Characterization of the joint effects of tridiphane and paraquat in soybean

Christopher Paul Dionigi
Iowa State University

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Characterization of the joint effects of tridiphane and paraquat in soybean

Dionigi, Christopher Paul, Ph.D.

Iowa State University, 1989
Characterization of the joint effects of tridiphane
and paraquat in soybean

by

Christopher Paul Dionigi

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Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Agronomy
Major: Crop Production and Physiology

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In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

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For the Graduate College

Iowa State University
Ames, Iowa

1989
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GENERAL INTRODUCTION

Background

Effective postemergence grass control in corn can be difficult to achieve, and often requires the application of triazine herbicides. To increase the efficacy of postemergence grass control with triazine herbicides, the herbicide tridiphane [2-(3, 5-dichlorophenyl)-2-(2,2,2-trichloroethyl)oxirane] was developed by the Dow Chemical Company.

Tridiphane is unique, as it is the only registered oxirane type herbicide and is the only herbicide marketed as a synergist of herbicide activity. The application of tridiphane with triazine herbicides, such as atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine], enhances the level of postemergence grass and broadleaf weed control above that achieved by application of atrazine alone (24). Although early investigations indicated that tridiphane may enhance weed control by increasing the absorption of atrazine by plant leaves (3), more extensive studies found that tridiphane does not increase the adsorption of atrazine by giant foxtail (Setaria faber) leaves; and the enhancement of weed control by tridiphane was due in part to the inhibition of detoxification of the atrazine (19).

In leaf tissue, glutathione-S-transferase (GST) (EC 2.5.1.18) detoxifies triazine herbicides by attaching the tripeptide glutathione (GSH) to the herbicide molecule (22), and both tridiphane and the GSH of conjugate of tridiphane were found to be potent inhibitors of GST (16). Only triphenyl tin chloride was found to be a more potent inhibitor of GST activity. By inhibiting GST activity, tridiphane can increase the potency
of triazine (19), and several other types of herbicides (10) and insecticides (17) that are detoxified by GST.

In addition to increasing the potency of other herbicides, tridiphane alone can inhibit the growth of certain grass species (1, 2, and 15). Although the mechanism of this growth inhibition is not fully known, Hillton and Pillai (15) found that exposure to tridiphane reduced the amount of low molecular weight thiols in plant leaf tissue, and that supplying exogenous cysteine increased low molecular weight thiol levels and reduced tridiphane-induced inhibition of plant growth. It was hypothesized that tridiphane inhibits plant growth by inhibiting the synthesis of cysteine or GSH. However, the level of low molecular weight thiol reduction associated with the addition of tridiphane was similar to the level associated with the application of atrazine, and therefore may have been due to the depletion of GSH levels by the formation of the GSH-tridiphane conjugate rather than inhibition of GSH synthesis. No evidence was provided concerning the effect of exogenous cysteine on the levels of tridiphane or metabolites of tridiphane in the plant tissue.

In addition to functioning in the detoxification of xenobiotics, GSH has several other functions in plant cells (18, 21) and is a component of the chloroplast's antioxidant system (12). The herbicide paraquat [1'1-dimethyl-4,4'bipyridinium ion] induces an oxidative stress in plant tissue by single electron transfer from photosystem I (9). Because paraquat is not detoxified by GST, it was hypothesized that if tridiphane increased the potency of paraquat-induced oxidative stress in leaf tissue, it would not be due to an inhibition of detoxification, but may be due instead to a
reduction in GSH levels (G. Ezra and J. H. Dekker, Iowa State University, personal communication, 1985). Field investigations were conducted to test this hypothesis, and results indicated an increase in the visual rating of percent oxidative leaf injury in plants exposed to tridiphane and paraquat compared to plants exposed to an equal dosage of paraquat alone (7, 8). It was hypothesized that exposure to tridiphane potentiates oxidative stress in leaf tissue treated with paraquat, possibly by an inhibition of the chloroplast's antioxidant system.

Objectives

The objectives of this research were to characterize the joint effects of tridiphane and paraquat in soybean and to determine the mechanisms that produce these effects.

Characterization of the Joint Effects of Herbicides

In order to characterize the joint effects of two herbicides, i.e., to determine if the joint effects are additive, antagonistic or synergistic, requires the use of a reference model. These models generate an expected response value for the combined application of two herbicides based upon the actual response produced by each herbicide when applied alone. If the observed combined response is less than, equal to, or greater than the expected combined response generated by the model, the joint effects are characterized as either antagonistic, additive, or synergistic, respectively (14). However, it is important to note that deviations between observed and expected values represent a deviation from a model-
generated expectation and may not be due to a toxicological interaction between the component herbicides (14).

There are two main types of reference models: multiplicative survival models (MSM), and additive dose models (ADM).

Multiplicative survival models are typified by the model derived by Gowing (13):

\[ E = A + B(100-A) \]

100

where \( E \) = the expected percent inhibition produced by herbicide A plus herbicide B; if \( A \) = the percent inhibition by herbicide A alone, and \( B \) = the percent inhibition by herbicide B alone.

This model was modified by Colby (5) so that the joint effects of two herbicides could be described in terms of percent of control rather than percent inhibition. The Colby MSM is the most widely used reference model in the herbicide interaction literature (11).

Additive dose models are typified by the analysis of variance (ANOVA) (23) model which in its most simplified form is:

\[ E = A + B - C \]

where \( E \) = the expected response value to the application of herbicide A plus herbicide B; if \( A \) = the response to herbicide a alone, \( B \) = the response to herbicide b alone, and \( C \) = a correction for the response of untreated controls, and in most cases \( C \) is equal to zero.

Multiplicative survival models and ADM will not produce the same expected values from the same data set (14), and thus reference model selection can confound the interpretation of results. However, MSM and
ADM will agree if one of the component herbicides of the mixture is applied at a dosage that on its own is ineffective (14). However, the joint effects of two herbicides may be different when both are applied at effective dosages. Therefore, both ineffective and effective dosages of the component herbicides should be investigated.

All dosage response data were subjected to a standard ANOVA procedure (20); and because regression analysis is considered the most appropriate and informative method for the analysis of factorial experiments in which the treatments are graded levels of a quantitative factor, such as several dosages of a herbicide (4, 23), regression analysis was used to provide first and second order polynomial dosage response models.
SECTION I.
EFFECTS OF TRIDIPHANE ON PARAQUAT-INDUCED LEAF INJURY,
INHIBITION OF GROWTH OF FIELD-GROWN SOYBEAN,
CORN, AND GIANT FOXTAIL PLANTS
Investigations were conducted to determine the effect of tridiphane on paraquat-induced injury in field-grown plants. Tridiphane was applied one day prior to paraquat and concurrently with paraquat to field plots containing soybean, corn, and giant foxtail plants. Visual ratings of percent necrotic leaf injury indicate that, in each species, the addition of tridiphane to paraquat increased leaf injury compared to the injury produced by an equal dosage of paraquat alone. This may indicate an increased level of oxidative stress in tridiphane treated leaf tissue.

Investigations of the effects of pretreatment with tridiphane on paraquat-induced inhibition of soybean plant growth indicate that, in 1986, under environmental conditions that inhibited growth in plant height, tridiphane increased the potency of paraquat-induced inhibition of height accumulation compared to the inhibition produced by paraquat applied alone. However, in 1987, environmental conditions did not inhibit soybean plant growth, and tridiphane did not increase the potency of paraquat-induced inhibition of soybean height or dry weight accumulation. These results indicate exposure to tridiphane may affect the potency of paraquat only under certain environmental conditions. Soybean plants exposed to tridiphane appeared to develop less new leaf tissue, and in 1987, accumulated plant height less rapidly than plants not exposed to tridiphane. This inhibition of new growth by tridiphane may have contributed to the increases in paraquat-induced leaf injury and inhibition of plant height accumulation.

Nomenclature: tridiphane, 2-(3, 5-dichlorophenyl)-2-(2,2,2-trichloroethyl)oxirane; paraquat, 1'1-
dimethyl-4,4′bipyridinium ion; giant foxtail, *Setaria faberii*; soybean, *Glycine max* L.; corn, *Zea mays* L.
INTRODUCTION

Tridiphane is a selective herbicide used in combination with triazine herbicides to enhance the control of grass and broadleaf weeds in crops such as corn. Tridiphane enhances weed control by the competitive inhibition of glutathione-S-transferase (E.C. 2.5.1.18) (GST) (9), which results in a reduced rate of detoxification of triazine (10) and certain other herbicides (7). In addition, tridiphane can inhibit plant growth in certain species (1, 2, 8). However, the mechanism of this growth inhibition has not been determined.

Paraquat is used for the nonselective control of plants. In chloroplasts, paraquat rapidly induces an oxidative stress by single electron transfer from photosystem I, resulting in the generation of potentially destructive free radicals (11). If not quenched, these free radicals rapidly destroy chloroplast and cell function (6).

Paraquat is not detoxified by GST (11). Therefore, tridiphane would not be expected to increase the potency of paraquat. However, field research indicates tridiphane may increase the herbicide injury in certain crop and weed species exposed to paraquat (3, 4, 5), but this research was not designed to specifically examine the combined effects of tridiphane and paraquat, and no data concerning plant growth were obtained.

The objective of this investigation was to determine the effect of tridiphane on paraquat-induced necrotic leaf injury in corn, soybean, and giant foxtail and inhibition of soybean plant growth under field conditions.
MATERIALS AND METHODS

The effects of tridiphane on leaf injury in giant foxtail, soybean, and corn plants exposed to paraquat were investigated in the field in 1986. In addition, a separate investigation of the effects of tridiphane on paraquat-induced inhibition of height and dry weight accumulation in field-grown soybean plants was conducted in 1986 and in 1987. Plant establishment and herbicide application methods used in these experiments are presented in Table 1, and the environmental conditions during these experiments are presented in Table 2.

Determination of Leaf Injury

The leaf injury experiment was conducted in a field near Napier, Iowa with Harps loam, Webster clay loam, and Okoboji silty clay loam soil types. The organic matter was 3.8% and the pH 6.7. Each plot contained two rows of corn, two rows of soybeans, a heavy natural infestation of giant foxtail, and a scattered population of other weed species. At the time of herbicide application, the plant species were in early reproductive growth stages.

Herbicide treatments

Herbicide treatments consisted of paraquat applied alone, paraquat applied following pretreatment with tridiphane, and paraquat applied concurrently with tridiphane. Paraquat was applied with 0.25% (v/v)
Table 1. Plant establishment and herbicide application methods used to investigate the effects of tridiphane on leaf injury, and paraquat-induced inhibition of height and dry weight accumulation.

<table>
<thead>
<tr>
<th>Leaf injury</th>
<th>Height and dry weight 1986</th>
<th>Height and dry weight 1987</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locations (Iowa county)</td>
<td>Napier (Story)</td>
<td>Ames (Story)</td>
</tr>
<tr>
<td>Experimental design (blocks)</td>
<td>RCB (3)</td>
<td>RCB (4)</td>
</tr>
<tr>
<td>Crop species (c.v.)</td>
<td>Glycine max (Corsoy 79)</td>
<td>G. max (Corsoy 79)</td>
</tr>
<tr>
<td>Zea mays (AP 391)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population (seeds/ha)</td>
<td>G. max (380,000)</td>
<td>G. max (383,000)</td>
</tr>
<tr>
<td>Z. mays (54,000)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Planting date</td>
<td>6/11/86</td>
<td>8/4/86</td>
</tr>
<tr>
<td>Weed control</td>
<td>None</td>
<td>Hand weeding</td>
</tr>
<tr>
<td>Row spacing (rows/plot)</td>
<td>76 cm (4)</td>
<td>76 cm (4)</td>
</tr>
<tr>
<td>Row direction</td>
<td>East/West</td>
<td>East/West</td>
</tr>
<tr>
<td>Tridiphane application date</td>
<td>7/15/86</td>
<td>9/3/86</td>
</tr>
<tr>
<td>Paraquat application date</td>
<td>7/16/86</td>
<td>9/4/86</td>
</tr>
<tr>
<td>Volume (L/ha)</td>
<td>187</td>
<td>187</td>
</tr>
<tr>
<td>Pressure (kPa)</td>
<td>152</td>
<td>152</td>
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</table>

Abbreviations: RCB - randomized complete block, c.v. - crop variety, and RH - relative humidity.
Table 1. Continued

<table>
<thead>
<tr>
<th></th>
<th>Leaf injury</th>
<th>Height and dry weight</th>
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<tr>
<td></td>
<td>1986</td>
<td>1987</td>
</tr>
<tr>
<td>Air temperature at application (°C)</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>RH at application (%)</td>
<td>75</td>
<td>60</td>
</tr>
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</table>
Table 2. Environmental conditions from the date of planting to the last data collection date for experiments to investigate the effects of tridiphane on leaf injury, and paraquat-induced inhibition of height and dry weight accumulation. Temperature data are followed by standard deviation of the mean.

<table>
<thead>
<tr>
<th>Locations (Iowa county)</th>
<th>Napier (Story)</th>
<th>Ames (Story)</th>
<th>Boone (Boone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dates of experiment</td>
<td>6/11/86 to 7/30/86</td>
<td>8/4/86 to 9/25/86</td>
<td>5/22/87 to 7/28/87</td>
</tr>
<tr>
<td>Temp (°C):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean min</td>
<td>17.9 (2.9)</td>
<td>13.8 (3.8)</td>
<td>16.4 (3.9)</td>
</tr>
<tr>
<td>Mean max</td>
<td>29.4 (2.6)</td>
<td>25.2 (3.3)</td>
<td>29.1 (3.9)</td>
</tr>
<tr>
<td>Mean ave</td>
<td>23.8 (3.4)</td>
<td>19.5 (3.2)</td>
<td>22.8 (3.6)</td>
</tr>
<tr>
<td>Precipitation (cm)</td>
<td>30.0</td>
<td>23.6</td>
<td>26.3</td>
</tr>
<tr>
<td>Accumulated GDD</td>
<td>1203.0</td>
<td>926.5</td>
<td>1503.5</td>
</tr>
</tbody>
</table>

*Abbreviations: temp = temperature, min = minimum, max = maximum, ave = average, and GDD = growing degree days [(max temp + min temp)/2.0]-50.
nonionic surfactant at 0.00, 0.07, 0.14, 0.28, and 0.56 kg ai/ha. Tridiphane was applied at 0.00 and 0.84 kg ai/ha. Pretreatments of tridiphane were applied one day before the paraquat alone and tridiphane plus paraquat mixture treatments. In addition to the above treatments, a 0.25% nonionic surfactant alone control treatment was also applied.

**Ratings of percent leaf injury**

Visual ratings of percent necrotic injury in leaf tissue were obtained 5 and 14 days after treatment (DAT) with paraquat. The change in percent leaf injury due to the presence of tridiphane was calculated by subtracting the percentage of leaf injury produced by paraquat applied alone from the percentage of injury produced by the tridiphane containing treatments at a comparable dosage of paraquat. For example:

\[
\% \text{ injury due to tridiphane in mixture} = (\% \text{ injury produced by paraquat-tridiphane treatment}) - (\% \text{ injury produced by the same dosage of paraquat applied alone}).
\]

Neither tridiphane nor the nonionic surfactant applied alone produced significant leaf injury compared to untreated controls, and these data were not used in the calculations. The standard error of the mean percent injury due to tridiphane in mixture was calculated for each treatment.

---

Determination of Growth Inhibition

In 1986, the growth inhibition experiments were conducted in a field near Ames, Iowa with Canisteo silty clay loam, Clarion loam, and Nicollet clay loam soil types. The organic matter was 4.7% and the pH 7.8. These experiments were repeated in 1987 in a field near Boone, Iowa with Clarion loam and Canisteo silty clay loam soil types. The organic matter was 3.6% and the pH 6.5.

Herbicide treatments

Herbicide treatments and dosages were the same as those used in the leaf injury investigation except that the nonionic surfactant alone and tridiphane applied concurrently with paraquat treatments were deleted.

Data analysis

Treatments were arranged as a complete factorial with herbicide treatment as the whole plot factor and time of sampling as the split plot factor. Heights were measured from the soil surface to the top of the plant canopy at 11, 18, and 21 DAT in 1986, and 9, 16, 21, and 23 DAT in 1987. Dry matter samples were obtained from within a subplot at 11, 18, and 21 DAT in 1986, and 9, 16, and 23 DAT in 1987. Each sample consisted of the above ground portions of ten plants and was dried to a constant weight at 60 C in a forced air oven. Once sampled, subplots were not sampled again. All data were subjected to factorial analysis of variance and regression analysis procedures.
RESULTS

Leaf Injury

At 14 days after treatment (DAT) with paraquat, exposure to tridiphane increased the percent leaf injury in at least one dosage of paraquat compared to the injury produced by an equal dosage of paraquat alone in each species tested (Figure 1). In addition, concurrent application of tridiphane and paraquat decreased leaf injury in corn compared to the injury produced by an equal dose of paraquat alone. Increases in leaf injury generally were more apparent at 14 DAT than at 5 DAT. Prior exposure to tridiphane increased paraquat-induced injury in more instances than did concurrent exposure with tridiphane.

Giant foxtail

In giant foxtail, concurrent application of tridiphane with paraquat did not affect percent leaf injury 5 DAT (Figure 1). However, an increase in injury was observed in plants receiving a pretreatment of tridiphane followed by 0.56 kg/ha paraquat at 5 DAT. The largest increases in injury were observed 14 DAT in plants receiving a concurrent application of tridiphane and 0.07 kg/ha paraquat and in plants receiving a pretreatment of tridiphane followed by 0.28 and 0.56 kg/ha paraquat.

Soybean

Of the species tested, the most consistent increases in leaf injury were observed 14 DAT in soybean plants receiving a pretreatment of tridiphane followed by 0.07, 0.14, and 0.28 kg/ha paraquat (Figure 1).
Figure 1. Effect of 0.84 kg/ha tridiphane on percent leaf injury in paraquat treated plants. Open bars = percent leaf injury produced by paraquat-tridiphane mixture treatments (mix% inj) minus percent injury produced by an equal dosage of paraquat alone (EDPA). Cross slashed bars = percent leaf injury produced by a pretreatment with tridiphane one day before paraquat (pretrt.% inj) minus percent injury produced by an EDPA. Data were obtained 5 and 14 days after treatment (DAT). Differences more than 2 SE units from the percent injury produced by an EDPA are indicated by an *. n=3
DIFFERENCE IN INJURY (%) vs PARAQUAT (kg/ha)
In addition, at 14 DAT, plants exposed concurrently to tridiphane and 0.07 kg/ha paraquat exhibited more leaf injury than plants exposed to a comparable dosage of paraquat alone. Examination of individual soybean plants indicated that plants treated with tridiphane and paraquat produced few new leaves and in some cases the plants appeared completely necrotic, whereas plants receiving paraquat alone exhibited some new leaf growth. At 5 DAT, neither pretreatment nor concurrent application of tridiphane affected the percentage of leaf injury relative that produce by an equal dose of paraquat alone.

Corn

Corn was the only species to exhibit a decrease in leaf injury due to exposure to tridiphane. For example, at 14 DAT, plants receiving tridiphane concurrently with 0.14 and 0.28 kg/ha paraquat exhibited less injury than did plants receiving an equal dose of paraquat alone (Figure 1). However, increases in leaf injury were observed in corn plants pretreated with tridiphane followed by 0.56 kg/ha paraquat at 5 and 14 DAT compared to plants receiving an equal dose of paraquat alone.

Height and Dry Weight Reduction

The effects of tridiphane

Several days after application, tridiphane alone produced a heart-shaped deformation in some of the uppermost leaves which was similar in appearance to the injury characteristic of an overdose of chloroactamide herbicide. This injury was not associated with lower plant dry matter
levels (Table 3). However, plants exposed to tridiphane had 5.5% and 11.3% lower plant heights in 1986 and 1987, respectively, than plants not exposed to tridiphane. In addition, in 1987, plants treated with tridiphane accumulated height less rapidly than plants not exposed to tridiphane (Table 3, Figure 2).

The effect of paraquat

Some of the plants exposed to the highest dosage of paraquat appeared completely necrotic, whereas plants exposed to lower dosages of paraquat exhibited less injury.

In both 1986 and 1987, plants exposed to paraquat were shorter and had less dry matter than plants not exposed to paraquat (Table 3, Figures 3 and 4). However, growing conditions were cooler and drier in 1986 compared to 1987 (Table 2), and plant growth and the response of plants to paraquat over time were somewhat different between these two years.

In 1986, as the dosage of paraquat was increased, the heights of plants decreased, but the plants were not growing taller during the experiment (Table 3), and plant height remained constant during the experiment (Figure 3). In 1986, plants not exposed to paraquat were accumulating dry matter, but exposure to increasing dosages of paraquat produced an increasing inhibition of growth, and the highest dosages of paraquat induced a loss of dry matter (Figure 3). In 1987, plants not exposed to paraquat accumulated both height and dry matter, and exposure to increasing dosages of paraquat produced an increasing inhibition of growth (Figure 4).
Table 3. Results of factorial analysis of variance of two time course investigations that were conducted in 1986 and 1987 to determine the effects of pretreatment with 0.00 or 0.84 kg/ha tridiphane on the height and dry weight accumulation in field-grown soybean plants exposed to 0.00, 0.07, 0.14, 0.28, and 0.56 kg/ha paraquat.

<table>
<thead>
<tr>
<th>Source</th>
<th>Height 1986</th>
<th>Height 1987</th>
<th>Dry weight 1986</th>
<th>Dry weight 1987</th>
</tr>
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<tbody>
<tr>
<td>Rep</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Tr</td>
<td>*</td>
<td>***</td>
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Figure 2. Height accumulation in field-grown soybean plants in 1987 averaged over 0.00, 0.07, 0.14, 0.28, and 0.56 kg/ha dosages of paraquat. Values followed by *** are significant at the 0.1% level. n=20
TIME AFTER PARAQUAT (DAYS)

HEIGHT (cm)

- O 0.00 TRIDIPHANE \( r^2 = 0.96^{***} \)
- • 0.84 TRIDIPHANE \( r^2 = 0.94^{***} \)
Figure 3. Height and dry weight accumulation in field-grown soybean plants in 1986 exposed to increasing dosages (kg/ha) of paraquat (PQ) averaged over 0.00 and 0.84 kg/ha tridiphane pretreatments. Dry weight samples contained ten plants each. Values followed by * are significant at the 5.0% level. Values followed by ** are significant at the 1.0% level, and *** at the 0.1% level. n=8
Figure 4. Height and dry weight accumulation in field-grown soybean plants in 1987 exposed to increasing dosages (kg/ha) of paraquat (PQ) averaged over 0.00 and 0.84 kg/ha tridiphane pretreatments. Dry weight samples contained ten plants each. Values followed by * are significant at the 5.0% level. Values followed by ** are significant at the 1.0% level, and *** at the 0.1% level. n=8
The combined effect of tridiphane and paraquat

In both 1986 and 1987, soybean plants exposed to tridiphane and paraquat appeared to develop less new leaf tissue than plants exposed to an equal dosage of paraquat alone. In 1986, when plants were not accumulating height (Table 3), pretreatment with tridiphane increased the potency of paraquat-induced inhibition of height accumulation at the intermediate dosages of paraquat compared to plants exposed to paraquat alone (Table 3, Figure 5). However, tridiphane did not affect the potency of paraquat-induced inhibition of dry matter accumulation in 1986 or height and dry weight accumulation in 1987 (Table 3).
Figure 5. Height of field-grown soybean plants in 1986 exposed to 0.0 or 0.84 (kg/ha) tridiphane one day before treatment with paraquat. Data are averaged over 9, 18, and 21 days after paraquat application sampling dates. Values followed by *** are significant at the 0.01% level. n=12
DISCUSSION

Leaf Injury

While tridiphane alone produces little visible leaf injury, the addition of tridiphane increased the percentage of necrotic injury produced by at least one dosage of paraquat in each plant species tested. These data support earlier reports of a possible potentiation of paraquat-induced injury by tridiphane (3, 4, 5) and may indicate an increase in the level of oxidative stress tridiphane treated plant tissue.

Height and Dry Weight Reduction

Examination of individual soybean plants indicates that tridiphane may inhibit upper leaf development which could reduce plant canopy height, whereas dry weight accumulation could continue from lateral leaf bud development and expansion. This could compensate for the effects of tridiphane on the more apical portions of the plant, and may be why exposure to tridiphane did not affect dry matter accumulation in either year, and why an increase in the potency of paraquat-induced inhibition of plant growth by tridiphane was only observed in plant height data.

This increase in herbicide potency was only observed under environmental conditions that inhibited growth in plant height, and was not observed when the plants were growing taller. This may indicate that tridiphane only increases the potency paraquat under certain environmental conditions.

In 1987, plants exposed to tridiphane accumulated plant height less rapidly than plants not treated with tridiphane, and in both years,
examination of individual soybean plants indicated an inhibition of new soybean leaf tissue in plants exposed to tridiphane and paraquat. This inhibition of new growth would become more apparent with time and may be why increases in leaf injury became more apparent at the latter evaluation date. In addition, an inhibition of new growth may have contributed to the increase in the potency of paraquat-induced inhibition of plant height accumulation.

Further research should be conducted to determine under what conditions and in what ways exposure to tridiphane affects the responses of plants to paraquat.
LITERATURE CITED


SECTION II.

CHARACTERIZATION OF THE COMBINED EFFECTS OF TRIDIPHANE AND PARAQUAT IN SOYBEAN (GLYCINE MAX)
ABSTRACT

Investigations were conducted to characterize the combined effects of tridiphane and paraquat in soybean leaves and whole plants. Chlorophyll bleaching and electrolyte leakage assays indicated that exposure to tridiphane does not induce an oxidative stress or affect the level of paraquat-induced oxidative stress in soybean leaf tissue. Whole plant fresh weight investigations indicate that the joint effects of tridiphane and paraquat are additive both in terms of tridiphane's effect on paraquat and paraquat's effect on tridiphane's potency. This research indicates that the increases in injury herbicide associated with addition of tridiphane to paraquat previously reported from the field were probably not due to an increased level of oxidative stress or herbicide potency.

Nomenclature: tridiphane, 2-(3, 5-dichlorophenyl)-2-(2,2,2-trichloroethyl)oxirane; paraquat, 1'1-dimethyl-4,4'bipyridinium ion; soybean, Glycine max L.
INTRODUCTION

Tridiphane is a selective herbicide used in combination with triazine herbicides to enhance grass and broadleaf weed control in crops such as corn, *Zea mays* L. Tridiphane enhances weed control by the competitive inhibition of glutathione-S-transferase (GST) (EC 2.5.1.18) (12), which reduces the rate of detoxification of triazine (13) and certain other herbicides (9). Tridiphane can also inhibit plant growth in certain species (2, 3, 11). However, the mechanism of growth inhibition by tridiphane has not been determined.

Paraquat is used for the nonselective control of plants. In chloroplasts, paraquat rapidly induces an oxidative stress by single electron transfer from photosystem I, resulting in the generation of free radicals (14). If not quenched, these free radicals rapidly destroy chloroplast and cell function (8).

Paraquat is not detoxified by GST (14), and, therefore, tridiphane would not be expected to increase the potency of paraquat. However, field research indicates that soybean, *Glycine max* L. plants exposed to tridiphane and paraquat exhibit a higher percentage of leaf necrosis than plants exposed to an equal dosage of paraquat alone, and under certain conditions, exposure to tridiphane can increase the potency of paraquat-induced inhibition of whole plant height accumulation.\(^1\) It has not been

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determined whether these increases in herbicide injury were due to an increase in oxidative stress, herbicide potency, or some other factor.

The level of paraquat-induced oxidative stress can be determined in the field using indices of chlorophyll bleaching (4), and electrolyte leakage can be used to determine the level of paraquat-induced oxidative stress under controlled environmental conditions (1).

To determine the combined effects of tridiphane and paraquat on herbicide potency, i.e., if the combined are antagonistic, additive, or synergistic, requires the selection and use of a reference model (10). However, reference model selection can alter the interpretation of results (10), and no clear criteria exist for the selection of reference models. This can be overcome by applying one of the herbicides at a noneffective dose, i.e., a dosage that alone does not produce a significant response.

The objectives of this investigation were to determine the effect of tridiphane on the potency of paraquat-induced oxidative stress in soybean leaf tissue and to determine the combined effects of these two herbicides on herbicide potency in soybean plants.
MATERIALS AND METHODS

In all the experiments, tridiphane and paraquat were applied sequentially one day apart. All experiments were replicated in time, and all data were subjected to factorial analysis of variance (14).

Determination of the Effects of Tridiphane on Paraquat-induced Oxidative Stress

Chlorophyll bleaching

Soybean "Corsoy 79" plants were established from seeds in 1986 near Napier, Iowa in a field containing Clarion, Nicollet, Webster, and Canisteo clay loam type soils with an organic matter content of 4.6% and a pH of 7.6. In 1987, the experiment was repeated near Ames, Iowa in a field containing Clarion and Canisteo silty clay loam type soils organic matter content of 3.6% and a pH of 6.5. Plant establishment and herbicide application methods used in these experiments are presented in Table 1.

Prior to herbicide application, a single soybean plant was randomly selected within each plot, and all other plants were removed. This was done to avoid shading of the treated plant tissue and to allow for a more consistent application of herbicides. Herbicide treatments were arranged as a complete factorial and applied when the plants were at the early pod fill stage. Tridiphane was applied in water with a CO₂ pressurized backpack sprayer at two dosage levels (0.0 and 0.8 kg ai/ha). Paraquat was applied by coating the adaxial surface of the center leaflet of the uppermost fully-expanded leaf with a latex foam paint brush saturated with
Table 1. Plot establishment and herbicide application methods used to determine the effects of tridiphane on paraquat-induced total chlorophyll bleaching in soybean leaf tissue

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*Abbreviations: RCB = randomized complete block, DBT = days before tridiphane application, and RH = relative humidity.*
either one mM paraquat plus 0.25% (v/v) nonionic surfactant$^2$ or 0.25% nonionic surfactant alone.

One day after paraquat application, six leaf disks (four mm in diameter) were excised from each treated leaflet, placed in 2 ml of 95% ethanol, and stored on ice in darkness. After one day of storage, no additional chlorophyll could be extracted with ethanol from the tissue, and total chlorophyll (chlorophyll a and b) levels of the ethanol extract were determined (15).

Electrolyte leakage

Soybean "Centennial 84" plants were established from seeds in a steam-pasteurized (74°C for 45 min) clay loam soil, coarse perlite, sphagnum peat moss (1:1:1, v/v) medium. Plants were watered as needed. Environment chamber conditions were photosynthetic photon flux density (PPFD) of 420 umol/m2/s, day length 24 h, temperature 25 C and RH 80%.

Treatments were arranged as a complete factorial within each of two completely randomized blocks. Pretreatments of either 2.3 kg ai/ha tridiphane, an equal amount of the tridiphane solvent blank$^3$, or no pretreatment were applied in water six days after planting when the unifoliate leaves of the soybean plants were unfolding and the first trifoliate leaflets were about three mm in length. After pretreatment, 40 disks (four mm in diameter) were excised from unifoliate leaves using a


$^3$The solvent blank of tridiphane was a gift from the Dow Chem. Co., Midland, MI.
cork borer, rinsed in four ml of 0.2 mM MES plus 0.1% (w/w) sucrose buffer with a pH of 6.5 three times, and placed in a plastic petri dish (60 mm in diameter) with four ml of fresh buffer. After one hour, the buffer solution was decanted and replaced with four ml of fresh buffer.

Paraquat was added in one ml of buffer under green safe lights (PPFD of less than 5 umol/m2/s) bringing the final volume in each petri dish to 5 ml and the final paraquat concentration to either 0.0, 5.0, 10.0, or 15.0 uM. Tissue disks were allowed to come to equilibrium with the buffer solutions in darkness for 12 h before raising light levels to a PPFD of 420 umol/m2/s. No leakage of electrolyte was observed during this dark period. Electroconductivity of the buffered media was determined after six and 12 h in the light using an Amber Science Inc., model 604 conductivity meter with a gold dip cell.

Determination of Combined Effects of Tridiphane and Paraquat

Determination of noneffective dosages

Centennial 84 soybean plants were established from seeds in 10 by 10 by 12 cm plastic pots filled with steam-pasteurized (70 C for 30 min) clay loam soil, coarse sand (3:1 v/v) medium. Greenhouse conditions were: temperature 18 to 35 C, RH ranged from 65% to 100%, and ambient sunlight was supplemented for 16 h each day by an additional PPFD of 220 umol/m2/s supplied by metal arc lamps.

^Methyl viologen from Sigma Chem. Co., St. Louis, MO.
Treatments were arranged as a complete factorial within four completely randomized blocks. Plants were exposed to a range of either tridiphane or paraquat dosages applied in 187 L/ha water at 240 kPa by an automated spray chamber six days after planting when the unifoliate leaves were unfolding and the first trifoliate leaves were about 3 mm in length. Fresh weight of the above soil portion of the soybean plants were obtained 7, 14, and 21 days after herbicide application. Paraquat was applied with 0.25% v/v nonionic surfactant, and the amount of tridiphane solvent blank was held constant among tridiphane treatments. The maximum dosage of each herbicide that could be applied and not reduce (P>0.05) plant fresh weight compared to untreated plants was determined for each herbicide.

Determination of the joint effects of tridiphane and paraquat Soybean plants were established and herbicides were applied using the same methods used for the noneffective dosage investigations. Pretreatments and second herbicide were applied six and seven days after planting, respectively. In the first experiment, tridiphane was applied at either 0.00 or 0.11 kg/ha, and in the second experiment tridiphane was applied at either 0.00 or 0.28 kg/ha one day before the application of 0.00, 0.01, 0.02, 0.03, 0.05, or 0.06 kg ai/ha paraquat. In the third experiment, paraquat was applied at either 0.00 or 0.01 kg/ha, and in the fourth experiment paraquat was applied at either 0.00 or 0.03 kg/ha one day before the application of 0.0, 0.3, 0.8, 1.4, 2.0, or 2.5 kg/ha tridiphane. Fresh weight data were obtained at 7, 14, and 21 days after the application of the second herbicide.
RESULTS

Joint Effects of Tridiphane and Paraquat in Soybean Leaf Tissue

Chlorophyll bleaching

Necrotic areas were observed on soybean leaves exposed to paraquat, but no necrosis was observed in leaves not exposed to paraquat. In 1987, exposure to paraquat reduced chlorophyll levels more than in 1986 (Figure 1). When averaged over all herbicide treatments, chlorophyll levels were 42% lower in 1987 than in 1986 (Table 2).

Exposure to tridiphane did not affect chlorophyll levels or increase the potency of paraquat-induced oxidative bleaching of chlorophyll (Table 2).

Electrolyte leakage

Leaf tissue not exposed to paraquat did not lose electrolytes and remained green during the experiment. After 6 h in the light, tissue exposed to paraquat lost electrolytes to the buffer; and after 12 h in the light, there was about 250% more electrolyte present in the buffer than after 6 h, and tissue exposed to paraquat appeared completely necrotic (Table 3, Figure 2).

Pretreatments of either tridiphane or the solvent blank of tridiphane did not result in the loss of electrolytes from the plant tissue and did not increase the potency of paraquat-induced electrolyte leakage (Table 3).
Table 2. Results of factorial analysis of variance of investigations to determine the effects of tridiphane pretreatment on chlorophyll a and b bleaching. Tridiphane was applied at 0.0 and 0.8 kg/ha, and paraquat at 0 and 1 mM to field-grown soybean leaves in 1986 and 1987.

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<td>Year<em>Tr</em>Pq</td>
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*Abbreviations: Rep = replication, Tr = tridiphane dosage level, Pq = paraquat dosage level, Pr>F = probability of a greater F, NS = more than 5%, and *** less than 0.1%.
Table 3. Results of factorial analysis of variance of a time course investigation to determine the effects of no pretreatment, pretreatment with tridiphane solvent blank, and pretreatment with tridiphane on paraquat-induced electrolyte leakage from soybean leaf disks. Data were obtained after six and 12 h in the light, and paraquat was applied at 0, 5, 10, 15 \text{uM}^a

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*Abbreviations: Rep = replication, Tr = tridiphane dosage level, Pq = paraquat dosage level, Time = h in the light, Pr>F = probability of a greater F, NS = more than 5%, * = less than 5%, and *** less than 0.1%. 


Figure 1. Paraquat-induced chlorophyll a and b bleaching in field-grown soybean leaflet tissue in 1986 and 1987. Data were averaged over 0.0 and 0.8 kg/ha dosages of tridiphane. Narrow bars are 2 SE of the mean. n=36
Figure 2. Electroconductivity-conductivity of five ml of buffer bathing 40, four mm in diameter soybean leaf disks exposed to paraquat. Data were obtained after six and 12 h in the light, and averaged over no tridiphane, 2.3 kg/ha tridiphane solvent blank, and 2.28 kg/ha tridiphane treatments. Narrow bars are 2 SE of the mean. n=12
Joint Effects of Tridiphane and Paraquat in Whole Soybean Plants

Noneffective dosages

The maximum dosage of herbicide that did not reduce (P>0.05) plant fresh weight compared to untreated plants was 0.11 and 0.01 kg/ha for tridiphane and paraquat, respectively.

The effect of tridiphane on paraquat

The highest dosages of paraquat produced nearly complete necrosis in the leaf tissue that came in contact with the spray droplets, whereas the lowest dosage of paraquat produced no visible necrotic injury.

Averaged over the 0.00 and 0.11 kg/ha pretreatment dosages of tridiphane, plants exposed to 0.01 kg/ha paraquat accumulated approximately the same amount of fresh weight as plants not exposed to paraquat (Figure 3). However, plants exposed to higher dosages of paraquat accumulated fresh weight less rapidly than plants exposed to 0.00 and 0.01 kg/ha paraquat (Table 4, Figure 3).

Averaged over 0.00 and 0.28 kg/ha pretreatment dosages of tridiphane, plants exposed to paraquat accumulated fresh weight less rapidly than plants not exposed to paraquat (Table 4, Figure 4).

Averaged over all other treatments, plants exposed to 0.11 kg/ha tridiphane accumulated 11% less fresh matter than plants not treated with tridiphane (Table 4). Plants exposed to 0.28 kg/ha tridiphane had 23% less fresh matter and accumulated fresh weight less rapidly than plants not exposed to tridiphane (Table 4, Figure 5).
Table 4. Results of factorial analysis of variance of two investigations to determine the effects of tridiphane on paraquat-induced inhibition of fresh weight accumulation in soybean. In number one, tridiphane was applied at 0.00 vs. 0.11 kg/ha, and in number two, at 0.00 vs 0.28 kg/ha. Paraquat was applied at 0.00, 0.01, 0.02, 0.03, 0.05, and 0.06 kg/ha. Data were obtained 7, 14, and 21 days after exposure to paraquat.

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Abbreviations: Rep = replication, Tr = tridiphane dosage level, Pq = paraquat dosage level, Time = days after exposure to paraquat, Pr>F = probability of a greater F, NS = more than 5%, and *** less than 0.1%.
Figure 3. The effects of a range of paraquat dosages on the rate of fresh weight accumulation in soybean plants. Data were averaged over 0.00 and 0.11 kg/ha pretreatments of tridiphane. All r2 values are significant at the 0.1% level.
TIME AFTER PARAQUAT (DAYS)

FRESH WEIGHT (g)

- △ 0.01 PQ $r^2=0.78$
- ○ 0.00 PQ $r^2=0.63$
- □ 0.02 PQ $r^2=0.80$
- ▼ 0.03 PQ $r^2=0.64$
- ◇ 0.05 PQ $r^2=0.73$
- ■ 0.06 PQ $r^2=0.49$
Figure 4. The effects of a range of paraquat dosages on the rate of fresh weight accumulation in soybean plants. Data were averaged over 0.00 and 0.28 kg/ha pretreatments of tridiphane. All r2 values are significant at the 0.1% level.
Figure 5. The effects of tridiphane on the rate of fresh weight accumulation in soybean plants. Data were averaged over 0.00 and 0.01, 0.02, 0.03, 0.05 and 0.06 kg/ha dosages of paraquat. All r² values are significant at the 0.1% level.
Neither the noneffective (0.11 kg/ha) nor the effective (0.28 kg/ha) dosages of tridiphane increased the potency of paraquat-induced inhibition of fresh weight accumulation (Table 4).

The effect of paraquat on tridiphane

The highest dosages of tridiphane produced a nearly complete inhibition of trifoliate leaf development, whereas the lowest dosage of tridiphane produced little visible injury. Exposure to tridiphane reduced the rate of plant fresh weight accumulation compared to plants not treated with tridiphane (Table 5, Figures 6 and 7).

Averaged over all other treatments, 0.01 kg/ha paraquat did not reduce the fresh weight of plants compared to plants not exposed to paraquat, but plants exposed to 0.03 kg/ha paraquat contained 14% less fresh matter than plants not exposed to paraquat (Table 5).

Neither the noneffective (0.01 kg/ha) nor the effective (0.03 kg/ha) pretreatment dosages of paraquat increased the potency of tridiphane-induced inhibition of fresh weight accumulation (Table 5).
Table 5. Results of factorial analysis of variance of two time investigations to determine the effects of paraquat pretreatment on tridiphane-induced inhibition of fresh weight accumulation in soybean. In number one, paraquat was applied at 0.00 vs. 0.01 kg/ha, and in number two, paraquat was applied at 0.00 or 0.03 kg/ha. Tridiphane was applied at 0.00, 0.3, 0.8, 1.4, 2.0, and 2.5 kg/ha. Data were obtained 7, 14, and 21 days after exposure to paraquat.

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<td>Time<em>Pq</em>Tr</td>
<td>NS</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: Rep = replication, Tr = tridiphane dosage level, Pq = paraquat dosage level, Time = days after exposure to paraquat, Pr>F = probability of a greater F, NS = more than 5%, and *** less than 0.1%.
Figure 6. The effects of a range of tridiphane dosages on the rate of fresh weight accumulation in soybean plants. Data were averaged over 0.00 and 0.01 kg/ha pretreatments of paraquat. All r² values are significant at the 0.1% level.
Figure 7. The effects of a range of tridiphane dosages on the rate of fresh weight accumulation in soybean plants. Data were averaged over 0.00 and 0.03 kg/ha pretreatments of paraquat. All r2 values are significant at the 0.1% level.
TIME AFTER TRIDIPHANE (DAYS)

FRESH WEIGHT (g)

○ ○ ○ 0.0 TR $r^2=0.45$

△ △ 0.3 TR $r^2=0.54$

□ □ □ 0.8 TR $r^2=0.55$

◇ ◇ ◇ 1.4 TR $r^2=0.47$

▽ ▽ ▽ 2.0 TR $r^2=0.47$

◆ ◆ ◆ 2.5 TR $r^2=0.40$
DISCUSSION

Reports of field investigations indicated that tridiphane can increase the level of paraquat-induced oxidative leaf injury, and under certain conditions, increase the potency of paraquat-induced inhibition of plant height accumulation. Chlorophyll bleaching and electrolyte leakage investigations indicate that tridiphane does not induce an oxidative stress or increase the potency of paraquat-induced oxidative stress in soybean leaf tissue under either field or controlled environmental conditions. In addition, investigations of the growth of whole soybean plants indicate that pretreatment with tridiphane does not increase the potency of paraquat, and pretreatment with paraquat does not increase the potency of tridiphane. Therefore, the increases in herbicide injury that have been previously reported were probably not due to an increased level of oxidative stress or an increase in herbicide potency.

Over the 21 day time course of the experiment, the combined effects of tridiphane and paraquat were additive. However, field experiments can be conducted for longer periods of time which would allow for the possible recovery of treated plants from herbicide injury. Tridiphane appeared to inhibit new leaf development, whereas paraquat induced necrosis in developed leaf tissue. In the field, plants exposed to the combined effects of tridiphane and paraquat may have been unable to recover from herbicide injury, and stress from competition or other environmental

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factors may have increased the mortality rate in these plants. Whereas, plants exposed to either herbicide alone may have a greater chance of recovery. Late season comparisons between the predicted response for the combined effects of tridiphane and paraquat (based upon data obtained from plants that may be recovering from exposure to either herbicide alone) with the observed response, obtained from nonrecovering plants, would fit the definition of a synergistic interaction (10), but in this case would not be the result of an increase in herbicide potency. Research should be conducted to test this hypothesis of a synergism that is not the result of an increase in herbicide potency.
LITERATURE CITED


SECTION III.

THE EFFECTS OF TRIDIPHANE ON THE GROWTH
AND STRUCTURAL MORPHOLOGY OF SOYBEAN LEAF TISSUE
ABSTRACT

The effects of tridiphane on leaf growth and structural morphology were investigated in developing first trifoliate and mature unifoliate leaf tissue of soybean plants grown in a controlled environment. Fourteen h after exposure to tridiphane, a disruption of parenchyma cells in the midrib of the trifoliate leaf was evident, but cells on the surface of the leaf lamella did not appear injured. At 44 h after exposure, treated trifoliate midrib and leaf lamella tissue appeared nearly completely disrupted. Treated plants did not accumulate new leaf tissue between 14 h and 44 h after exposure. After ten days, exposure to tridiphane resulted in a heart-shaped leaf margin in some of the trifoliate leaves which resembled injury produced by chloroacetamide herbicides. Although tridiphane produced necrotic areas on the surface of the unifoliate leaves, unifoliate leaf growth and midrib tissue were not affected. Results indicate that tridiphane inhibits soybean leaf growth and development and may have a mechanism of herbicide action similar to that of the chloroacetamide herbicides. Nomenclature: tridiphane, 2-(3, 5-dichlorophenyl)-2-(2,2,2-trichloroethyl)oxirane; soybean, Glycine max L.
INTRODUCTION

Tridiphane is a selective herbicide used in combination with triazine herbicides to enhance the control of grass and certain broadleaf weed species in crops such as corn. Tridiphane enhances weed control by inhibiting the detoxification of triazine (8) and certain other herbicides (6). In addition, tridiphane can inhibit the growth of certain grass species (1, 2, 7). However, the mechanism of growth inhibition by tridiphane has not been determined.

Paraquat (1'1-dimethyl-4,4'bipyridinium ion) is an oxidative stress-inducing herbicide (5) that is not detoxified by plant tissue (10). Therefore, tridiphane would not be expected to inhibit the detoxification of paraquat. However, field research indicates the addition of tridiphane to paraquat can increase percent leaf injury on soybean leaves and can increase inhibition of plant height accumulation, compared to the injury produced by an equal dosage of paraquat alone. It was hypothesized by Dionigi and Dekker (4) that these effects observed in the field may be due to a retardation of plant recovery from herbicide injury resulting from the additive effects of a paraquat-induced oxidative stress in mature leaf tissue and tridiphane-induced inhibition of new leaf development. While the effects of paraquat-induced oxidative stress in leaf tissue have been determined (5), the effects of tridiphane on leaf growth and structural morphology has not been investigated in any broadleaf species.

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The objective of this investigation was to determine the effects of tridiphane on the growth and structural morphology of soybean leaf tissue.
MATERIALS AND METHODS

Plant Establishment

Eight soybean *Glycine max* L. "Centennial 84" seeds were planted in 10 by 10 by 12 cm plastic pots filled with a steam-pasteurized (70 °C for 30 min) clay loam soil, coarse sand (3:1, v/v) medium and placed in a controlled environmental chamber. Plants were watered as needed and thinned to four plants per pot after emergence. Environmental conditions were as follows: photosynthetic photon flux density (PPFD) 210 umol/m²/s, day length 24 h, temperature 28 °C, and RH from 75% to 100%.

Treatments were arranged in completely randomized blocks, and all experiments were replicated in time.

Tridiphane application

Seven days after planting, either 2.3 kg ai/ha tridiphane, an equal amount of tridiphane solvent blank² or no herbicide was applied to each plant using an atomizer. At the time of herbicide application, the middle leaflet of the first trifoliate leaf was about six mm in length and unifoliate leaves were completely unfolded.

Tissue and data collection

Leaf tissue samples and length measurements were obtained at 14 h and from different plants at 44 h after herbicide application.

²Tridiphane solvent blank was a gift from the Dow Chemical Co., Midland, MI.
Two to three mm long samples of tissue were obtained from the tip of the middle leaflet of the first trifoliate leaf with a razor blade. The length of the trifoliate leaf tissue was measured from the apex of the first trifoliate leaf to the axis of the unifoliate leaves.

Four mm in diameter disks of tissue were obtained from the unifoliate leaf midrib at about four mm from the leaf tip using a cork borer. Tissue was also obtained from other locations on the unifoliate leaf and the leaf apex. The length of the unifoliate leaf was measured from the apex of the unifoliate leaf to the axis of the unifoliate leaf.

**Tissue Preparation**

After excision, tissue samples were immediately placed in a buffered (pH 7.2) fixative solution containing 4% glutaraldehyde, 2% paraformaldehyde, 84% 0.1M sodium phosphate buffer (v/v/v). After one day in fixative at 4°C, samples were washed three times for ten min each in 0.1M sodium phosphate buffer (pH 7.2), immersed in a 1% osmium tetroxide 99% 0.1M sodium phosphate buffer solution (w/v) for two h at 4°C, and then washed three times for ten min each with double distilled water.

Dehydration was by graded ethanol series (70%, 80%, 85%, 90%, 95%, 100%, 100%, and 100% ethanol). Transition to acetone was by 100% acetone, 100% ethanol (1:1, v/v) followed by two changes to 100% acetone. Dry tissue was embedded in resin (II), sectioned (two um thick) using a Reichert ultramicrotome with a glass knife, and stained with a warm aqueous solution of 1% methylene blue and 1% azure II (v/v/v).
Microscopy

Tissue sections were photographed on a bright field using a light microscope.
RESULTS

The Effect of Tridiphane on Trifoliate Leaf Tissue

At 14 h after exposure, tridiphane had no effect on the length of the trifoliate leaf tissue compared to the untreated tissue (Figure 1). However, parenchyma cells along the midrib were more irregular in shape than untreated parenchyma cells [Figure 2, A (P) vs. B (P)], yet cells on the surface of the treated leaflet lamella did not exhibit an irregular shape [Figure 2, A (P)].

At 44 h after exposure, treated plants had about the same amount of trifoliate leaf tissue that was present 14 h after exposure, whereas untreated plants grew an additional 16 mm of trifoliate leaf tissue between 14 h and 44 h after exposure (Figure 1). At 44 h after exposure, most cells in the midrib and lamella of the treated leaflets appeared necrotic, while the untreated tissue appeared uninjured (Figure 2, C vs. D).

At ten days after exposure, some of the treated trifoliate leaves exhibited a heart-shaped leaflet margin, whereas untreated leaflets were ovate (Figure 3, A vs. B). Treated plants exhibited less internode elongation than untreated plants (Figure 3, C vs. D).

Compounds used in the formulation of tridiphane did not affect either leaf growth or structural morphology (data not presented).

The Effect of Tridiphane on Unifoliate Leaf Tissue

The unifoliate leaves did not grow in length between 14 h and 44 h after exposure, and tridiphane did not affect the length of unifoliate
Figure 1. The length from the apex of the first trifoliate leaf to node of the unifoliate leaf (trifoliate leaf) and the length from the unifoliate node to the apex of the unifoliate leaf (unifoliate leaf) of soybean plants at 14 h and 44 h after exposure to either 0.0 or 2.3 kg/ha tridiphane. Narrow bars are ± two SE of the mean. n=8
TRIFOLIATE LEAF

UNTREATED

TREATED

UNIFOLIATE LEAF

UNTREATED

TREATED

LEAF LENGTH (mm)

TIME AFTER TRIDIPHANE (h)
Figure 2. Cross sections through the midrib near the apex of the middle leaflet of the first trifoliate leaf of soybean. Tridiphane dosage was either 0.0 or 2.3 kg/ha. A - tissue obtained 14 h after exposure to tridiphane; note the irregular shape of the parenchyma cells (p). B - untreated tissue obtained 14 h after application of tridiphane. C - tissue obtained 44 h after exposure to tridiphane; note necrotic cells. D - untreated tissue obtained 44 h after application of tridiphane. Bars=40 \( \mu \text{m} \)
Figure 3. Trifoliate leaves and whole soybean plants ten days after application of either 0.00 or 2.3 kg/ha tridiphane. A - treated trifoliate leaf; note heart-shaped leaf margin, bar = 10 mm. B - untreated trifoliate leaf, bar = 20 mm. C - treated plant; note stunted internodes, bar = 70 mm. D - untreated plant, bar = 70 mm
leaves either 14 h or 44 h after exposure (Figure 1). Treated unifoliate midrib tissue appeared similar to untreated midrib tissue at both 14 h (Figure 4, A vs. B) and 44 h (Figure 4, C vs. D) after exposure. At ten days after exposure, necrotic areas were observed on the surface of the treated unifoliate leaves, but the shape of the treated leaf appeared similar to that of the untreated leaf (Figure 4, E vs. F).

Tissue obtained from nonnecrotic areas on the unifoliate leaf and at the leaf apex appeared similar to untreated tissue (data not presented).
Figure 4. Cross sections of soybean unifoliate leaf midrib tissue. Tridiphane dosage was either 0.0 or 2.3 kg/ha. A - tissue obtained 14 h after exposure to tridiphane, bar = 40 µm. B - untreated tissue obtained 14 h after application of tridiphane, bar = 40 µm. C - tissue obtained 44 h after exposure to tridiphane, bar = 40 µm. D - untreated tissue obtained 44 h after application of tridiphane, bar = 40 µm. E - treated unifoliate leaf ten days after exposure to tridiphane, note necrotic areas, bar = 10 mm. F - untreated unifoliate leaf ten days after application of tridiphane, bar = 10 mm.
DISCUSSION

Injury was first observed in parenchyma cells in the midrib of the treated trifoliate leaves. Whereas, cells on the surface of the leaf lamella, that may have had more direct exposure to the herbicide spray droplets, appeared unaffected. This may indicate that midrib parenchyma cells are more sensitive to tridiphane than other cells. In addition, tridiphane may become concentrated in these cells. Tridiphane can volatilize from leaf tissue (8). Parenchyma cells in the interior of the leaf may retain more of the herbicide than cells on the leaf surface, or tridiphane may preferentially translocate to these cells.

Cellular expansion around necrotic tissue at the apex of the trifoliate leaflets produced a heart-shaped deformation in the treated leaflets which appeared similar to injury produced by chloroacetamide herbicides. In addition, tridiphane inhibited internode elongation, and a similar inhibition of growth was observed in pea hypocotyl following exposure to chloroacetamide herbicides (3). Although the biochemical mechanism of growth inhibition by the chloroacetamide herbicides and tridiphane has not been determined (9), the similarity between the injury symptoms suggests that tridiphane and chloroacetamide herbicides may have similar mechanisms of action.

It has been hypothesized that the additive effects of paraquat-induced oxidative stress in mature leaf tissue and tridiphane-induced inhibition of leaf development may inhibit recovery from herbicide injury in field-grown soybean plants (4). Results of this investigation indicate tridiphane primarily affects growing and developing leaf tissue and
support the hypothesis that tridiphane can inhibit the growth and retard
the recovery of soybean plants from herbicide injury.
LITERATURE CITED


GENERAL SUMMARY

Section I

Investigations were conducted to determine the effect of tridiphane on paraquat-induced injury in field-grown plants. Tridiphane was applied one day prior to paraquat and concurrently with paraquat to field plots containing soybean, corn, and giant foxtail plants. Visual ratings of percent necrotic leaf injury indicate, in each species, the addition of tridiphane to paraquat increased leaf injury compared to the injury produced by an equal dosage of paraquat alone. This may indicate an increased level of oxidative stress in tridiphane-treated leaf tissue.

Investigations of the effects of pretreatment with tridiphane on paraquat-induced inhibition of soybean plant growth indicate, in 1986, under environmental conditions that inhibited growth in plant height, tridiphane increased the potency of paraquat-induced inhibition of height accumulation compared to the inhibition produced by paraquat applied alone. However, in 1987, environmental conditions did not inhibit soybean plant growth, and tridiphane did not increase the potency of paraquat-induced inhibition of soybean height or dry weight accumulation. These results indicate exposure to tridiphane may affect the potency of paraquat only under certain environmental conditions. Soybean plants exposed to tridiphane appeared to develop less new leaf tissue, and in 1987, accumulated plant height less rapidly than plants not exposed to tridiphane. This inhibition of new growth by tridiphane may have contributed to the increases in paraquat-induced leaf injury and inhibition of plant height accumulation.
Section II

Investigations were conducted to characterize the combined effects of tridiphane and paraquat in soybean leaves and whole plants. Chlorophyll bleaching and electrolyte leakage assays indicated that exposure to tridiphane does not induce an oxidative stress or affect the level of paraquat-induced oxidative stress in soybean leaf tissue. Whole plant fresh weight investigations indicate the joint effects of tridiphane and paraquat are additive both in terms of tridiphane’s effect on paraquat and paraquat’s effect on tridiphane’s potency. This research indicates the increases in injury herbicide associated with addition of tridiphane to paraquat previously reported from the field were probably not due to an increased level of oxidative stress or herbicide potency.

Section III

The effects of tridiphane on leaf growth and structural morphology were investigated in developing first trifoliate and mature unifoliate leaf tissue of soybean plants grown in a controlled environment. Fourteen h after exposure to tridiphane, a disruption of parenchyma cells in the midrib of the trifoliate leaf was evident, but cells on the surface of the leaf lamella did not appear injured. At 44 h after exposure, treated trifoliate midrib and leaf lamella tissue appeared nearly completely disrupted. Treated plants did not accumulate new leaf tissue between 14 h and 44 h after exposure. After ten days, exposure to tridiphane resulted in a heart-shaped leaf margin in some of the trifoliate leaves which resembled injury produced by chloroacetamide herbicides. Although
tridiphane produced necrotic areas on the surface of the unifoliolate leaves, unifoliolate leaf growth and midrib tissue were not affected. Results indicate that tridiphane inhibits soybean leaf growth and development and may have a mechanism of herbicide action similar to that of the chloroacetamide herbicides.
REFERENCES CITED


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