip 72 hrs. after time of treatment	29
13. Cross-section of pea root 3 mm from tip 0.1 ppm dicamba - 24 hrs. after treatment	31
14. Cross-section of untreated pea root 3 mm from tip 24 hrs. after time of treatment	33
15. Cross-section of pea root 3 mm from tip 0.1 ppm dicamba - 48 hrs. after treatment	36
16. Cross-section of untreated pea root 3 mm from tip 48 hrs. after time of treatment	38
17. Cross-section of pea root 3 mm from tip 0.1 ppm dicamba - 72 hrs. after treatment	40
18. Cross-section of untreated pea root 3 mm from tip 72 hrs. after time of treatment	42

LIST OF APPENDIXES

Appendix	Page
A. Pea root elongation determinations	49
B. Pea root nuclear volume measurements of meristematic regions.	50

ABSTRACT

Anatomical Effects of Dicamba on Pea Root Tissues

by

Brent George Ovard, Master of Science

Utah State University, 1974

Major Professor: Dr. J. LaMar Anderson
Department: Plant Science

Peas (Pisum sativum L. var Alaska) were allowed to absorb calcium and magnesium chloride for 8 hours and then were germinated in a potassium phosphate buffer pH 6.5 for 40 hours. Peas were then treated with 0, 0.1, 0.3, or 0.5 ppm dicamba (3,6-dichloro-o-anisic acid) and harvested at 24, 48, and 72 hour intervals. The following determinations were recorded: root elongation, nuclear volume, and anatomical modifications.

Root elongation recordings showed that dicamba prevented normal root elongation. Treatments induced very short thick primary roots.

Measurements of nuclear volume indicated that all herbicide concentrations were able to reduce the total volume. Several other compounds, (chloramphenicol, actinomycin D and colchicine), were introduced to better characterize the actions of dicamba. Chloramphenicol and colchicine were responsible for nuclear volume reductions.

Dicamba induced major anatomical alterations of treated pea roots. In the region 1 millimeter from the root tip, cortical cells were induced to divide more profusely. The diameter of treated roots exceeded that of untreated root tips.

In the region 3 millimeters from the root tip, excessive cellular division and swelling resulted in cortical damage.

(51 pages)

INTRODUCTION

A group of synthetic weed killers commonly known as the "auxin herbicides" have intrigued researchers since the initial development of 2,4-D [(2,4-dichlorophenoxy)acetic acid]. The selectivity of these herbicides varies but generalities do exist.

One of the later developed auxin herbicides, dicamba (3,6-dichloro-o-anisic acid), has greater selectivity, persists in the soil longer, and is more effective against several perennial weeds than 2,4-D. The success of dicamba may be attributed to either its mobility in the plant's vascular system or to the inability of sensitive plants to detoxify it. Recent studies support the contention that toxic effects are a result of unmetabolized herbicide localized in the key areas in the plant (31). Twenty-four hours after treatment, the highest concentration was localized in an area 0-3 millimeters from the root tip.

This research was designed to study in detail the anatomical and morphological effects of the herbicide dicamba on pea (Pisum sativum L.) root tissue. The area of study was limited to the root tip which has been shown to contain the highest concentration of herbicide after initial treatment.

REVIEW OF LITERATURE

Dicamba persists longer in both loam and sandy loam soils than 2,4-D (10). It moves readily in the soil. When the herbicide is mixed with water and added to the soil, the chemical follows slightly behind the front of the water as it moves through the soil (10, 15). The persistence and detoxification of dicamba is influenced by soil type, soil temperatures (5, 14), activity of micro-organisms (10), and acidity of soil (14).

Chemical residues of dicamba found in soils have prevented new growth from rhizomes of quackgrass and controlled growth more effectively than foliar sprays (36).

Mobility of dicamba within plant tissues enables dicamba to control many perennial weeds. When applied to the leaves of Canada thistle, the herbicide was translocated to other points within the plant. The greatest concentration as observed by injury symptoms occurred within the meristematic regions. Safflower seedlings (Carthamus tinctorius L.) as a biological assay indicated the presence of dicamba in the soil. Canada thistle roots evidently exuded small amounts of herbicide into the soil. Dicamba was thought to be translocated in both xylem and phloem (8). Other researchers (17, 22) have noted that the mobility of dicamba is influenced by concentration, temperature and sensitivity of the plant.

¹⁴C-labeled dicamba was transported via the symplast when applied to a mature leaf of Johnsongrass [Sorghum halapense (L.) Pers.] or a primary leaf of a bean (Phaseolus vulgaris L.). Leakage of the herbicide from the phloem resulted in a uniform distribution of the radioactive

label to all leaves above the site of application. Basipetal transport was restricted in both plants. Application applied to the roots resulted in slow translocation to the shoots (17). When the herbicide was applied to the leaves of purple nutsedge, both acropetal and basipetal movement occurred. The greatest accumulations were found in the meristematic regions (22).

Bluegrass and wheat treated with dicamba yielded both a major and minor metabolite. The major metabolite which constituted 90% of total metabolic products was 5-hydroxy 2-methoxy 3,6-dichlorobenzoic acid. The minor metabolite was 3,6-dichloro-salicylic acid (4).

Conversions from benzoic acid to salicylic acid and Beta-d-glucoside have been discovered in potatoes and peas which are very sensitive in dicamba (21). However, the amount of dicamba the plant can convert to metabolites and the ability to immobilize the product seems to differ between tolerant and sensitive plants. Application of dicamba to the leaves of corn (Zea mays L.) resulted in little movement from the point of application (6).

A reduction of root elongation has been shown in soybean (Glycine max L.) corn (6), and pea seedlings (31) when germinated in varying concentrations of dicamba. These observations were very similar to the early results with 2,4-D (39).

While root elongation was inhibited, radial enlargement occurred because of the unmetabolized herbicide at the point of activity. Lateral root initiation was greatly enhanced (31).

Cereal crops, wheat (Triticum aestivum L.) and barley (Hordeum vulgare L.), sprayed with dicamba produced irregular growth. Seedlings

germinated in 1, 5, 10, and 100 ppm dicamba resulted in abnormal chromosome clumping. This suggested that normal spindle fiber behavior had been interrupted. The reduction of dividing cells in treated root tip smears was further evidence of inhibition of mitosis by dicamba (11).

Weeds sprayed in wheat and barley should be treated with dicamba at an earlier stage of growth than what is recommended for 2,4-D to avoid herbicide damage. Treatment prior to the 3-leaf stage introduced bending of the internodes of the main culms. At the 4-leaf stage, tiller, floret, and kernel development were affected. Barley was more sensitive than wheat to all treatments (11).

Growth regulating characteristics of dicamba have been mentioned (6, 31). These were initially reported, however, for benzoic acids and aldehydes as early as 1950 (2, 23, 24, 41). Promotion of growth as measured by the *Avena* coleoptile test was reported with 2,3,6-trichlorobenzoic-aldehyde (2). Cell elongation and tissue proliferation were initiated with 2,3,6-trichlorobenzoic acid (41). Other substitutions on the heterocyclic were responsible for root swelling, leaf curvatures and modifications, galls (24) and inhibition of growth in bean seedlings (23). These responses were similar in many respects to those induced by the phenoxy herbicides (3, 9, 24, 32, 34, 38). The benzoic acids were later included in the group of herbicides exhibiting auxin-like activities (1, 16).

MATERIALS & METHODS

Alaska pea seeds (Pisum sativum L.) were surface sterilized for 10 minutes in a 10% solution of sodium hypochlorite, rinsed in distilled water, and placed on germinating paper (Kimpac) moistened with one millimole of calcium chloride and two millimoles magnesium chloride. After 8 hours they were then transferred to paper that had previously been moistened with 0.7 M pH 6.5 potassium phosphate buffer. Forty hours later seeds were placed in various herbicide concentrations. The process from surface sterilization until treatment required 48 hours and will be referred to as the "conditioning period."

Root elongations: Following the 48-hour conditioning period peas were placed in containers which had germinating paper moistened with 0, 0.1, 0.3, 0.5 ppm dicamba. Thirty peas were removed from each concentration 24, 48, and 72 hours after treatment and root lengths were measured.

Nuclear volume: Peas that had gone through the conditioning period were placed in the following treatment solutions: actinomycin D, 10 micrograms/milliliter; chloramphenicol, 2 mg/ml; colchicine, 2% solution; and dicamba at 0, 0.1, 0.3, and 0.5 ppm. Three peas were removed from each compound at 24-hour intervals up to 72 hours. The root tips were excised one centimeter from the tip and fixed in Formalin-acetic acid. Following fixation they were dehydrated in a standard tertiary butyl alcohol series and embedded in paraplast. Medium longitudinal sections, 10 microns in thickness, were stained with safranin and fast green (18).

Nuclei in the meristematic region of the root tip were measured with an ocular micrometer in two directions. These two directions were averaged to obtain one diameter for each cell. The volume of each cell was computed using the formula for a sphere, $1/6\pi d^3$. Ten cells were measured from each root tip and their volumes were expressed in cubic microns.

Anatomical studies: Peas were removed from the germinating paper moistened with dicamba 0, 0.1, 0.3, and 0.5 ppm at 24, 48, and 72 hours after the conditioning treatment. The pea roots were excised 1 cm from the tip, fixed and stained. Sections 1 and 3 mm from the root tip were examined. These sections were selected because it had been earlier established that the greatest concentration of dicamba after 24 hours was in the 0 to 3 mm region (31). Diameters of the root tips at 1 mm region were determined measuring with the ocular micrometer in two directions and calculating the average. Three sections were measured from each slide and three separate root tips were used to calculate the average diameter for treatment and time. Actual counts in two directions were made of the cells across the diameter of the 1 mm region. The number of cells were added to obtain an average.

The diameter of the 3 mm region was initially measured and cell counts were taken, but after 24 hours it was determined that cellular breakdown and tearing would influence the accuracy of the data. Therefore the 48 and 72 hour period data were not recorded.

Tissue observations were made and recorded with a Zeiss Photomicroscope.

RESULTS & DISCUSSION

Root elongation: All three concentrations of dicamba, (0.1, 0.3, and 0.5 ppm), were equally effective in preventing root elongation as compared to the control (Figure 1).

The complex process of elongation was inhibited with the initial exposure. Subsequent removal intervals bore out more vividly the effectiveness of dicamba in preventing root elongation. The root elongation process has been studied in detail, but the mechanisms involved are not fully understood at this time. The most generally accepted theory is that elongation is the result of enlargement of new cells that are constantly being formed by cell division in the apical meristem (35).

Messenger-RNA (ribonucleic acid) and protein synthesis are essential for the process of elongation to proceed (19, 20, 28, 37). Formation of these compounds presumably is influenced by auxins. The mere presence of an auxin, however, does not always encourage formation of compounds essential for plant growth. It must be understood that auxins are active in extremely small amounts and may vary chemically in different plant species.

The presence of dicamba in the apical meristem could possibly do the following:

- (1) Prevent natural biological processes;
- (2) Induce the cell to follow a natural degradation process. This would occur after normal growth induced by any auxin;
- (3) Inhibit the chemical process of auxin synthesis.

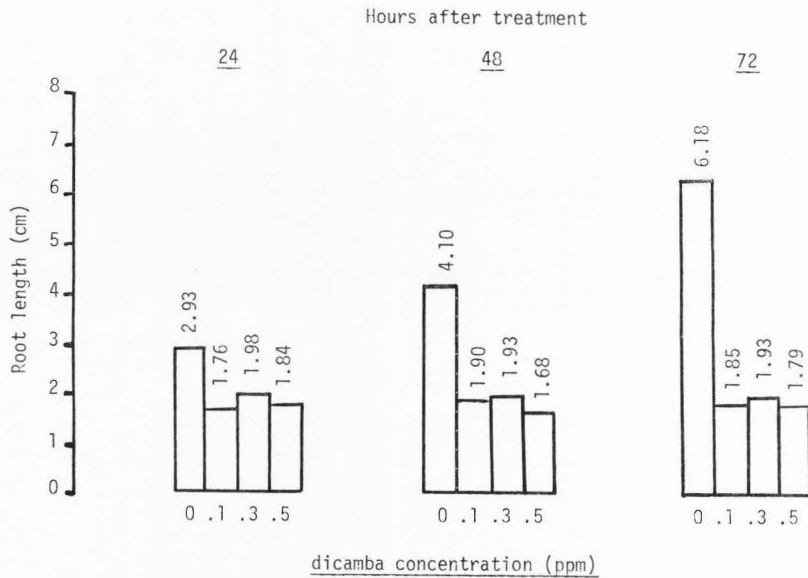


Figure 1. Pea root elongation following dicamba treatment

The exact natures of its herbicidal action may not be known until the natural process of growth is fully understood.

The difference between the length of untreated and dicamba-treated root tips was significant ($P < .01$). However, there was not a significant difference among dicamba treatments (Appendix A). This would indicate that the major difference was between the treated and untreated root tips.

Complete inhibition of root elongation is not a property unique to dicamba alone, but has been documented for other auxin herbicides such as 2,4-D (7, 30, 31, 39, 40) and picloram (31, 40).

Nuclear volume: Nuclear volumes were computed during the first 24 and 48 hour intervals of the conditioning period to determine the activity of the cell. A series of changes occur in the nucleus before cell division is possible. The necessary precursors must be gathered into the nucleus so that the new daughter cells will be similar. Nuclear volumes, therefore, indicate the activity of the cell.

Nuclear volumes recorded (Figure 2) during the 24 and 48 hour intervals of the conditioning period were the largest for the entire experiment. Following herbicide treatment the nuclei in the meristematic area were smaller than those of the control (Figure 3), thus indicating that one or more of the stages of the mitotic cycle had been altered or inhibited.

It has been reported (in 1973) that a relationship exists between nuclear volumes and responses to auxin herbicides (25). In general, susceptible weeds had a smaller nuclear volume than resistant species. Dicamba was one of the auxin herbicides tested.

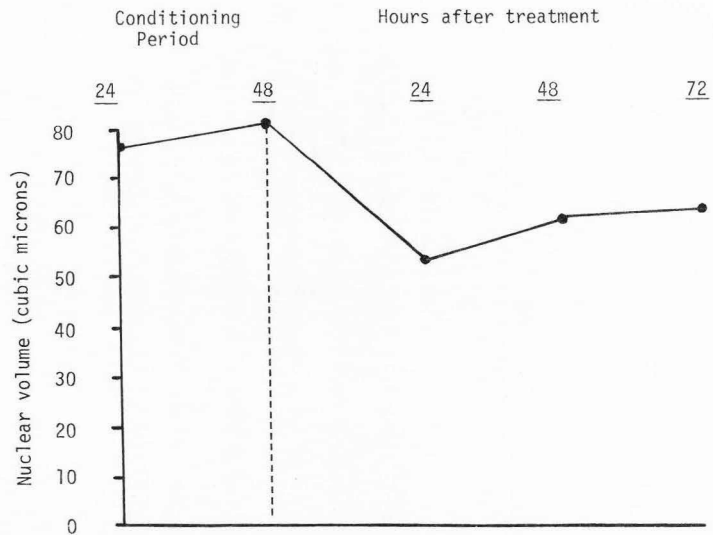


Figure 2. Nuclear volume of untreated pea root meristem cells

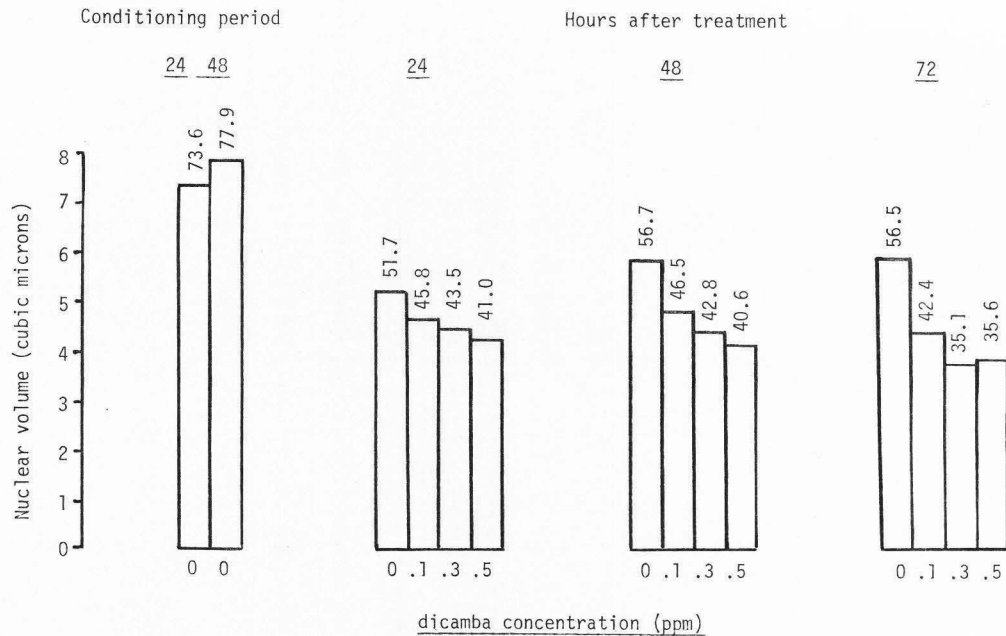


Figure 3. Nuclear volume of pea root meristem cells

Peas are very sensitive to dicamba and all treatments in this study reduced the nuclear volumes of root tip cells as compared with the untreated (Figure 3); however, the results were not significant (Appendix B).

Pea roots, removed 48 and 72 hours after treatment of dicamba, had nuclear volumes which did not vary significantly from those of the 24 hour period. It was concluded that further contact with the herbicide did not vary nuclear volume. The mitotic process most likely was affected immediately following the initial herbicide contact.

Nuclear volumes from meristems of untreated tissues 48 hours after treatment time, however, were greater than the nuclear volumes of treated tissue. This same pattern was also true of the 72-hour level (Figure 3).

A closer examination of the treated means indicated that two means were similar and one mean falling outside the accepted level of Duncan's multiple range test.

Reduction of nuclear volume with dicamba had not been previously reported, but it had been shown that plants with small nuclear volumes are susceptible to auxin herbicides (25). However, a sharp reduction in the number of dividing cells was suggested as the concentration of dicamba increased. There was also evidence of chromosome clumping and formation of multinucleate cells, indicating that normal spindle fiber behavior was upset (11).

An active cell which is rapidly dividing should have a larger nuclear volume than one that is inactive. Dicamba treatment reduced the nuclear volume of the cells in the root meristem. Cell division also ceased or was reduced.

Since nuclear volumes were reduced following herbicide treatment, it was decided to treat the pea roots with known inhibitors of cellular processes. Actinomycin D inhibits root elongation by preventing the synthesis of messenger-RNA (12, 19, 20, 36) and consequently protein (27). Chloramphenicol decreases fresh weight (36) and prevents auxin-induced growth by inhibiting formation of enzymes and specifically the binding of ^{14}C amino acids into proteins (27). Colchicine has been used for many years in plant breeding to induce polyploidy and is referred to as a mitotic poison (28).

Chloramphenicol at 2 mg/ml and a 2% solution of colchicine reduced nuclear volumes at 24, 48, and 72 hours following treatment (Figure 4). The reduced volumes were comparable to dicamba treatment (Figures 3 and 4). Peas treated with actinomycin D at 10 $\mu\text{g}/\text{ml}$, possessed increased nuclear volumes in the meristematic region of their root tips 24 hours after treatment. These volumes were larger than those of untreated tissue, but volumes decreased sharply (less than control) by 48 and 72 hours (Figure 4). The delayed reaction of actinomycin D was unexplainable.

The fact still remains that these chemicals were responsible for nuclear reductions very similar to the dicamba treatment. The possibility exists that dicamba may have effected some of the same chemical processes within the cell as standard growth inhibitors. These processes may be the prevention of protein formation, as caused by chloramphenicol (25, 26, 29), or the inhibition of RNA, as induced by actinomycin D (12, 19, 20, 27, 36).

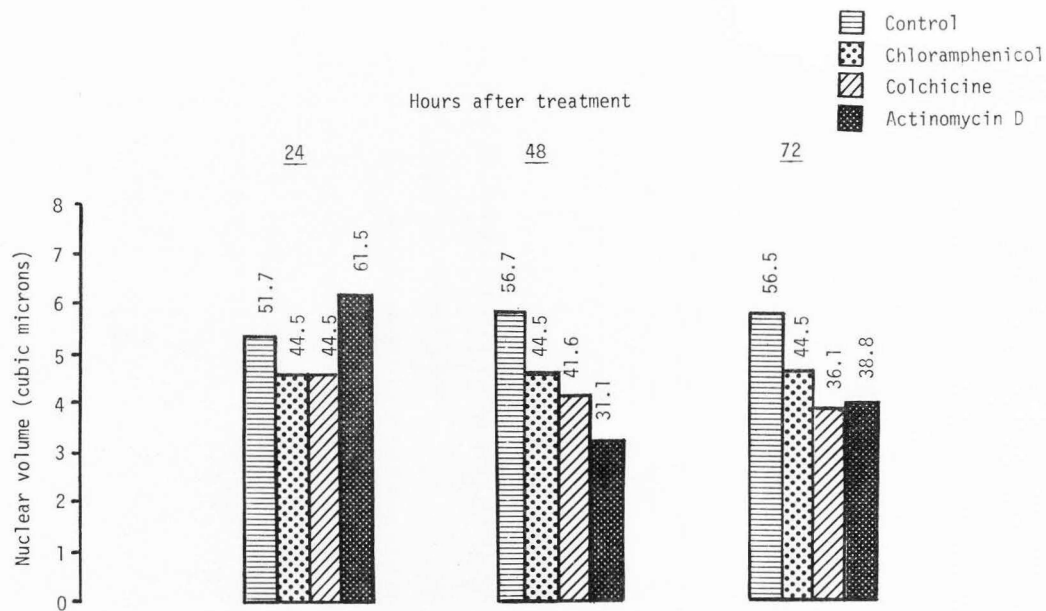


Figure 4. Nuclear volume of pea root meristem cells

Anatomical studies: In the root elongation study, all three concentrations of dicamba were effective in arresting root elongation or cell expansion in the vertical plane. Cross sections made one millimeter from the root tip show that roots treated with 0.1, 0.3, and 0.5 ppm dicamba concentrations exhibited the same degree of growth; consequently, only those root tips from the 0.5 ppm rate will be discussed in comparison with control.

Twenty-four hours following treatment, little, if any, differences were observed either in the cortical or vascular regions of the photomicrographs when compared to the control (Figures 5 and 6). Only slight differences were evident following diameter measurements of the 1 mm region (Figure 7). These data would appear to be in agreement with the small root volume changes recorded by Scott and Morris (31). The number of cells recorded across the diameter of the 1 mm region of the root tip also supported the above data (Figure 8).

After 48 hours the cortical parenchyma cells of the treated root tips appeared to have divided both periclinally, and anticlinally (Figures 9 and 10). The cells exhibiting rapid division in both planes resembled tetra cell condition (Figures 9 and 10). The tetrads would result from extremely rapid cell division. New cell walls either were not present or were not very prominent. It appeared at first that there was but a single cell with four nuclei. New activity was not limited to the cortical area. The pericycle region was also very active (Compare Figures 9 and 10).

The root tip in cross-section showed extreme crowding as a result of cell division. The average diameter of this region (88.8 microns compared

Figure 5. Cross-section of pea root 1 mm from tip
0.5 ppm dicamba - 24 hrs. after treatment

A. Cortical region (80x)

B. Steler region (50x)

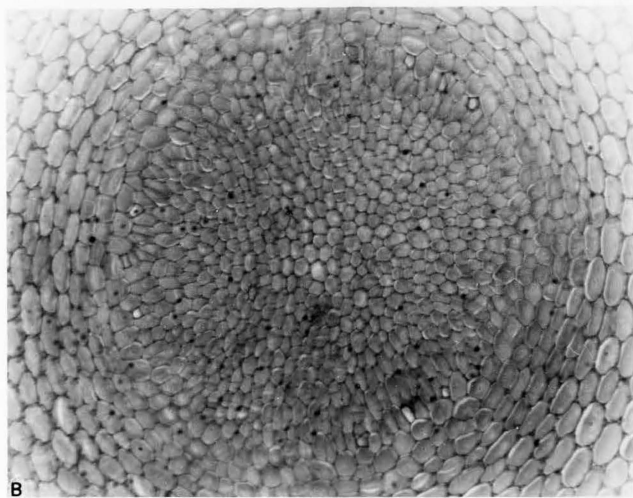
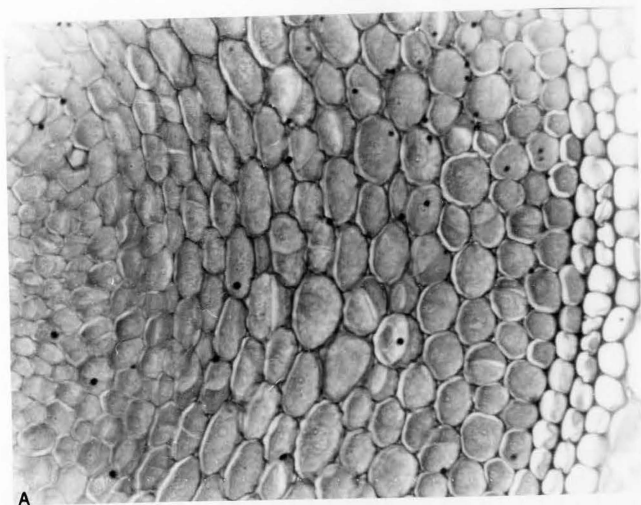
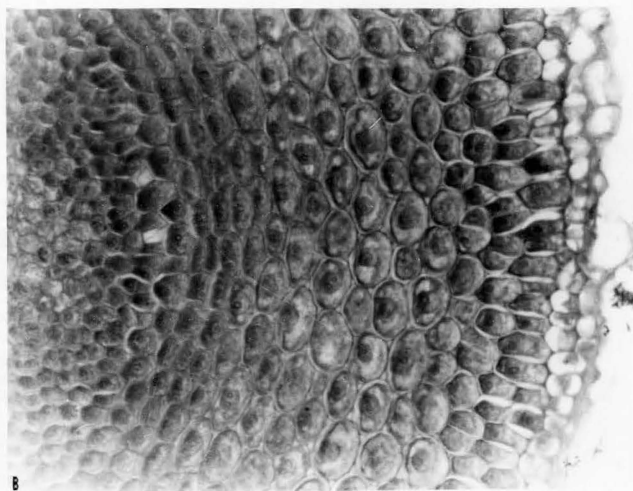
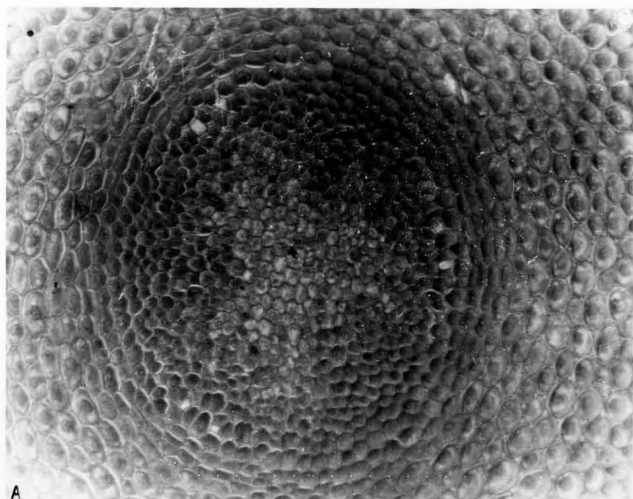


Figure 6. Cross-section of untreated pea root 1 mm from tip
24 hrs. after time of treatment

- A. Cortical region (50x)
- B. Stealer region (80x)



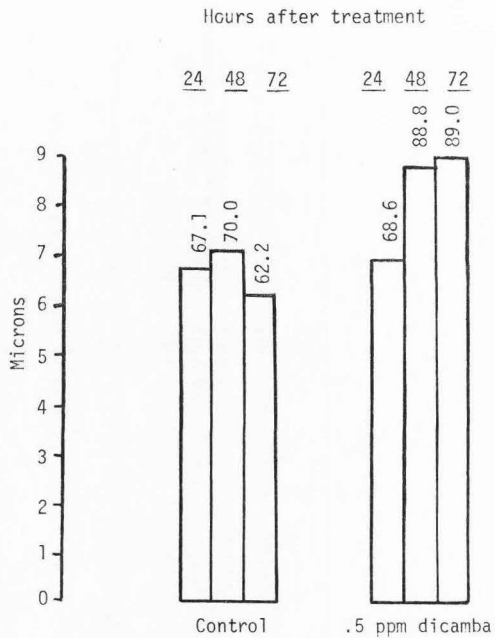


Figure 7. Diameter of root tip one millimeter from root end

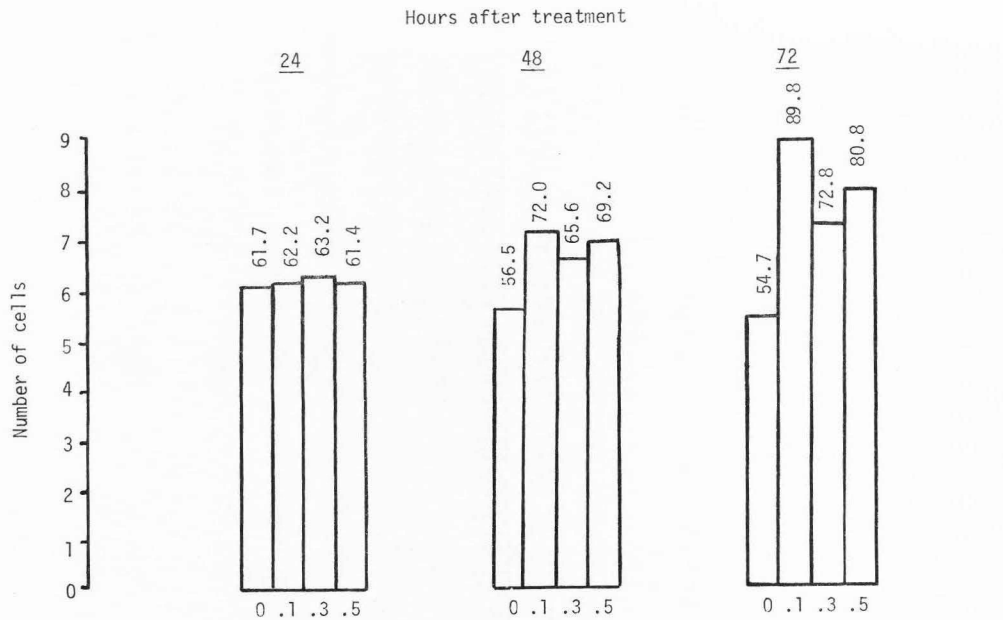


Figure 8. Diameter of pea root tip as measured by number of cells 1 mm above root end

Figure 9. Cross-section of pea root 1 mm from tip
0.5 ppm dicamba - 48 hrs. after treatment

A. Cortical region (80x)

B. Entire region (20x)

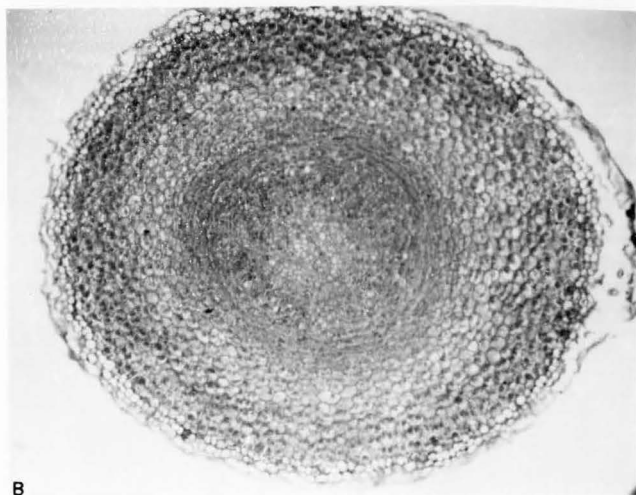
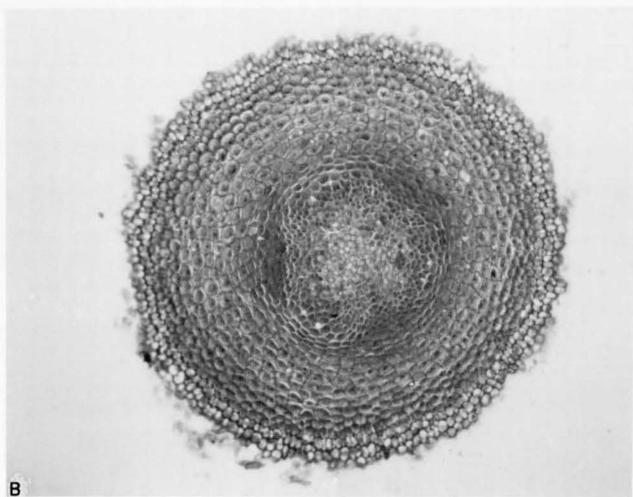
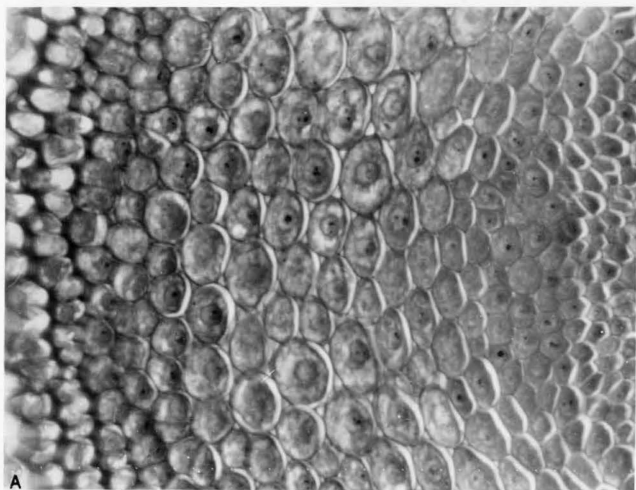


Figure 10. Cross-section of untreated pea root 1 mm from tip
48 hrs. after time of treatment

A. Cortical region (20x)

B. Entire region (7.9x)



to 70.9 microns of control [Figure 7]), indicated crowding and swelling had increased the volume of the root tip, but breakdown of cell walls had not occurred. However, additional data (Figure 8) showed that the increase in diameter was primarily due to new cell formation, (Compare control and treated).

At the conclusion of the experiment 72 hours after treatment in the 1 mm region crowding was still evident as a result of cellular division. The diameter of the root tip had changed little from the 48-hour period (Figure 7), but there appeared to be some breakdown in the walls of the cells near the epidermal region (Figures 11 and 12). New cells were formed (Figure 8) in treated root tips, and contributed to cellular breakdown.

An examination of the region 3 mm from the root tip illustrated that the 3 concentrations, 0.1, 0.3, and 0.5 ppm, were again equal in the response which was produced. The main difference between the 3 mm and 1 mm region was that the 3 mm region showed cellular division 24 hours after treatment. Cellular activity was localized within the triarch regions of the root tip. (Compare Figures 13 and 14). The alterations due to treatment become more evident when comparing the two polarized light photomicrographs. The treated root tip exhibited more secondary wall development and appeared to be much more mature than the control. Vessel elements were also more prominent. As the region matured, more lignin, fibers, and cell walls became evident. The cortical region of treated root tips, however, showed some wall deterioration, but the breakdown was not severe at this stage of development (Figure 13).

Figure 11. Cross-section of pea root 1 mm from tip
0.5 ppm dicamba - 72 hrs. after treatment

A. Cortical region (50x)

B. Entire region (20x)

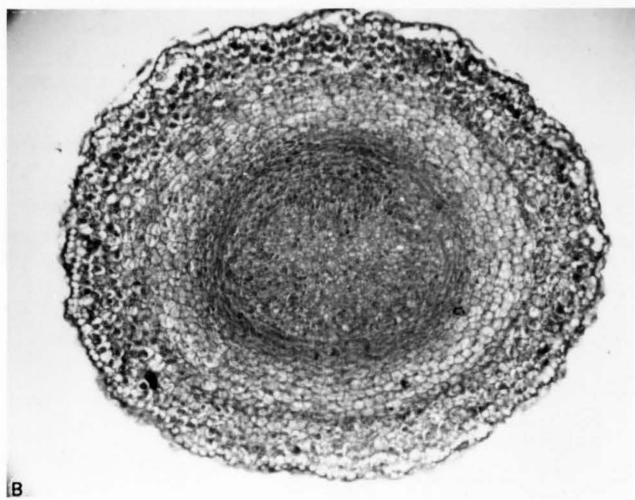
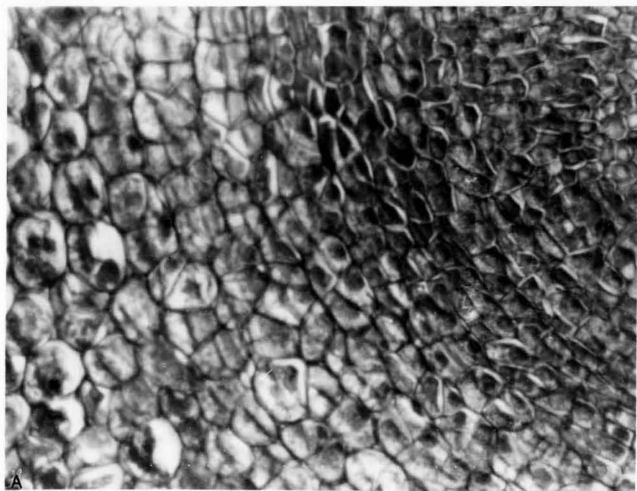
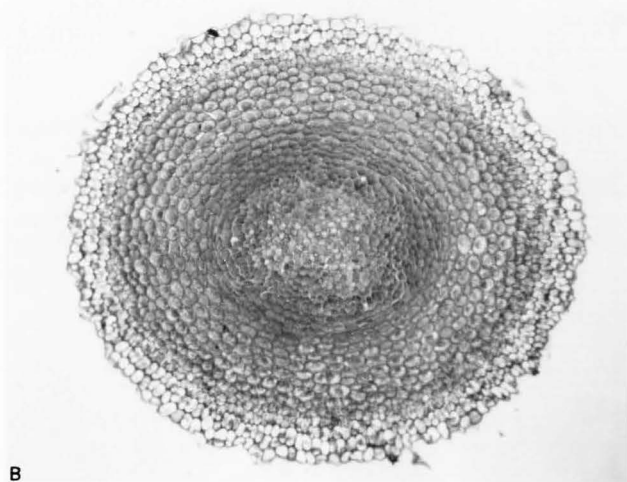
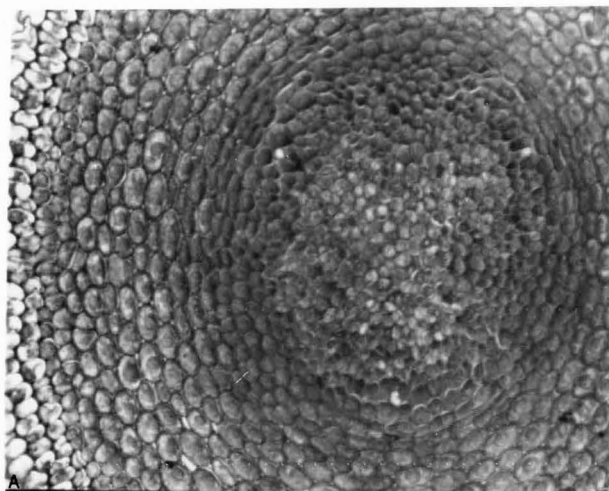


Figure 12. Cross-section of untreated pea root 1 mm from tip
72 hrs. after time of treatment

- A. Cortical region (20x)
- B. Entire region (10x)



B

Figure 13. Cross-section of pea root 3 mm from tip
0.1 ppm dicamba - 24 hrs. after treatment

A. Tri-arch region (25.6x)

B. Polarized tri-arch region (50x)

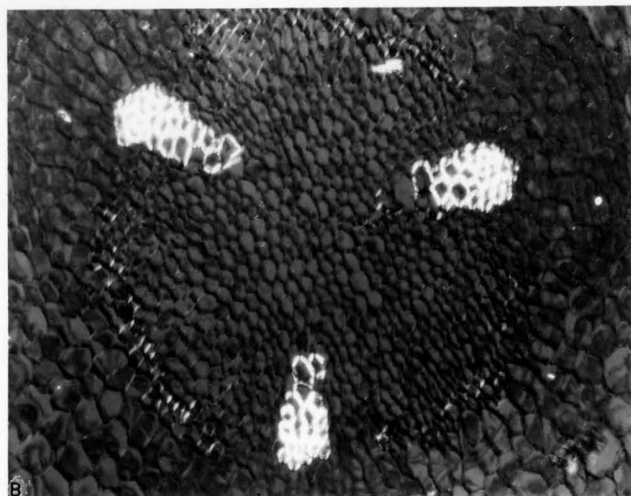
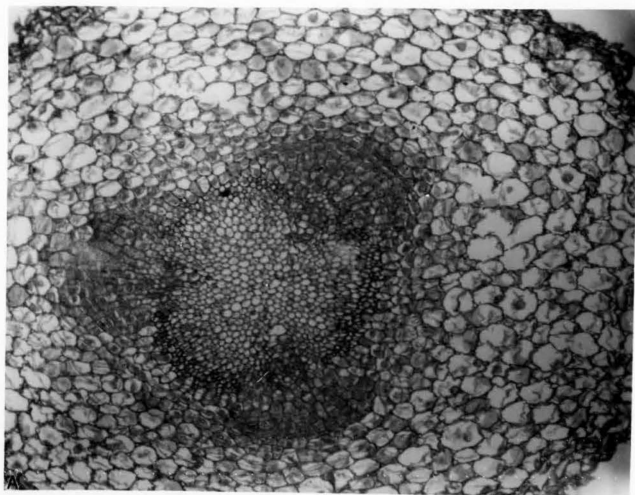
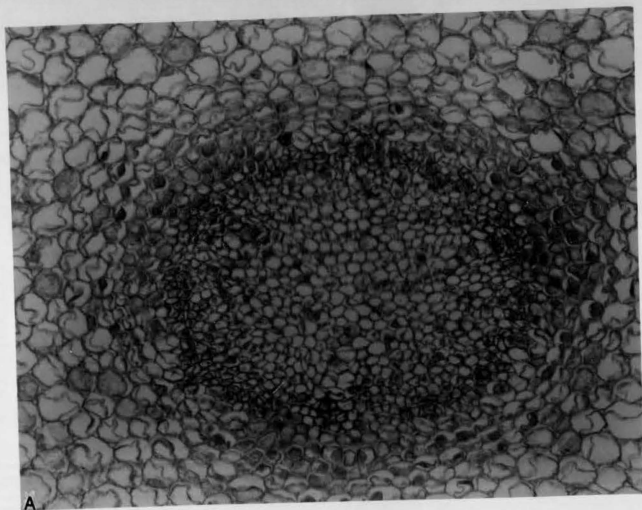


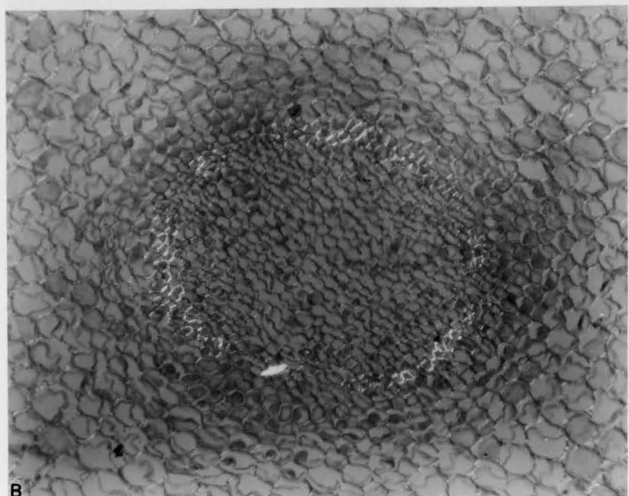
Figure 14. Cross-section of untreated pea root 3 mm from tip
24 hrs. after time of treatment

A. Tri-arch region (50x)

B. Polarized tri-arch region (50x)



A



B

Forty-eight hours following treatment of herbicide the pericycle appeared extremely active. The recently divided cells of the pericycle was possibly being crowded into the cortical area and might have been responsible for breakdown of cell walls in this region. The cortex parenchyma cells had not divided as was apparent in the 1 mm region. The possibility exists that these cells in the 3 mm region were older and no longer had the ability to divide (Figures 15 and 16).

After 72 hours the 3 mm region contained excessive cortical damage (Figures 17 and 18). Most of the cortex parenchyma cells had deteriorated leaving the cells in the stele and the epidermal cells intact. Prominent breaks, however, were observed in the epidermis. These epidermal breaks could be seen at the time of sampling without magnification.

Cellular deterioration could not readily be observed 24 hours after treatment in the 1 mm regions, but did appear in the 3 mm region. Deterioration might have been due to internal pressures which exerted a strong force on adjacent cells. Weaker cell walls might show the results of the stress.

Deterioration might also have been due to the plasma membrane breaking or becoming more soluble. Cell contents would easily be lost. 2,4-D and high concentrations of indole acetic acid (natural plant auxin) have been shown to influence cellular breakdown or deterioration (9, 13, 35). Cellular deterioration is primarily accomplished by the rapid growth of cells which disrupt and crush normal cells.

Figure 15. Cross-section of pea root 3 mm from tip
0.1 ppm dicamba - 48 hrs. after treatment

- A. Steler region (32x)
- B. Entire region (5.0x)

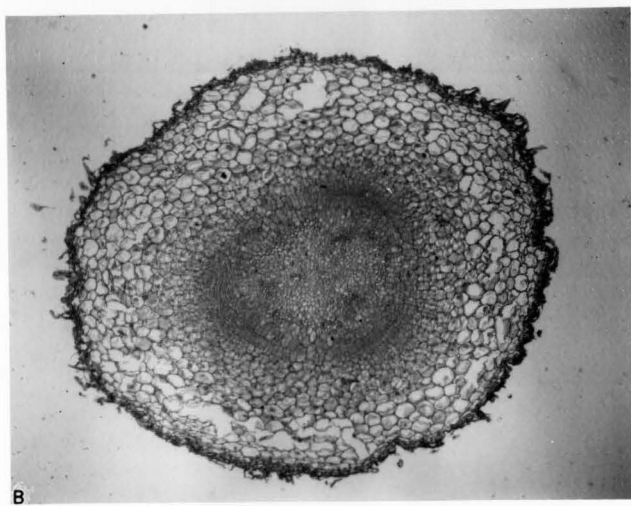
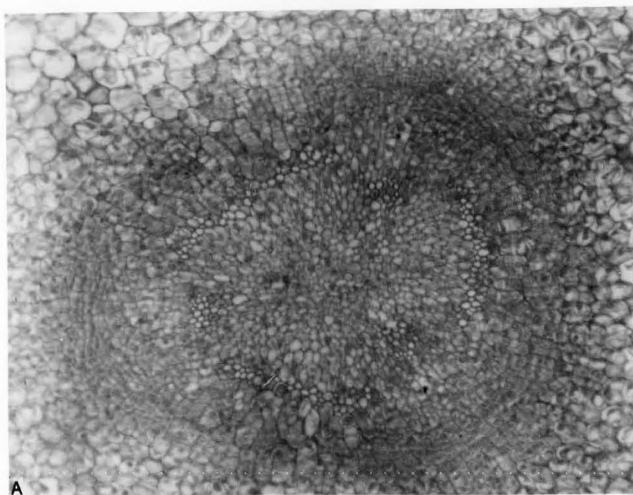


Figure 16. Cross-section of untreated pea root 3 mm from tip
48 hrs. after time of treatment

A. Cortical region (32x)

B. Entire region (10x)

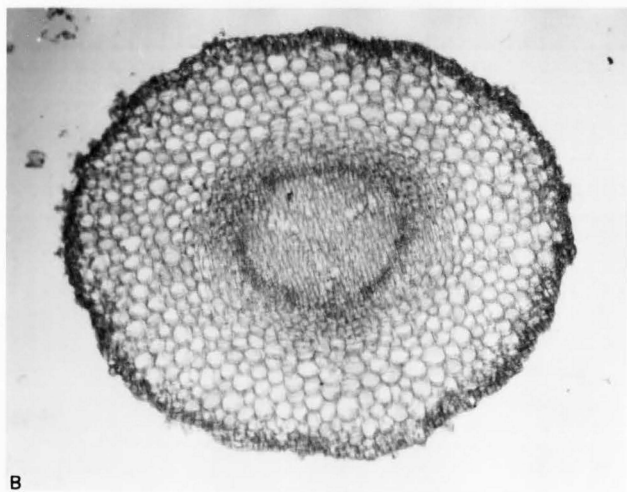
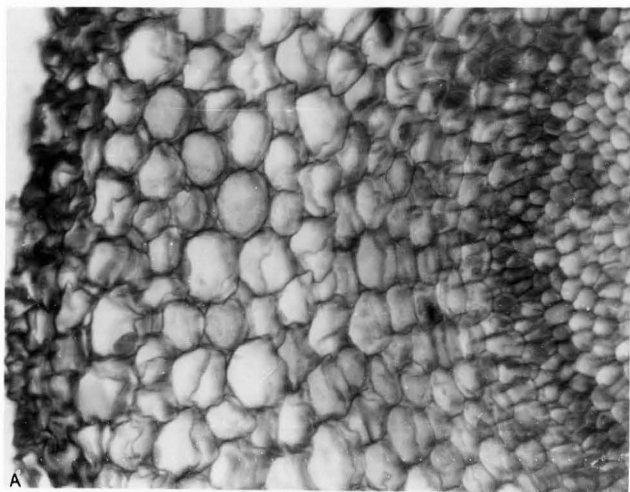


Figure 17. Cross-section of pea root 3 mm from tip
0.1 ppm dicamba - 72 hrs. after treatment

A. Pericycle and cortex (80x)

B. Entire region (4.0x)

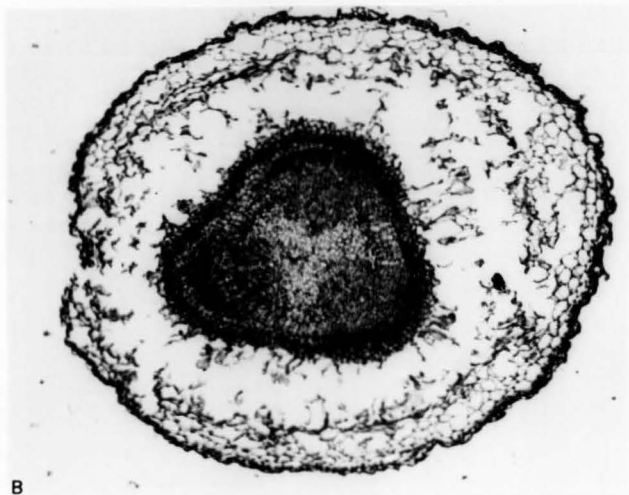
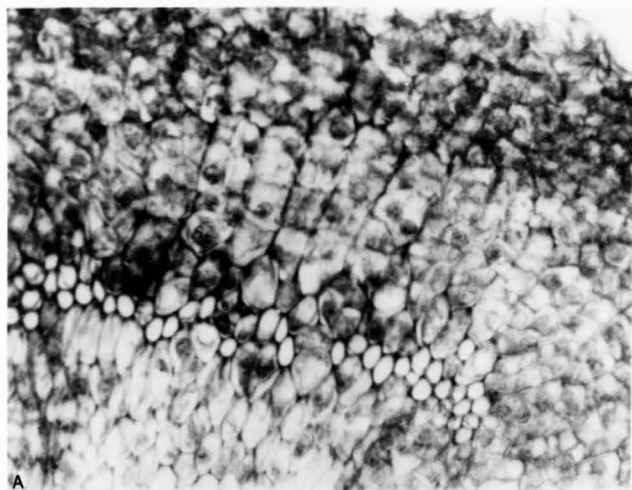
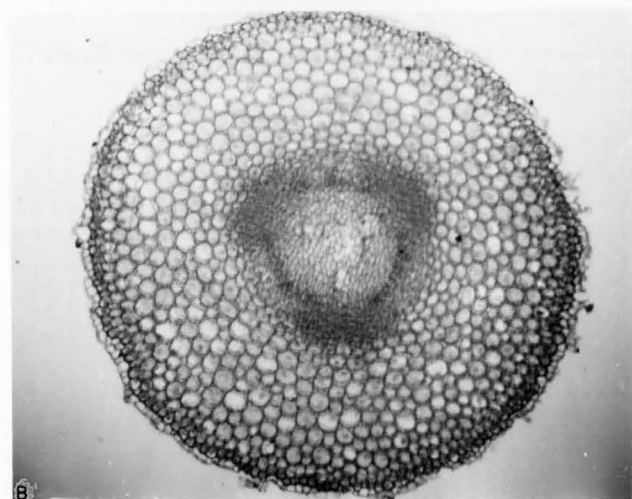
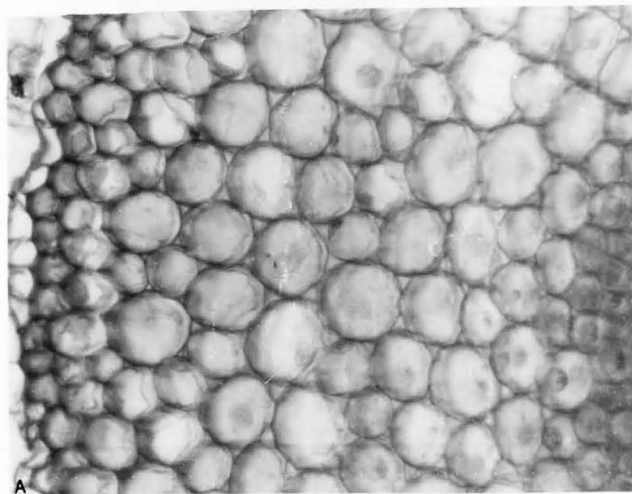


Figure 18. Cross-section of untreated pea root 3 mm from tip
72 hrs. after time of treatment

- A. Cortical region (32x)
- B. Entire region (7.9x)



SUMMARY AND CONCLUSION

When pea seeds were germinated in direct contact with dicamba the first observable symptoms of the herbicide were abnormal root development. Root elongation was inhibited.

Root expansion in the lateral plane was greatly increased by cellular swelling and division. The end result was a very short thick primary root. Suppression of normal root development is well documented by several auxin herbicides.

Treated root tips were examined to determine if the herbicide was affecting the nucleus of the cell. The results showed that all concentrations were responsible for nuclear volume reductions.

Since nuclear volumes had been reduced, several chemicals that had been shown to inhibit or affect certain cell processes were tested for comparison. In general, actinomycin D, chloramphenicol, and colchicine were all responsible for nuclear reductions. It is possible then that dicamba may affect the synthesis of RNA and proteins and prevent the formation of specific enzymes similar to the action of these known inhibitors.

Dicamba was responsible for considerable anatomical moderations. In the region 1 mm from the root tip, the cortical parenchyma cells began to divide rather profusely. The newly divided cells greatly enlarged the entire region as compared with untreated roots.

In the 3 mm region, cellular division seemed to be localized in the tri-arch regions. The pericycle becomes active and new cells are pushed into the cortex. The result here is that many cells have collapsed, leaving large holes in this area.

It would appear that cells are formed very rapidly, but the process is prevented which influences normal cell maturation.

Growth processes are complex and it is difficult to pinpoint various chemical reactions; however, this study shows that dicamba affected root elongation, nuclear volume, and apical modifications.

LITERATURE CITED

1. Andus, L.J. 1964. The physiology and biochemistry of herbicides. Academic Press, London and New York. 555 p.
2. Bentley, A.J. 1950. Growth-regulating effect of certain organic compounds. *Nature* 165:449.
3. Bond, L. 1948. Response of pea roots to application of certain growth-regulating substances. *Bot. Gaz.* 109:435-447.
4. Broadhurst, N.A., M.L. Montgomery and V.A. Freed. 1966. Metabolism of 2-methoxy 3,6-dichlorobenzoic acid (dicamba) by wheat and blue-grass plants. *J. Agric. Food Chem.* 14:585-588.
5. Burnside, O.C., and T.L. Lavy. 1966. Dissipation of dicamba. *Weeds* 14:211-214.
6. Cain, P.S. 1966. An investigation of the herbicidal activity of 2-methoxy 3,6-dichlorobenzoic acid. PhD Thesis, Univ. Illinois. 138 p. (Original not seen; abstracted in *Diss. Abstr.* 27:3757-3758 B).
7. Cardenas, J., F.W. Slife, J.B. Hanson, and H. Butler. 1968. Physiological changes accompanying the death of cocklebur plants treated with 2,4-D. *Weed Sci.* 16:96-100.
8. Chang, F.Y. and W.H. Vandeborn. 1968. Translocation of dicamba in Canada thistle. *Weed Sci.* 16:176-181.
9. Eames, A.J. 1950. Destruction of phloem in young bean plants after treatment with 2,4-D. *Amer. J. Bot.* 37:840-847.
10. Friesen, H.A. 1965. The movement and persistence of dicamba in soil. *Weeds* 13:30-33.
11. Friesen, H.A., H. Baenziger and C.H. Keys. 1964. Morphological and cytological effects of dicamba on wheat and barley. *Can. J. of Plant Sci.* 44:288-294.
12. Fujisawa, H. 1966. Role of nucleic acid and protein metabolism in the initiation of growth of germination. *Plant and Cell Physiol.* 7:185-197.
13. Haber, A.H. 1962. Effects of indoleacetic acid on growth without mitosis on mitotic activity in absence of growth by expansion. *Plant Physiol.* 37:18-26.
14. Hahn, R.R., O.C. Burnside and T.L. Lavy. 1969. Dissipation and phytotoxicity of dicamba. *Weed Sci.* 17:3-8.

15. Harris, C.I. 1964. Movement of dicamba and diphenamid in soils. *Weeds* 12:112-115.
16. Hilton, J.L., L.L. Jansen and H.M. Hull. 1963. Mechanisms of herbicide action. *Ann. Rev. Plant Physiol.* 14:353-384.
17. Hull, R.J. and M.R. Weisenberg. 1967. Translocation and metabolism of dicamba in *Sorghum halepense* and *Phaseolus vulgaris*. *Plant Physiol.* 42:s-49.
18. Jensen, W.A. 1962. Botanical histo-chemistry. W.H. Freeman and Co., San Francisco, Calif. 408 p.
19. Key, J.L. 1964. Ribonucleic acid and protein synthesis as essential process for cell elongation. *Plant Physiol.* 39:365-370.
20. Key, J.L. and J.C. Shannon. 1964. Enhancement by auxin on RNA synthesis in excised soybean hypocotyl tissue. *Plant Physiol.* 39: 360-364.
21. Klambt, H.D. 1962. Conversion in plants of benzoic acid to salicylic acid and its Bd-glucoside. *Nature* 196:491.
22. Magalhaes, A.C., F.M. Ashton and C.L. Foy. 1968. Translocation and fate of dicamba in purple nutsedge. *Weed Sci.* 16:240-245.
23. Miller, J.M. 1952. Plant hormone activity of substituted benzoic acids and related compounds. *Weeds* 1:185-188.
24. Minarik, C.E., D. Ready, A.G. Norman, H.E. Thompson and J.F. Owings, Jr. 1951. New growth regulating compounds II. Substituted benzoic acids. *Bot. Gaz.* 113:135-146.
25. Mohandas, T., and W.F. Grant. 1973. A relationship between nuclear volume and response to auxin herbicides for some weed species. *Can. J. Bot.* 51:1133-1136.
26. Morton, R.K. and J.K. Raison. 1964. The separate incorporation of amino acids into storage and soluble proteins catalyzed by two independent systems isolated from developing wheat endosperm. *Biochem. J.* 91:528-539.
27. Nooden, L.D. and K.V. Thimann. 1965. Inhibition of protein synthesis and of auxin-induced growth by chloramphenicol. *Plant Physiol.* 40: 193-201.
28. Nooden, L.D. and K.V. Thimann. 1966. Action of inhibitors of RNA and protein synthesis on cell enlargement. *Plant Physiol.* 41:157-164.
29. Poehlman, J.M. 1959. Breeding field crops. Holt, Rinehart & Winston, Inc., N.Y. 427 p.

30. Rojas, G., M. and T. Kommedahl. 1958. The effects of 2,4-dichlorophenoxyacetic acid on radicle development and stem anatomy of soybean. *Weeds* 6:49-51.
31. Scott, P.C. and R.O. Morris. 1970. Quantitative distribution and metabolism of auxin herbicides in roots. *Plant Physiol.* 46:680-684.
32. Sun, C.N. 1955. Anomalous structure in the hypocotyl of soybeans following treatment with 2,4-dichlorophenoxyacetic acid. *Science* 121:641.
33. Sun, C.N. 1956. Histological changes induced in soybean roots by 2,4-dichlorophenoxyacetic acid. *Science* 123:1129-1130.
34. Swanson, C.P. 1946. Histological responses of the kidney bean to aqueous sprays of 2,4-dichlorophenoxyacetic acid. *Bot. Gaz.* 107:522-531.
35. Torrey, J.G. 1956. Physiology of root elongation. *Ann. Rev. Plant Physiol.* 7:237-266.
36. VandenBorn, W.H. 1965. The effects of dicamba and picloram on quackgrass, bromgrass, and Kentucky blue-grass. *Weeds* 13:309-312.
37. Walton, D.C. 1966. Germination of Phaseolus vulgaris I. Resumption of axis growth. *Plant Physiol.* 41:298-302.
38. Watson, D.P. 1948. An anatomical study of the modification of bean leaves as a result of treatment with 2,4-D. *Amer. J. Bot.* 35:543-555.
39. Wilde, M.H. 1951. Anatomical modification of bean roots following treatment with 2,4-D. *Amer. J. Bot.* 38:79-91.
40. Wu, C.C., T.T. Kozlowski, R.F. Evert and S. Sasaki. 1971. Effects of direct contact of Pinus resinosa seeds and seedlings with 2,4-D or picloram on seedling development. *Can. J. Bot.* 49:1737-1741.
41. Zimmerman, P.W. and A.E. Hitchcock. 1951. Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. *Contrib. Boyce Thompson Inst.* 16:209.

<u>Source of variation</u>	<u>Degrees of freedom</u>	<u>Sum of squares</u>	<u>Mean square</u>
Replication	2	.01	.005
Treatment	3	451.69	150.560*
Replication x treatment	6	3.19	.865
Dates	2	40.59	20.295
Treatment x dates	6	120.93	20.155
Replication x dates	4	.70	.175
Replication x treatment x dates	12	129.57	10.797
Samples	9	2.17	.241
Treatment x samples	27	7.74	.287
Dates x samples	18	3.02	.168
Treatment x dates x samples	54	139.61	2.587
Remainder	216	2048.43	9.483

*Highly significant ($P < .01$)

Appendix A. Pea root elongation determinations

<u>Source of variation</u>	<u>Degrees of freedom</u>	<u>Sum of squares</u>	<u>Mean square</u>
Replication	2	388.68	194.34
Treatment	3	13,969.50	4,656.50*
Replication x treatment	6	2,152.95	358.82
Dates	2	1,150.30	575.15
Treatment x dates	6	263.71	43.95
Replication x dates	4	3,847.58	961.90
Replication x treatment x dates	12	15,247.89	1,270.66
Samples	9	1,656.60	184.07
Treatment x samples	27	9,368.45	346.98
Dates x samples	18	12,282.75	682.38
Treatment x dates x samples	54	42,651.62	789.84
Remainder	216	772,151.59	3,574.78

*Not significant ($P < .05$)

Appendix B. Pea root nuclear volume measurements of meristematic regions

VITA

Brent George Ovard

Candidate for the Degree of
Master of Science in Weed Science

Thesis: Anatomical Effects of Dicamba on Pea Root Tissues

Major Field: Weed Science & Weed Control

Biographical Information:

Personal Data: Born June 20, 1944, in Coalville, Utah, son of Joseph W. and Retta Fowler Ovard; married Cheryl Ann Glissmeyer April 20, 1967; one daughter - Leena.

Education: Attended elementary school in Henefer, Utah; graduated from North Summit High School in 1962; received a Bachelor of Arts degree from Weber State College, with a major in botany, in 1969; received language credit from Brigham Young University.

Professional Experience: 1971-1974, County Agricultural Agent, Lewis County, Idaho, with the University of Idaho; 1971, six months with University of Idaho, Aberdeen Experiment Station.