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THE RELATIONSHIP BETWEEN METRIBUZIN INJURY AND PEROXIDASE IN TOMATO CULTIVARS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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The Ohio State University
1976

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Tomato seedlings showing symptoms of metribuzin injury.
A comparison on the development of secondary roots in 7-day old seedlings of H-1370 treated with 0.28 and 0.56 kg/ha of metribuzin.
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A diagrammatic representation of the isozyme components of peroxidase in the seedlings of two cultivars of tomatoes treated (pre-emergence) as affected by 70 ppm IAA.
INTRODUCTION

Metribuzin is a triazine herbicide. Many of the toxicity symptoms induced by metribuzin are similar to those induced by other triazine herbicides.

The mechanism of action of metribuzin is not well understood. In 1973, Minami (38) observed that tomato cultivars exhibited differential response to metribuzin when applied (pre-emergence) at the rate of 0.56 and 1.12 kg per hectare. The tolerant cultivar exhibited no toxicity symptoms while the susceptible cultivars were poorly developed and dwarfed.

This suggested that a mechanism of tolerance operated among tomato cultivars. The mechanism may be different from those exhibited by triazine-tolerant plants such as corn (Zea mays L.) and sorghum (Sorghum bicolor L.).

Direct seeding of tomatoes is becoming increasingly important in commercial canning crop tomatoes. With this trend, there are many new cultivars being introduced (28). The sensitivity of these cultivars needs to be evaluated.

Metribuzin has been reported as effective against velvetleaf (Abutilon theophrasti Medic.), cocklebur (Xanthium sp.), ragweed (Ambrosia sp.) and other large-seeded broadleaf weeds which are not selectively controlled by other herbicides in direct-seeded tomatoes. It also suppresses the growth of weeds that are difficult to control such
as the common purslane (*Portulaca oleracea* L.), barnyard grass (*Echinochloa* sp.) and the yellow nutsedge (*Cyperus esculentus* L.) reducing early competition with young tomato plants. Thus, it seems that metribuzin is one of the most promising herbicides to date on direct-seeded tomatoes.

Therefore, a study was conducted at The Ohio State University, Department of Horticulture laboratory, greenhouse, and Lane Avenue farm with the following objectives:

1. To study the performance of two tomato cultivars which are tolerant and susceptible, respectively to metribuzin under field conditions.

2. To determine the relationship between the enzyme peroxidase and metribuzin injury in the tolerant and susceptible tomato cultivars.

3. To study the influence of hormone treatments on metribuzin injury in tolerant and susceptible tomato cultivars.
REVIEW OF LITERATURE

The triazines are used extensively against annual weeds in corn and sorghum (54). This group of compounds is also used effectively in other crops such as potatoes (Solanum tuberosum L.) and soybeans (Glycine max L.). The triazines inhibit growth of plants but this is considered one of the many known effects caused by an inhibition of photosynthesis (3). At herbicide concentration, the triazines cause foliar chlorosis followed by death of the leaf. Other leaf effects include loss of membrane integrity and destruction of the chloplast structure. At sub-lethal concentration, increased greening and more vigorous growth of plants may occur (3, 32).

This group of herbicides is rapidly absorbed through the roots and is readily translocated through the transpirational stream (3).

The rate by which the triazines are degraded into non-toxic forms varies greatly with the different species (3). In tolerant species, these compounds are degraded rapidly; while in susceptible species the triazines are degraded slowly. Thus, the primary basis of selectivity appears to be the rate by which the triazines are transformed into non-toxic materials (32, 54). The processes involved are hydroxylation, dechlorination, demethylthiolation, or demethoxylation depending on the substitution involved in the parent compound.

The mechanism of action of the triazines involves a severe inhibition of the Hill reaction in photosynthesis (41, 55), thus
reducing the fixation of carbon dioxide in plants (54, 59, 67, 68, 74). The total herbicide effect may be more complex than the above. It has been postulated that the mode of action may involve the interaction of light, the chlorophyll and the triazine compound resulting in a secondary phytotoxic substance (2). Therefore, other metabolites may be involved.

In corn, tolerance to the triazines has been attributed to the non-enzymatic mechanism by the formation of the hydroxy form of the herbicide (8, 23, 26, 42) if the material has been absorbed through the roots (55). It is catalyzed by a cyclic hydraxamate, identified later as a benzoaxaxinone (25, 26, 68).

More recently, glutathione conjugation has been found to be the factor of selectivity when the triazine material is absorbed through the leaves of corn, sorghum, and sugar cane (Saccharum officinarum L.). Many workers attributed the glutathione conjugation to a very active enzymatic pathway of a soluble enzyme, glutathione-s-transferase (33, 34, 55, 56, 57). Atrazine and three closely related 2-chloro-s-triazines such as propazine, simazine, and cyperazine are rapidly metabolised into water-soluble metabolites. Atrazine, particularly become detoxified into an atrazine-glutathione conjugate, GS-atrazine (55, 56). In excised leaves of corn, the conjugation was so rapid that other factors such as absorption, translocation, and penetration of the substrate into the site of metabolism may have been the limiting factors (33).

Metribuzin, an asymmetrical triazine, has been used as a selective herbicide in soybeans. Several workers reported that soybean cultivars exhibited differential tolerance to the herbicide (9, 29). Other crops
also exhibited similar differential tolerance to metribuzin. This has been reported in tomatoes (1, 7, 17) and potatoes (22).

The mechanism of detoxification in metribuzin-tolerant plants is different from those exhibited by atrazine and other 2-chloro-s-triazine tolerant plants. The major metabolite found in soybean cultivars was a deaminated diketo metribuzin (DADK), 6-tert-butyl-as-triazin-3-5(2H,4H)dione. It was found as a glucose conjugate in mature plants (65). However, there were indications that another metabolite, a deaminated metribuzin (DA), 6-tert-butyl-3-(methylthio)-as-triazin-5-(4H)one and a possible third metabolite, a diketo metribuzin (DK), 4-amino-6-tert-butyl-as-triazin-3,5-(2H,4H)dione could also play a role in metribuzin detoxification (65). These two products of metribuzin degradation were found to be similar to DADK in solubility characteristics.

According to Smith and Wilkinson (60), both the tolerant and the susceptible soybean cultivars readily absorbed and translocated metribuzin. Greater amounts of the metribuzin metabolite were found in the stem and roots of the tolerant soybean cultivar than the susceptible soybean cultivar, indicating that this could be a major mechanism of herbicide detoxification.

The phytotoxicity of metribuzin is influenced by environmental conditions. Fortino and Splittstoesser (16) reported that tomato plants were more susceptible to the herbicide when grown under high humidity and high temperature. Similar observations were reported in tomato plants grown under low light conditions before herbicide application (45).
Soil conditions such as low organic matter content and high pH could increase herbicidal injury (40), especially when the compound has been applied at concentration higher than 0.56 kg per hectare.

The mechanism of tolerance of tomato cultivars to metribuzin may involve other metabolic sites of degradation besides those reported among soybean cultivars. Only approximately 4% of the herbicide was reported to be absorbed in tolerant as well as in susceptible tomato cultivars (16), a much lower rate than those reported among soybean cultivars (60). Furthermore, increased herbicide injury under low light conditions could not be overcome by exogenous application of sucrose (16), which could increase glucose conjugation with the parent compound.

Observations by previous workers (7, 16) on metribuzin injury indicated that death of the affected plant resulted if the appearance of toxicity symptoms occur during any stage of the cotyledonary leaf expansion. However, if the symptoms of metribuzin injury occur after the formation of the first pair of true leaves, the affected plant survived but would be dwarfed. The affected plants were thus similar to those reportedly treated with a growth retardant such as AMO 1618 (2-isopropyl-4-dimethyl-amino-5-methylphenyl-1-piperidinecarboxylate methyl chloride) (39). These plants had a higher peroxidase activity and could be overcome by hormone treatments (24, 36). Therefore, other metabolic pathways may be affected and may involve the participation of oxidative enzymes.

Among the enzymes involved in growth and development which have been implicated with dwarfism and slow rate of growth of plants are the
peroxidases, which acts through auxin destruction, a fact that has been well documented (37, 46, 47, 64, 73).

Peroxidase may participate in the indoleacetic acid (IAA) oxidase system (47) resulting in the reduction of the endogenous auxin and which may be reflected in slow growth rate. Furthermore, peroxidase is involved in lignin synthesis (6, 35) while IAA inhibits it (43, 61). Therefore, a reduction in endogenous IAA could result in abnormal lignification of juvenile tissues.
MATERIALS AND METHODS

The herbicide

Metribuzin is an asymmetrical triazine. Chemically, it is known as 4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5-(4H)-one.

The chemical structure is as follows:

\[
\begin{align*}
S & \quad CH_3 \\
C & \quad N_1 \quad 2 \\
N_2 & \quad 3 \\
C & \quad 4 \quad - \quad NH_2 \\
C & \quad N_6 \\
CH_3 & \quad C \quad CH_3 \\
CH_3 & \quad \end{align*}
\]

The herbicide formulation used was a wettable powder with a 50% active ingredient.

The cultivars

The two tomato cultivars used were Campbell 28 (C-28) and Heinz 1370 (H-1370), representing the tolerant and the susceptible cultivars, respectively, to metribuzin. Both cultivars have determinate type of growth. The seeds of the two cultivars were obtained from Harris Seed Company.

C-28 was developed at Mississauga, Ontario, Canada from a cross involving two breeding lines, Z59 and Z110. Z59 was derived from Scotia.
and three breeding lines. Z110 was derived from two breeding lines. The other named cultivars in the parentage of C-28 included Devon Surprise, Porter, Fireball, Nagcarlang, VR Moscow, and C-135.

C-28 has been known for its earliness, concentrated fruit setting and ripening, firm and crack-resistant fruits which remain on the vine in good condition for extended periods of time, excellent characteristics for mechanical or once-over harvest.

H-1370 was developed from a cross involving Cornell 55-539 and Eastern States 54-1878-B. H-1370 is productive and the fruits are firm and crack-resistant.

The experiment was conducted in three parts; namely, field, greenhouse, and laboratory studies.

A. Field studies

The experiment was conducted at The Ohio State University, Department of Horticulture Lane Avenue Farm to study the influence of metribuzin on the growth and yield of the two tomato cultivars, C-28 and H-1370 under field conditions. The experiment was undertaken in the 1974 and 1975 planting seasons on Brookston silty clay loam soil with 3% organic matter.

The field was first mechanically cultivated and prepared before planting.

The cultivars were direct-seeded in 9-meter rows, 2 cm deep. Metribuzin was applied at the rate of 0.28 and 0.56 kg per hectare immediately after planting in 234 liters of water per hectare.

A split plot design was used with four replications, with the different rates of metribuzin as the main plots and the tomato cultivars
as the sub-plots. An untreated and cultivated check was provided. Standard practices in the production of tomato were followed until the termination of the experiment. The following data were taken:

a. Emergence count—the number of seedlings that emerged was counted twice. The first count was taken 10 days after planting. The second count was taken 5 days after the first.

b. Final stand count—the final stand count was taken 21 days after emergence.

c. Plant height—the height of 10 seedlings from every replicate and treatment was taken at random after thinning to approximately 30 cm between hills in a row, 35 days after emergence and was measured from the ground level to the tip of the youngest fully developed leaf.

d. Dry weight—10 of the seedlings that were thinned were taken at random, dried at 70°C for 48 hours, and weighed.

e. Percentage of fruit set.

f. Yield—the fruits from 5 plants taken at random were harvested, graded into marketable and non-marketable fruits. The marketable fruits were further graded into ripe and mature green. The non-marketable fruits were separated into immature green fruits and culls. Each class was weighed separately.

B and C. Greenhouse and laboratory studies

1. Determination of peroxidase activity.—Seedflats measuring 7.6 x 35 x 56 cm in size were filled with pulverized Brookston silty clay loam soil, levelled and prepared before planting. Thirty seeds of each cultivar per row were directly planted in the seedflat. There were 6 rows
per seedflat; 3 rows for each cultivar. Each row represented a replication. A split plot design was used with the different rates of metribuzin; namely, 0.28 and 0.56 kg per hectare as the main plots with the tomato cultivars as the sub-plots.

After planting, the seedflats were brought to the Lane Avenue Farm where the different rates of metribuzin were applied with a special herbicide applicator. The seedflats were then brought back to the greenhouse where similar care was given regardless of treatment.

The temperature in the greenhouse was taken at approximately 20 cm above the ground floor by means of a portable thermograph. The mean weekly day and night temperatures are found in Appendix Table 1.

The following data were taken:

a. Emergence count—the number of seedlings that emerged were counted twice. The first count was taken 7 days after planting and the second count was taken 5 days later.

b. Final stand count—the final stand count was taken 21 days after emergence.

c. Plant height—the height of 5 plants taken at random was measured from the ground level to the top of the youngest fully developed leaf, from every replicate and treatment.

d. Dry weight—5 plants were taken at random from every replicate and treatment, dried at 70°C for 48 hours, and weighed.

Plant height and weight measurements were done when the seedlings were 21 days old from emergence.

e. Peroxidase levels—peroxidase was assayed from seedlings that were untreated with metribuzin at 1, 2, 3, and 4 weeks after emergence.
Similar assay was done from seedlings that were treated with metribuzin 3 weeks after emergence.

The plant samples were prepared and assayed for peroxidase as follows:

**Sample preparation**

In all experiments, the samples were prepared following the method prescribed by Evans and Alldridge (14).

Hypocotyl sections were taken, washed with distilled water and blotted dry. One-half gram samples to which 2 ml of distilled water was added were crushed by means of a mortar and pestle. The tissue homogenates were then centrifuged at 4500 rpm for 5 minutes by means of the SORVALL Superspeed centrifuge. The supernatant was dialyzed with four changes of distilled water at 3-4°C. At the end of 48 hours the extract was again centrifuged at 1600 rpm for 3 minutes and then stored in small vials at 3-4°C. The samples were assayed for peroxidase within 24-48 hours after preparation, although samples prepared as previously described could be assayed without loss of activity even after one week but not later than two weeks.

**Peroxidase assay by electrophoresis**

Electrophoresis is a protein separation technique that involves the migration of large molecules and small aggregates of molecules under the influence of an electric field applied to a medium in which the particles are suspended (70). Separation of the charged molecules occurs because of their different mobilities (31).

The discontinuous system of electrophoresis used in this study was the method prescribed by Davis (11).
The quantitative determination of peroxidase was done by taking 0.3 ml of the previously prepared sample and mixing it with 0.7 ml of a 20% (w/v) sucrose solution. A 0.2 ml of the mixture was then layered onto the top of the acrylamide gels (2.5% stacking gel, 10.5% running gel) that were prepared previously.

Tris-glycine buffer at pH 8.3 was used in both the upper and lower reservoir. A few drops of 0.01% (w/v) Bromphenol Blue were added to the upper buffer as a marker dye. A continuous flow of water, cooled at 2-3°C inside the lower buffer reservoir jacket was maintained. The power supply was then connected and 2-4 ma per tube was applied. The power supply was turned off when the marker dye reached approximately one cm from the tip of the tubes.

After each electrophoretic run which usually took 2.5-3.0 hours, the tubes were removed and the gels were taken with a syringe having a flat-tipped needle by forcing cool distilled water along the sides of the tubes.

Zymogram patterns were developed by staining for 10 minutes with a Benzidine-H₂O₂ as prescribed by Scandalios (53). The gels were destained with 7% (v/v) acetic acid for 5 minutes, washed in adequate amounts of distilled water and then stored in individually labelled test tubes containing distilled water.

The zymograms were read by means of the JOYCE LOEBL Chromoscan MK 11, Double Beam Recording and Integrating Densitometer with a gear ratio of 1:3 using Cam #5077-D. Quantitation of the peroxidase enzyme in the samples was based on the standard horseradish peroxidase preparation.
Duplicate determinations for each sample were done in all analyses.

2. IAA and GA experiments.—The results of the previous experiment and various literature indicated that the triazines and metribuzin in particular influence growth and development by affecting enzyme levels which may in turn influence hormonal levels in plants.

Therefore, a study was conducted to determine the influence of hormones (IAA and GA) on metribuzin injury in tolerant and susceptible tomato cultivars.

The preparation and type of the soil used, method of planting the seeds of the two cultivars, and the application of the different rates of metribuzin were done similarly as in the previous greenhouse experiments.

IAA and GA were each applied 7 times to seedlings at the rate of 5 and 10 ppm each time with a mist sprayer until run-off occurred. The first application was done on the fourth day after seedling emergence and every 2 days thereafter until a total of 35 and 70 ppm for each hormone was reached.

A split-split plot design was used with the different rates of metribuzin as the main plot, the tomato cultivars as the sub-plot and the hormone treatments as the sub-sub-plot. There were 3 replications for each treatment.

The following data were taken:

a. Emergence count—the number of seedlings that emerged were counted as previously described.
b. Percentage of final stand—the final stand count was taken 4 weeks after emergence. The percentage of final stand of the seedlings was computed as the number of seedlings that survived over the number of seedlings that emerged multiplied by 100.

c. Plant height—the height of 5 seedlings taken at random was measured as previously described and was done when the seedlings were 5 weeks old.

d. Dry weight—five 5-week old seedlings were carefully harvested at random, washed with tap water to remove the soil in the roots, dried at 70°C for 48 hours, and weighed.

e. Peroxidase levels—peroxidase was assayed from 70 ppm of IAA treatment alone since it was considered the best hormone treatment. The method used was described previously. Zymograms were developed using the method described previously.
RESULTS

Metribuzin Injury

The injury due to metribuzin was observed to occur in any stage of cotyledonary leaf expansion. The first discernable symptom of injury was the presence of cotyledonary leaves which had turned light green. Within 24 to 48 hours the affected leaves became seemingly bleached, wrinkled and dry, while the stem remained in the upright position. Often times the symptom of injury appeared after cotyledonary leaf expansion (Fig. 1).

Seedlings with well-developed first pair of true leaves when affected recovered without any apparent injury. However, when the injury occurred earlier, the affected seedling recovered but was stunted and dwarfed.

The roots of 7-day old affected seedlings revealed some differences in secondary root development (Figs. 2 and 3). Secondary root development in the affected seedlings was practically absent along the entire length of the primary root in H-1370, except at the basal region of the primary root. This condition in secondary root formation was not as distinct in the C-28 seedlings.

Growth and Development

On emergence

The data indicated that at the rates used in this study, metri-
Fig. 1. Tomato seedlings showing symptoms of metribuzin injury.
Fig. 2. A comparison on the development of secondary roots in 7-day old seedlings of H-1370 treated with 0.28 kg (0.25 lb/A) and 0.56 kg/ha (0.50 lb/A) of metribuzin.
Fig. 3. A comparison on the development of secondary roots in 7-day old seedlings of C-28 treated with 0.28 kg (0.25 lb/A) and 0.56 kg/ha (0.50 lb/A) of metribuzin.
buzin did not interfere with the emergence of seedlings of the two cultivars of tomato (Table 1).

**TABLE 1. THE EMERGENCE OF SEEDLINGS OF TWO CULTIVARS OF TOMATOES IN THE GREENHOUSE AND FIELD TREATED WITH METRIBUZIN**

<table>
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<tr>
<td></td>
<td>C-28 (%)</td>
<td>H-1370 (%)</td>
<td>C-28 (%)</td>
<td>H-1370 (%)</td>
<td>C-28 (%)</td>
<td>H-1370 (%)</td>
<td>C-28 (%)</td>
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<tr>
<td>0</td>
<td>84.66</td>
<td>90.00</td>
<td>285.25</td>
<td>265.25</td>
<td>189.00</td>
<td>192.00</td>
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<td>0.28</td>
<td>85.33</td>
<td>86.66</td>
<td>306.50</td>
<td>248.25</td>
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<td>0.56</td>
<td>83.33</td>
<td>88.66</td>
<td>255.00</td>
<td>207.00</td>
<td>193.75</td>
<td>198.50</td>
<td></td>
</tr>
</tbody>
</table>

1 Actual count.
No statistical significance observed among the differences at 5% level.

On seedling stand

There were reductions in the stand of seedlings in the greenhouse and field as a result of metribuzin application (Table 2).

**TABLE 2. THE SEEDLING STAND OF TWO CULTIVARS OF TOMATOES TREATED WITH METRIBUZIN IN THE GREENHOUSE AND FIELD**

<table>
<thead>
<tr>
<th>Rate (kg/ha)</th>
<th>Greenhouse Cultivars 2</th>
<th>Field 1</th>
<th>1974</th>
<th>1975</th>
<th>Field 1</th>
<th>1974</th>
<th>1975</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-28 (%)</td>
<td>H-1370 (%)</td>
<td>C-28 (%)</td>
<td>H-1370 (%)</td>
<td>C-28 (%)</td>
<td>H-1370 (%)</td>
<td>C-28 (%)</td>
</tr>
<tr>
<td>0</td>
<td>98.31 c</td>
<td>98.62 c</td>
<td>264.00 a</td>
<td>277.75 a</td>
<td>184.75 a</td>
<td>191.75 b</td>
<td></td>
</tr>
<tr>
<td>0.28</td>
<td>86.87 b</td>
<td>75.28 b</td>
<td>194.75 a</td>
<td>176.25 a</td>
<td>194.00 a</td>
<td>248.25 c</td>
<td></td>
</tr>
<tr>
<td>0.56</td>
<td>23.86 a</td>
<td>23.58 a</td>
<td>73.75 a</td>
<td>82.00 a</td>
<td>138.50 a</td>
<td>84.50 a</td>
<td></td>
</tr>
</tbody>
</table>

1 Actual count.

2 Means within column with similar letter are not significantly different at 5% level by L.S.D.
In the greenhouse, the application of metribuzin at 0.28 kg/ha significantly reduced the seedling stand of C-28 and H-1370 by 11.44% and 23.34% respectively. Increasing the herbicide rate to 0.56 kg/ha significantly reduced the seedling stand of C-28 and H-1370 by 74.45% and 75.04% respectively.

In the field in 1974, metribuzin at either rate of application did not affect seedling stand.

In the field in 1975, metribuzin at 0.28 and 0.56 kg/ha did not affect seedling stand of C-28. The seedling stand of H-1370 was significantly reduced by the application of 0.56 kg/ha of metribuzin.

**On seedling height**

There were changes in seedling height of C-28 and H-1370 as a result of metribuzin application (Table 3).

**TABLE 3. THE INFLUENCE OF METRIBUZIN ON SEEDLING HEIGHT OF TWO CULTIVARS OF TOMATOES IN THE GREENHOUSE AND FIELD (cm)**

<table>
<thead>
<tr>
<th>Rate (kg/ha)</th>
<th>Greenhouse Cultivars</th>
<th>1974</th>
<th>Field</th>
<th>1974</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-28</td>
<td>H-1370</td>
<td>C-28</td>
<td>H-1370</td>
<td>C-28</td>
</tr>
<tr>
<td>0</td>
<td>8.68 b</td>
<td>9.86 c</td>
<td>22.50 a</td>
<td>26.50 a</td>
<td>26.40 a</td>
</tr>
<tr>
<td>0.28</td>
<td>8.58 b</td>
<td>8.65 b</td>
<td>20.45 a</td>
<td>23.05 a</td>
<td>25.35 a</td>
</tr>
<tr>
<td>0.56</td>
<td>6.31 a</td>
<td>6.18 a</td>
<td>13.20 a</td>
<td>14.90 a</td>
<td>24.90 a</td>
</tr>
</tbody>
</table>

1Means within column with similar letter are not significantly different at 5% level by L.S.D.
In the greenhouse, the seedling height of C-28 was not affected by the application of 0.28 kg/ha of metribuzin. Increasing the herbicide rate to 0.56 kg/ha produced significantly shorter C-28 seedlings. On the other hand, H-1370 seedlings were significantly shorter as a result of metribuzin application at 0.28 kg/ha. At 0.56 kg/ha of metribuzin, H-1370 seedlings were significantly reduced further in height.

In the field in 1974, no significant differences in seedling height were observed in the C-28 and H-1370 resulting from metribuzin treatment.

In the field in 1975, the seedling height of C-28 was not affected by metribuzin treatments. However, there were significant reductions in seedling height of H-1370 as a result of the application of 0.28 and 0.56 kg/ha of metribuzin.

On dry weight of seedlings

There were changes in the dry weight of seedlings of the two cultivars of tomatoes as a result of metribuzin treatments (Table 4).

In the greenhouse, the application of 0.28 kg/ha of metribuzin significantly reduced the dry weight of C-28 seedlings. Increasing the rate of the herbicide to 0.56 kg/ha further reduced the seedling dry weight of C-28. Similar significant trend was observed with the H-1370 seedlings.

In the field in 1974, no significant changes in dry weight measurements were recorded.

In the field in 1975, the dry weight of seedlings of C-28 was not affected by metribuzin at either rate of application. However, the
application of 0.56 kg/ha of metribuzin significantly reduced the dry weight of seedlings of H-1370.

TABLE 4. THE INFLUENCE OF METRIBUZIN ON DRY WEIGHT OF SEEDLINGS OF TWO CULTIVARS OF TOMATOES IN THE GREENHOUSE AND FIELD

<table>
<thead>
<tr>
<th>Rate (kg/ha)</th>
<th>Greenhouse Cultivars(^1) (g/5 seedlings)</th>
<th>Field (^1) (g/10 seedlings)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.200 c</td>
<td>0.210 c</td>
</tr>
<tr>
<td>0.28</td>
<td>0.180 b</td>
<td>0.180 b</td>
</tr>
<tr>
<td>0.56</td>
<td>0.160 a</td>
<td>0.120 a</td>
</tr>
</tbody>
</table>

\(^1\)Means within column with similar letter are not significantly different at 10% level by L.S.D.

On fruit set

In the field in 1974, no significant changes in the percentage of fruit set were observed as a result of metribuzin application (Table 5).

In the field in 1975, the percentage of fruit set of C-28 was not affected by the application of 0.28 and 0.56 kg/ha of metribuzin. However, at similar rates of metribuzin application, the percentage of fruit set of H-1370 was significantly reduced.

On marketable fruit yield

In the field in 1974, the application of 0.28 and 0.56 kg/ha of metribuzin did not produce significant changes in marketable fruit yield of C-28 and H-1370 (Table 6).
### TABLE 5. THE PERCENTAGE OF FRUIT SET OF TWO CULTIVARS OF TOMATOES TREATED WITH METRIBUZIN

<table>
<thead>
<tr>
<th>Rate (kg/ha)</th>
<th>1974</th>
<th>1975</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cultivars 1</td>
<td>Cultivars 1</td>
</tr>
<tr>
<td></td>
<td>C-28</td>
<td>H-1370</td>
</tr>
<tr>
<td>0</td>
<td>93.32 a</td>
<td>91.10 a</td>
</tr>
<tr>
<td>0.28</td>
<td>93.64 a</td>
<td>94.58 a</td>
</tr>
<tr>
<td>0.56</td>
<td>93.47 a</td>
<td>93.06 a</td>
</tr>
</tbody>
</table>

*Means within column with similar letter are not significantly different at 15% level by L.S.D.*

### TABLE 6. THE MARKABLE FRUIT YIELD OF THE TWO CULTIVARS OF TOMATOES TREATED WITH METRIBUZIN (kg/5 plants)

<table>
<thead>
<tr>
<th>Rate (kg/ha)</th>
<th>1974</th>
<th>1975</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cultivars 1</td>
<td>Cultivars 1</td>
</tr>
<tr>
<td></td>
<td>C-28</td>
<td>H-1370</td>
</tr>
<tr>
<td>0</td>
<td>6.10 a</td>
<td>5.82 a</td>
</tr>
<tr>
<td>0.28</td>
<td>11.56 a</td>
<td>11.70 a</td>
</tr>
<tr>
<td>0.56</td>
<td>10.50 a</td>
<td>9.47 a</td>
</tr>
</tbody>
</table>

*Means within column with similar letter are not significantly different at 10% level by L.S.D.*

In the field in 1975, significant changes in the marketable fruit yield of C-28 were recorded at 0.28 and 0.56 kg/ha of metribuzin application. No significant changes in marketable fruit yield were obtained from H-1370.
On non-marketable fruit yield

The data indicated that the non-marketable fruit yield of C-28 and H-1370 were not affected by metribuzin application (Table 7).

TABLE 7. THE NON-MARKETABLE FRUIT YIELD OF THE TWO CULTIVARS OF TOMATOES TREATED WITH METRIBUZIN (kg/5 plants)

<table>
<thead>
<tr>
<th>Rate (kg/ha)</th>
<th>Cultivars</th>
<th>1974</th>
<th></th>
<th>Cultivars</th>
<th>1975</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-28</td>
<td>H-1370</td>
<td></td>
<td>C-28</td>
<td>H-1370</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.25</td>
<td>1.24</td>
<td>5.36</td>
<td>6.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.28</td>
<td>1.57</td>
<td>2.55</td>
<td>3.21</td>
<td>3.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.56</td>
<td>1.45</td>
<td>1.68</td>
<td>4.84</td>
<td>4.54</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No statistical significance observed among differences at 5% level.

On Peroxidase Levels

In untreated seedlings

There was a decreasing trend in peroxidase levels in the C-28 seedlings (Table 8). In H-1370, an increasing trend in peroxidase levels was recorded.

TABLE 8. THE PEROXIDASE LEVELS IN THE SEEDLINGS OF TWO CULTIVARS OF TOMATOES AT 1, 2, 3, AND 4 WEEKS AFTER EMERGENCE (iu/g fresh weight)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-28</td>
</tr>
<tr>
<td>1</td>
<td>42.79</td>
</tr>
<tr>
<td>2</td>
<td>45.65</td>
</tr>
<tr>
<td>3</td>
<td>41.37</td>
</tr>
<tr>
<td>4</td>
<td>39.95</td>
</tr>
</tbody>
</table>
There were changes in the isozyme components of peroxidase in the seedlings of two cultivars at 1, 2, 3, and 4 weeks after emergence (Fig. 5). The $R_f$ values of each component are found in Table 9).

At one week after emergence, the enzyme peroxidase of C-28 and H-1370 had the same number of isozyme components with similar $R_f$ values. Similar results were obtained at 2 weeks after emergence. Three weeks after emergence, the fastest migrating isozyme band in both cultivars disappeared but a slower migrating isozyme band appeared between isozyme numbers 5 and 6 in C-28 and thus was called isozyme number 5a. Therefore, the number of isozymes in C-28 remained the same but was one less in H-1370.

At 4 weeks after emergence, isozyme number 5a appeared in H-1370 and another isozyme appeared and was called isozyme number 8. This isozyme band was considered the slowest and was absent in C-28 at 4 weeks after emergence.

**Influence of metribuzin on peroxidase levels**

There were changes in peroxidase levels in the two cultivars of tomatoes treated with metribuzin (Table 10).

The application of 0.28 kg/ha of metribuzin increased the peroxidase levels of C-28 and H-1370. When the rate of the herbicide was increased to 0.56 kg/ha, peroxidase level of C-28 was increased but not as much as those obtained from H-1370 seedlings.

There were changes in the isozyme components of peroxidase in the 3-week old seedlings of the two cultivars as a result of metribuzin treatment (Fig. 6). The $R_f$ values of each component are found in Table 11.
Fig. 4. The standard curve for the horseradish peroxidase preparation.
Fig. 5. A diagrammatic representation of the isozyme components of peroxidase in the two cultivars of tomatoes at 1, 2, 3, and 4 weeks after emergence.
### TABLE 9. THE Rf VALUES OF THE DIFFERENT ISOZYME COMPONENTS OF PEROXIDASE IN THE TWO CULTIVARS AT 1, 2, 3, AND 4 WEEKS AFTER EMERGENCE

<table>
<thead>
<tr>
<th>Isozyme Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-28 H-1370</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.85</td>
<td>0.85</td>
<td>0.83</td>
<td>0.83</td>
</tr>
<tr>
<td>2</td>
<td>0.72</td>
<td>0.72</td>
<td>0.71</td>
<td>0.71</td>
</tr>
<tr>
<td>3</td>
<td>0.65</td>
<td>0.65</td>
<td>0.64</td>
<td>0.64</td>
</tr>
<tr>
<td>4</td>
<td>0.58</td>
<td>0.58</td>
<td>0.57</td>
<td>0.57</td>
</tr>
<tr>
<td>5</td>
<td>0.53</td>
<td>0.53</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>5a</td>
<td></td>
<td></td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td>6</td>
<td>0.41</td>
<td>0.41</td>
<td>0.41</td>
<td>0.41</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td>0.37</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
</tbody>
</table>

1. Untreated plants.
2. Arranged from the fastest to the slowest moving isozyme band.

### TABLE 10. THE PEROXIDASE LEVELS OF SEEDLINGS OF THE TWO CULTIVARS OF TOMATOES TREATED WITH METRIBUZIN (iu/g fresh weight)

<table>
<thead>
<tr>
<th>Rate (kg/ha)</th>
<th>Cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-28</td>
</tr>
<tr>
<td>0</td>
<td>41.37</td>
</tr>
<tr>
<td>0.28</td>
<td>57.07</td>
</tr>
<tr>
<td>0.56</td>
<td>66.34</td>
</tr>
</tbody>
</table>
Fig. 6. A diagrammatic representation of the isozyme components of peroxidase in the two cultivars of tomatoes at 3 weeks after emergence as influenced by metribuzin.
TABLE 11. THE R<sub>f</sub> VALUES OF THE DIFFERENT ISOZYME COMPONENTS OF PEROXIDASE IN THE TWO CULTIVARS OF TOMATOES TREATED WITH METRIBUZIN<sup>1</sup>

<table>
<thead>
<tr>
<th>Isozyme Number&lt;sup&gt;2&lt;/sup&gt;</th>
<th>C-28</th>
<th>H-1370</th>
<th>0.28</th>
<th>H-1370</th>
<th>0.56</th>
<th>C-28</th>
<th>H-1370</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.67</td>
<td>0.67</td>
<td>0.71</td>
<td>0.71</td>
<td>0.70</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.63</td>
<td>0.63</td>
<td>0.65</td>
<td>0.65</td>
<td>0.62</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.56</td>
<td>0.56</td>
<td>0.59</td>
<td>0.59</td>
<td>0.56</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.48</td>
<td>0.48</td>
<td>0.52</td>
<td>-</td>
<td>0.50</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>5a</td>
<td>0.43</td>
<td>-</td>
<td>0.46</td>
<td>0.46</td>
<td>0.42</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
<td>0.34</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Samples for peroxidase analysis were taken at 3 weeks from emergence.

<sup>2</sup>Arranged from the fastest to the slowest moving isozyme band.

At 0, 0.28, and 0.56 kg/ha of metribuzin, isozyme number 1 was absent in C-28 and in H-1370. At 0.28 kg/ha of metribuzin, isozyme number 5 was present in C-28 but was not found in H-1370. Another isozyme appeared with an R<sub>f</sub> value of 0.30. This isozyme could not be found in C-28. At 0.56 kg/ha of metribuzin, isozyme number 5 which was absent in H-1370 at 0.28 kg/ha of metribuzin, appeared having an R<sub>f</sub> value of 0.50. Isozyme 5a in H-1370 disappeared. Isozyme 7 appeared in C-28 with an R<sub>f</sub> value of 0.30.
Hormone Treatments and Metribuzin Injury

On seedling stand

The data indicated that the seedling stand of C-28 and H-1370 treated with metribuzin (pre-emergence) at 0.56 kg/ha were significantly improved with the application of 35 and 70 ppm IAA (Table 12). GA at either concentration did not have any effect.

TABLE 12. THE PERCENTAGE OF FINAL STAND OF SEEDLINGS OF TWO CULTIVARS OF TOMATOES TREATED WITH PRE-EMERGENT APPLICATION OF METRIBUZIN AS AFFECTED BY HORMONE TREATMENTS

<table>
<thead>
<tr>
<th>Rate (kg/ha)</th>
<th>Cultivars</th>
<th>0 ppm</th>
<th>IAA 35 ppm</th>
<th>IAA 70 ppm</th>
<th>GA 35 ppm</th>
<th>GA 70 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>C-28</td>
<td>99.98 a</td>
<td>98.81 a</td>
<td>99.68 a</td>
<td>100.00 a</td>
<td>99.39 a</td>
</tr>
<tr>
<td></td>
<td>H-1370</td>
<td>99.14 a</td>
<td>99.74 a</td>
<td>98.20 a</td>
<td>99.44 a</td>
<td>99.78 a</td>
</tr>
<tr>
<td>0.28</td>
<td>C-28</td>
<td>99.80 a</td>
<td>96.15 a</td>
<td>98.86 a</td>
<td>99.66 a</td>
<td>99.46 a</td>
</tr>
<tr>
<td></td>
<td>H-1370</td>
<td>98.67 a</td>
<td>99.72 a</td>
<td>97.67 a</td>
<td>98.36 a</td>
<td>97.91 a</td>
</tr>
<tr>
<td>0.56</td>
<td>C-28</td>
<td>16.88 a</td>
<td>67.50 b</td>
<td>83.10 c</td>
<td>21.91 a</td>
<td>16.16 a</td>
</tr>
<tr>
<td></td>
<td>H-1370</td>
<td>4.98 a</td>
<td>49.74 b</td>
<td>61.73 b</td>
<td>13.74 a</td>
<td>15.58 a</td>
</tr>
</tbody>
</table>

1Means within row with similar letter are not significantly different at 5% level by L.S.D.

The hormone treatments did not affect the seedling stand of either cultivar with metribuzin (pre-emergence) application at 0.28 kg/ha.

On seedling height

The application of IAA at 35 and 70 ppm to C-28 seedlings with 0.28 kg/ha of metribuzin (pre-emergence) treatment did not result in increased plant height. GA at 35 and 70 ppm produced significantly
taller C-28 plants; on the other hand, IAA at 70 ppm produced significantly shorter H-1370 seedlings. GA produced taller seedlings of H-1370 at 35 and 70 ppm.

The application of IAA at 35 and 70 ppm to C-28 seedlings treated with 0.56 kg/ha of metribuzin (pre-emergence) did not affect seedling height. GA at 35 and 70 ppm produced taller C-28 plants. However, IAA at 35 and 70 ppm produced taller H-1370 seedlings. Taller H-1370 plants were produced with the application of 35 and 70 ppm of GA.

TABLE 13. THE INFLUENCE OF HORMONE TREATMENTS ON SEEDLING HEIGHT OF TWO CULTIVARS TREATED WITH PRE-EMERGENT APPLICATION OF METRIBUZIN (cm)

<table>
<thead>
<tr>
<th>Rate (kg/ha)</th>
<th>Cultivars</th>
<th>Hormone Treatments¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 ppm</td>
</tr>
<tr>
<td>0</td>
<td>C-28</td>
<td>11.11 a</td>
</tr>
<tr>
<td></td>
<td>H-1370</td>
<td>13.62 a</td>
</tr>
<tr>
<td>0.28</td>
<td>C-28</td>
<td>12.33 a</td>
</tr>
<tr>
<td></td>
<td>H-1370</td>
<td>15.00 b</td>
</tr>
<tr>
<td>0.56</td>
<td>C-28</td>
<td>10.01 a</td>
</tr>
<tr>
<td></td>
<td>H-1370</td>
<td>6.23 a</td>
</tr>
</tbody>
</table>

¹Means within row with similar letter are not significantly different at 5% level by L.S.D.

On dry weight of seedlings

There were changes in the dry weight of seedlings of C-28 and H-1370 treated with metribuzin (pre-emergence) with the application of IAA and GA (Table 14).

The C-28 seedlings treated with 0.28 kg/ha of metribuzin (pre-emergence) had lower dry weight when IAA at 70 ppm was applied. IAA at
35 ppm did not have any effect. GA showed similar significant trend. The application of 35 and 70 ppm IAA reduced the dry weight of seedlings of H-1370. GA at 35 ppm produced H-1370 seedlings with significantly greater dry weight but not with 70 ppm GA.

The C-28 seedlings treated with 0.56 kg/ha of metribuzin (pre-emergence) did not change in dry weight when 35 and 70 ppm IAA were applied. GA at 35 ppm produced C-28 seedlings with lesser dry weight. When GA was increased to 70 ppm, the dry weight of C-28 seedlings was significantly greater. Both IAA and GA each at 35 and 70 ppm produced significantly greater dry weight of seedlings of H-1370.

TABLE 14. THE DRY WEIGHT OF SEEDLINGS OF TWO CULTIVARS OF TOMATOES TREATED WITH PRE-EMERGENT APPLICATIONS OF METRIBUZIN AS AFFECTED BY HORMONE TREATMENTS (g/5 seedlings)

<table>
<thead>
<tr>
<th>Rate (kg/ha)</th>
<th>Cultivars</th>
<th>Hormone Treatments</th>
<th>0 ppm</th>
<th>IAA 35 ppm</th>
<th>IAA 70 ppm</th>
<th>GA 35 ppm</th>
<th>GA 70 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>C-28</td>
<td></td>
<td>0.36 c</td>
<td>0.30 b</td>
<td>0.25 a</td>
<td>0.30 b</td>
<td>0.29 a</td>
</tr>
<tr>
<td></td>
<td>H-1370</td>
<td></td>
<td>0.38 b</td>
<td>0.35 a</td>
<td>0.33 a</td>
<td>0.40 b</td>
<td>0.35 a</td>
</tr>
<tr>
<td>0.28</td>
<td>C-28</td>
<td></td>
<td>0.32 b</td>
<td>0.29ab</td>
<td>0.26 a</td>
<td>0.30 b</td>
<td>0.26 a</td>
</tr>
<tr>
<td></td>
<td>H-1370</td>
<td></td>
<td>0.40 b</td>
<td>0.33 a</td>
<td>0.34 a</td>
<td>0.41 b</td>
<td>0.33 a</td>
</tr>
<tr>
<td>0.56</td>
<td>C-28</td>
<td></td>
<td>0.26ab</td>
<td>0.27bc</td>
<td>0.23 a</td>
<td>0.19 a</td>
<td>0.30 c</td>
</tr>
<tr>
<td></td>
<td>H-1370</td>
<td></td>
<td>0.13 a</td>
<td>0.28bc</td>
<td>0.26 b</td>
<td>0.33 d</td>
<td>0.31cd</td>
</tr>
</tbody>
</table>

¹Means within row with similar letter are not significantly different at 5% level by L.S.D.
Peroxidase activity

Peroxidase levels in the C-28 and H-1370 seedlings treated (pre-emergence) with 0.56 kg/ha of metribuzin were reduced as a result of IAA application at 70 ppm (Table 15).

TABLE 15. THE PEROXIDASE LEVELS IN THE SEEDLINGS OF TWO CULTIVARS OF TOMATOES TREATED (PRE-EMERGENCE) WITH 0.56 KG/HA OF METRIBUZIN AS AFFECTED BY THE APPLICATION OF 70 PPM OF IAA (iu/g fresh weight)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>C-28</th>
<th>H-1370</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>38.57</td>
<td>39.30</td>
</tr>
<tr>
<td>0 kg/ha metribuzin + 70 ppm IAA</td>
<td>24.28</td>
<td>26.43</td>
</tr>
<tr>
<td>0.56 kg/ha metribuzin</td>
<td>60.00</td>
<td>74.30</td>
</tr>
<tr>
<td>0.56 kg/ha metribuzin + 70 ppm IAA</td>
<td>37.14</td>
<td>42.85</td>
</tr>
</tbody>
</table>

The isozyme component 5a which was absent in the H-1370 seedlings treated (pre-emergence) with 0.56 kg/ha of metribuzin, appeared when IAA at 70 ppm was applied (Fig. 7). Thus, similar isozyme components of peroxidase were found in the C-28 and H-1370 seedlings at 3 weeks after emergence.
Fig. 7. A diagrammatic representation of the isozyme components of peroxidase in the two cultivars of tomatoes treated (pre-emergence) with 0.56 kg/ha of metribuzin as affected by 70 ppm of IAA.
DISCUSSION

In the light of the results obtained in this study, the discussion on the response of the tomato cultivars to metribuzin will be considered in two ways.

The first type of response to metribuzin was the stimulation of growth. The increases in dry weight measurements in the 1974 and 1975 seasons with the application of 0.28 kg/ha of metribuzin (Table 4), follows the observations reported by various workers on triazine effects in different plants (13, 15, 22, 50, 51). These workers indicated that the increase in dry weight measurements correlated with the increase in nitrogen fractions. The marketable fruit yield was increased similarly at 0.28 kg/ha of metribuzin (Table 6). The response to sub-lethal concentration of triazine application could be explained as the effect on the physiological and biochemical events which favored more utilization of carbohydrate materials for nitrate reduction and synthesis of amino acids and proteins. In sweet corn and peas, the sub-lethal application of various s-triazines caused an increase in the activities of different enzyme systems such as starch phosphorylase, pyruvic kinase, cytochrome oxidase, and glutamate dehydrogenase (72). Apparently, similar enzyme stimulation occurred in this study. It would be of considerable interest to determine if the same enzyme activities are indeed affected.

The second type of response to metribuzin was the suppression in growth which resulted in dwarfness or death. The results presented
in this paper clearly showed that metribuzin at all levels of application stimulated the increase in peroxidase levels in tomato hypocotyl sections (Table 10). The increase in peroxidase levels could be considered as a function of rate of metribuzin used and the cultivar upon which the herbicide was applied. This indicated that the enhancement of enzyme production may be a part of a chain of events that could lead eventually to injury and death of susceptible cultivars.

Various workers have reported that the mechanism of action of the triazines, and specifically the herbicide metribuzin, is on the inhibition of photosynthesis. The particular site of action is the oxygen evolution step in photosystem II (3, 32, 41, 54, 69).

However, the speed by which the affected seedlings die, imply that the herbicidal activity involves more than just the inhibition of photosynthesis. Moreover, reports from previous workers indicated that exogenous sucrose application did not counteract herbicidal injury (16). In this study, the observations obtained showed that the affected seedlings did not simply die of starvation for lack of photosynthate.

The timing of the appearance of the response and the rate by which the injury resulted in death showed that these were effects rather than the cause of metribuzin toxicity.

Numerous other effects were reported, that includes the swelling of the intergranal thylakoids, disruption of the tonoplast and chloroplast envelopes, and the thylakoid membranes (2, 3, 32).

In this study, the hypocotyl sections of seedlings of the two cultivars had similar peroxidase levels at 1, 2, 3, and 4 weeks after emergence (Table 8). However, the peroxidase levels were increased
with metribuzin concentration and the inhibition in seedling growth was parallel to these increases. Therefore, in the light of these results, it can be considered that the increase in peroxidase levels was an earlier response to herbicide treatment.

The physiological roles of peroxidase has been well documented (5, 10, 12, 20, 30, 43, 48, 63, 66). The exact roles of the enzyme are not well known but morphogenetic roles have been suggested by its action in producing (49) and inactivating auxins (18), stimulate the lignification of tissues (58), and the oxidation of important metabolic compounds such as the nicotinamide adenine dinucleotide and its phosphate (71). It would be interesting to find if these effects could be true in metribuzin susceptible tomato cultivars.

In this study, only the effect of peroxidase on auxin degradation was considered and explored. Peroxidase may have reduced endogenous auxin levels considerably as a result of metribuzin application. If this mechanism of action operated in cultivars with differential tolerances, then the exogenous application of IAA and GA to young seedlings should be able to counteract or prevent metribuzin injury, thereby improve seedling stand and restore normal growth.

The results of the study on the effect of hormone treatments (Tables 12, 13, and 14) indicated that this was true, except for GA. The hormones IAA and GA were each applied exogenously as spray mist to the point of run-off at two concentrations, hopefully to compensate for any loss in auxin activity.

IAA at either concentration had a profound effect in increasing seedling stand and growth of both cultivars, with C-28 having a greater
response compared with H-1370. The effect could be due to the reduction in peroxidase levels resulting from the IAA treatments (Table 15). It is of interest to find that the differential response of C-28 and H-1370 paralleled the results obtained in the degree of increase in peroxidase levels in each cultivar. It therefore appears that IAA deficiency in very young seedlings as a result of increased degradation by metribuzin-induced increase in enzyme levels was involved in herbicidal toxicity. Furthermore, the observation in this study, in which seedlings with well-developed first pair of true leaves could very well escape injury presumably due to increased auxin production, should add more truth to this contention.

GA at either concentration did not counteract metribuzin toxicity. It did not eliminate the visual symptoms of herbicide injury. However, GA stimulated the growth of seedlings that survived metribuzin injury. Therefore, the stimulation of growth by GA appears to be independent of metribuzin injury. Since exogenous GA did not cause a recovery or improvement in seedling stand, it is unlikely that the herbicidal action is mediated through the interference of endogenous GA levels. Although GA is involved in the promotion of auxin production (4), it is likely that the auxin so produced was not enough to counteract metribuzin injury.
SUMMARY

Experiments in the greenhouse and field were conducted with the following objectives: (1) to determine the performance of two tomato cultivars, C-28 and H-1370, with differential tolerance to metribuzin under field conditions; (2) to determine the relative levels of the enzyme peroxidase in the two cultivars as influenced by the application of metribuzin; and (3) to determine the influence of hormone treatments on the injury caused by metribuzin. In line with above objectives, measurements on germination, stand, height, dry weight, fruit set, yield in terms of marketable and non-marketable fruits based on processing grades, enzyme assay for peroxidase and visual observations were recorded. The summary of results are as follows:

1. Metribuzin did not affect seedling emergence.

2. There were reductions in seedling stand in the greenhouse and field for both cultivars.

3. Seedling height of C-28 was reduced by metribuzin but not as much as H-1370.

4. The dry weight of seedlings of C-28 and H-1370 were affected by 0.56 kg/ha of metribuzin. The seedlings tended to have greater dry weight with 0.28 kg/ha of metribuzin application.

5. The percentage of fruit set was not affected for the C-28 in the field in 1974 and 1975. However, H-1370 showed a lower percentage of fruit set resulting from metribuzin application.
6. There was a trend to increase marketable yield. C-28 had more marked increases in marketable yield than H-1370 with the increase in the rate of metribuzin application.

7. The non-marketable yield of both cultivars was not affected by metribuzin treatments.

8. There was a decreasing trend in peroxidase levels at 1, 2, 3, and 4 weeks after emergence in the C-28 seedlings. H-1370 seedlings showed an increasing trend in peroxidase levels. Significant changes in isozyme components of peroxidase in the two cultivars were observed.

9. Peroxidase levels increased with the increase in the rate of metribuzin application in both cultivars. However, the increase in peroxidase levels in C-28 seedlings was not as much as that obtained from H-1370 with the application of 0.56 kg/ha of metribuzin. Significant changes in isozyme patterns were observed in both cultivars resulting from the herbicide treatments.

10. IAA at 35 and 70 ppm improved the seedling stand of C-28 and H-1370 treated (pre-emergence) with 0.56 kg/ha of metribuzin.

11. Taller plants were produced resulting from GA treatments on those that survived in both cultivars. IAA at 35 and 70 ppm produced taller H-1370 seedlings treated with 0.56 kg/ha metribuzin.

12. IAA at 70 ppm had a tendency to reduce dry weight of the C-28 seedlings treated with 0.28 kg/ha of metribuzin. IAA at 35 ppm produced greater dry weight of H-1370 seedlings treated (pre-emergence) with 0.56 kg/ha of metribuzin.

13. IAA at 70 ppm reduced peroxidase levels in C-28 and H-1370 seedlings treated (pre-emergence) with 0.56 kg/ha metribuzin.
14. Similar isozyme components were observed in C-28 and H-1370 seedlings treated (pre-emergence) with 0.56 kg/ha metribuzin when IAA at 70 ppm was applied.

Based on the information obtained from this study, it appeared that peroxidase-auxin interaction might be involved in the series of events that eventually lead to injury as a result of metribuzin toxicity. The results and observations gathered from this study cast doubt on the inhibition of photosynthesis as the major mechanism of action of metribuzin.
### APPENDIX

**TABLE 1. THE MEAN WEEKLY DAY AND NIGHT TEMPERATURE IN THE GREENHOUSE**

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
</tr>
<tr>
<td>December 5-11, 1975</td>
<td>24.9</td>
</tr>
<tr>
<td>December 12-18, 1975</td>
<td>25.0</td>
</tr>
<tr>
<td>December 19-25, 1975</td>
<td>26.4</td>
</tr>
<tr>
<td>January 2-8, 1976</td>
<td>25.0</td>
</tr>
<tr>
<td>January 9-15, 1976</td>
<td>26.1</td>
</tr>
<tr>
<td>January 16-22, 1976</td>
<td>22.1</td>
</tr>
<tr>
<td>January 23-29, 1976</td>
<td>26.3</td>
</tr>
<tr>
<td>January 30-February 5; 1976</td>
<td>26.9</td>
</tr>
<tr>
<td>February 6-12, 1976</td>
<td>21.3</td>
</tr>
<tr>
<td>February 13-19, 1976</td>
<td>23.1</td>
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<tr>
<td>February 20-26, 1976</td>
<td>22.4</td>
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<tr>
<td>February 27-March 4, 1976</td>
<td>24.4</td>
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<tr>
<td>May 4-10, 1976</td>
<td>20.6</td>
</tr>
<tr>
<td>May 11-16, 1976</td>
<td>21.9</td>
</tr>
<tr>
<td>May 17-23, 1976</td>
<td>21.5</td>
</tr>
<tr>
<td>May 24-June 1, 1976</td>
<td>21.4</td>
</tr>
</tbody>
</table>

1. Taken 20 cm above the ground floor.
1. C-28 no treatments 3. C-28 with 70 ppm IAA
2. H-1370 no treatments 4. H-1370 with 70 ppm IAA
5. C-28 with 0.56 kg/ha metribuzin (pre-emergence)
6. H-1370 with 0.56 kg/ha metribuzin (pre-emergence)
7. C-28 with 0.56 kg/ha metribuzin (pre-emergence) + 70 ppm IAA
8. H-1370 with 0.56 kg/ha metribuzin (pre-emergence) + 70 ppm IAA

Appendix Fig. 1. The isozyme components of peroxidase in C-28 and H-1370 seedlings at 3 weeks from emergence.
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