Photosynthetic and growth responses of *Zea mays* L and four weed species following post-emergence treatments with mesotrione and atrazine†

J Earl Creech,1 Thomas A Monaco2∗ and John O Evans1

1Utah State University, Plants, Soils, and Biometeorology Department, 4820 Old Main Hill, Logan, UT 84322-4820, USA
2USDA-ARS Forage and Range Research Laboratory, Utah State University, Logan, UT 84322-6300, USA

Abstract: We compared photosynthesis and growth of *Zea mays* L (corn) and four weed species, *Setaria viridis* (L) Beauv (green foxtail), *Echinochloa crus-galli* (L) Beauv (barnyardgrass), *Abutilon theophrasti* Medic (velvetleaf), and *Amaranthus retroflexus* L (redroot pigweed), following foliar applications with atrazine, mesotrione, or a combination of atrazine and mesotrione in two greenhouse experiments. Plant responses to the three herbicide treatments were compared with responses of untreated plants (control). Photosynthesis on day 14 and dry mass of *Z m a y s* was not reduced by any of the herbicide treatments. Photosynthesis and dry mass of *E crus-galli, A retroflexus* and *A theophrasti* were significantly reduced by mesotrione and atrazine alone and in combination. Photosynthesis on day 14 and dry mass of large *S viridis* plants were not suppressed by either herbicide applied alone. The mesotrione plus atrazine treatment was the most effective treatment for grass weed control because plants did not regain photosynthetic capacity and had significantly lower dry mass. Shoot dry mass of broadleaf weeds was significantly reduced by all three herbicide treatments, except for *A retroflexus* treated with mesotrione alone.

© 2004 Society of Chemical Industry

Keywords: atrazine; mesotrione; photosynthesis; plant size; post-emergence; root growth

1 INTRODUCTION

Mesotrione is a selective pre-emergence (PRE) and post-emergence (POST) herbicide that controls most broadleaf and some grass weeds in *Zea mays* L (corn).1−3 Mesotrione belongs to the triketone class of chemicals, a group derived from the natural plant product leptospermine.4 Triketones inhibit ρ-hydroxyphenylpyruvate dioxygenase5,6 (HPPD, EC 1.13.11.27), an enzyme in the pathway that converts the amino acid tyrosine to plastoquinone and α-tocopherol.7 As a cofactor for the enzyme phytene desaturase, plastoquinone is essential for carotenoid biosynthesis.8 Loss of carotenoids leads to destruction of chloroplast membranes and other plastid components by the oxidative activity of free radicals (active oxygen species) that are generated during photosynthesis.9 Three to 5 days after mesotrione treatment, initial bleaching symptoms become visible in susceptible plants, and about two weeks are required for bleaching and necrosis to appear throughout the plant.2

Since its introduction in the late 1950s, atrazine has been more widely used than any other herbicide in *Z m a y s* production.10 Atrazine can be PRE or POST applied and is effective for the control of many broadleaf and grass weeds.11 Atrazine inhibits photosynthetic electron transport by competitively binding to the plastoquinone (Qb) binding site of the D1 protein of photosystem II (PS II).12 The carotenoids are unable to quench the influx of free radicals generated by the blockage of PS II, leading to cell membrane destruction and eventual plant death.12 Symptoms of injury become visible in susceptible plants within a few days following foliar atrazine treatment.9

One reason for the widespread use of atrazine is its ability to complement other herbicides and improve the spectrum of weed control. Nearly all presently registered herbicides for *Z m a y s* benefit from combination with atrazine.10 Research has shown that atrazine addition to mesotrione can improve control of some grass and broadleaf weeds compared to mesotrione

...
applied alone. Johnson et al reported increased control of Xanthium strumarium L (common cocklebur), Ipomoea hederacea L Jacq (ivyleaf morningglory) and Cyperus esculentus L (yellow nutsedge) by the addition of atrazine to mesotrione. Mesotrione activity on Ambrosia artemisiifolia L (common ragweed), Solanum carolinense L (horsenettle), and Ipomoea lacunosa L (pitted morningglory) has also been enhanced by adding atrazine.

Plants rely on their ability to assimilate carbon in photosynthesis for their growth and overall vigor. Herbicide-treated plants may be impacted physiologically in a way that is not immediately apparent from visual evaluations. Plant vigor may be determined objectively following herbicide treatment by measuring photosynthetic variables. Sulcotrione, a triketone similar to mesotrione, inhibits photosynthetic electron transport via HPPD, while atrazine inhibits PSII directly. Shortly after the cessation of electron transfer, photosynthesis is terminated. Consequently, we hypothesized that photosynthesis can objectively detect and quantify mesotrione and atrazine activity, and that effects on photosynthesis will closely mirror growth responses. The objective of this research was to examine the efficacy of foliar applications of mesotrione and atrazine under greenhouse conditions in Z mays and four weeds commonly found in Z mays production fields by measuring photosynthetic and growth responses.

2 MATERIALS AND METHODS

Seeds of Z mays (DKC63-03; Monsanto Co, DEKALB Genetics Corp, St Louis, MO, USA) were planted 2.5 cm deep and seeds of Setaria viridis (L) Beauv, Echinocloa crus-galli (L) Beauv, Abutilon theophrasti Medic and Amaranthus retroflexus L (Valley Seed Service, Fresno, CA, USA) were planted 1 cm deep in 8-liter pots filled with a Preston fine sand (mixed, mesic, Typic Xeropsamments) collected in Cache County, UT, USA. Seedlings were thinned to three uniformly sized individuals three weeks after emergence. Plants were watered daily to field capacity and fertilized weekly with Miracle Gro® (Scotts-Sierra Horticultural Co, Marysville, OH, USA). Pots were randomly arranged in a greenhouse located in Logan, UT, USA. The greenhouse temperature was controlled with an evaporative cooler. Daytime temperatures ranged from 17 to 26 °C. To maintain daytime temperatures within this range, solar radiation was reduced by applying a neutral density chalk solution (Kool Ray, Liquid Shade White, 9.7% solution, The Continental Products Co, Euclid, OH, USA) to the exterior of the greenhouse. Supplemental lighting was not used. Night-time temperatures ranged from 12 to 17 °C. Photosynthetically active radiation (PAR) at midday on cloudless days was periodically checked and averaged 600 μmol m⁻² s⁻¹ during the experimental period. The experiment was conducted twice between July to October 2002. Experiment replications are designated as experiment 1 and experiment 2.

Atrazine was used as 480 g liter⁻¹ SC (Aatrex 4L, Syngenta) and mesotrione as 480 g liter⁻¹ SC (Calisto, Syngenta). Four pots of each species were randomly assigned to each of the following herbicide treatments: (1) atrazine (280 g AI ha⁻¹), (2) mesotrione (105 g AI ha⁻¹), (3) atrazine (280 g AI ha⁻¹) plus mesotrione (105 g AI ha⁻¹), and (4) untreated control. All treatments were applied with crop oil concentrate (COC; Crop-Surf® Universal Cooperatives, Inc, Minneapolis, MN, USA) at 10 ml liter⁻¹ (1% v/v) and 32% urea ammonium nitrate (UAN) at 25 ml liter⁻¹ (2.5% v/v). We evaluated only one dose for each herbicide treatment to test our hypothesis; however, this approach is not suitable to determine dose-response effects on herbicide selectivity. Herbicides were applied to individual pots using a custom precision table sprayer equipped with a Teejet® 8001E flat fan nozzle tip (Spraying Systems Co, Wheaton, IL, USA) calibrated to deliver 179 liter ha⁻¹ at 207 kPa. Pots containing control plants and treated plants were handled identically, but control pots were sprayed with water instead of herbicides and surfactants. Plant heights at the time of herbicide application are summarized in Table 1.

Measurements of leaf photosynthesis, stomatal conductance and leaf transpiration were taken on each of the three plants in each pot prior to herbicide applications (day 0) and on days 1, 3, 7, and 14. These measurements were taken on one leaf per plant with a portable photosynthesis system (LI-6400, Lincoln, NE, USA) and averaged for each pot. The leaf measured on each plant was of similar size and stage of development to those on other plants of the same species. The same leaf was measured on each sampling date. The leaf chamber environment was maintained at the following settings for all photosynthesis measurements: block temperature 24 °C, carbon dioxide concentration 400 μmol mol⁻¹, air flow 500 μmol s⁻¹ and PAR 600 μmol m⁻² s⁻¹.

**Table 1.** Plant height of Zea mays and four weed species at the time of herbicide treatment for experiments 1 and 2

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Z mays (cm)</th>
<th>A theophrasti (cm)</th>
<th>A retroflexus (cm)</th>
<th>E crus-galli (cm)</th>
<th>S viridis (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.6 ± 0.5</td>
<td>16.4 ± 0.7</td>
<td>9.3 ± 0.5</td>
<td>16.2 ± 0.8</td>
<td>15.3 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>38.6 ± 1.1</td>
<td>25.3 ± 1.5</td>
<td>29.2 ± 0.9</td>
<td>38.4 ± 1.4</td>
<td>37.1 ± 1.4</td>
</tr>
</tbody>
</table>

*Values are means (±1 SEM) of 64 plants.
All measurements were taken between 1100 and 1300 h when the intensity of solar radiation was most consistent. PAR was set to a consistent level and measurements were taken within the same timeframe each sampling date to avoid the effects of variable light intensity on photosynthetic rates that may be encountered when measuring photosynthesis over time. Immediately following measurements on day 14 of each experiment, plants were clipped at the soil surface and entire shoots were oven-dried for 48 h at 60°C to determine shoot dry mass. Soil from the pots was sieved (2 mm) to separate and collect the roots, which were subsequently oven-dried in a similar manner to the shoots. Extremely fine roots of the broadleaf weeds (A theophrasti and A retroflexus) prevented accurate estimates of root mass and, thus, were not quantified.

The effects of atrazine and mesotrione on photosynthesis through time for both experiments were assessed using an analysis of variance (ANOVA) for a four-way factorial using a repeated measures design for each of five species separately. Herbicide treatment and experiment replication were considered as fixed-effects in a completely randomized design. Time was considered a fixed-effect, repeated-measure factor. The covariance structure for the repeated measures was specified as a first-order autoregressive mode.22 Pots (n = 4) were treated as replications, and a mean for the three plants was computed for each pot and used as the data value in the ANOVA. Residuals were examined to evaluate assumptions of normality and homogeneity of variance. No transformations of data were needed to adequately meet these assumptions. Many herbicide-treated plants of A retroflexus, Echinochloa crus-galli, Abutilon theophrasti and Setaria viridis died before the end of the experiment (day 14). Consequently, data for these species were analyzed only for the first 7 days. Models were fit using PROC MIXED (SAS Institute Inc, Cary, NC, USA).

Determination of photosynthetic differences in response to herbicide treatments during the course of the experiment were compared using contrasts. The contrasts statistically tested differences between treatments for both experiments through time. Differences in dry mass responses were determined with Tukey’s (HSD) procedure when ANOVA tests of the effects of treatment and experiment replication were significant using PROC GLM (SAS Institute Inc, Cary, NC, USA). Responses were referred to as ‘significant’ only when $P < 0.05$ for contrasts and Tukey tests.

3 RESULTS AND DISCUSSION
3.1 Photosynthetic responses

3.1.1 Mesotrione

Photosynthesis, conductance, and transpiration data consistently complemented each other, thus we present only photosynthesis data for brevity. Mesotrione significantly reduced photosynthesis relative to the untreated control in all species except Zea mays (experiments 1 and 2) and S viridis (experiment 2) (Fig. 1). The suppression of photosynthesis persisted until plant death in E crus-galli, A theophrasti and A retroflexus, but did not for S viridis in experiment 1. Negative photosynthetic rates indicate that, under the stress of herbicide damage, respiratory carbon loss exceeds carbon assimilation. Reductions in photosynthesis due to mesotrione in plants in experiment 1 occurred earlier and were prolonged relative to those of plants in experiment 2 within the same species. Greater sensitivity of plants to mesotrione in experiment 1 than 2 is likely due to size differences at the time of application. Plants in experiment 1 were smaller than those of experiment 2 (Table 1) and herbicide activity usually decreases as plant size increases.12 In susceptible plants treated with mesotrione, a reduction in photosynthesis did not become apparent before day 3. Extensive field studies have shown Z mays to be highly tolerant to mesotrione with only slight injury occurring under certain environmental conditions.1,2 Zea mays escapes damage from mesotrione through a combination of low foliar absorption and high

![Figure 1. Photosynthesis (carbon assimilation) of Zea mays, Setaria viridis, Echinochloa crus-galli, Abutilon theophrasti, and Amaranthus retroflexus for experiments 1 and 2 measured prior to treatment (day 0), and following treatment (days 1, 3, 7 and 14) with atrazine, mesotrione or atrazine + mesotrione. Individual data points represent the mean of four measurements. Vertical bars represent one standard error of the mean.](image-url)
JE Creech, TA Monaco, JO Evans

metabolic removal or dissipation of herbicide toxins. Previous research indicated that *S. viridis* was less sensitive to mesotrione than other species. In the field, *E. crus-galli* was effectively controlled by mesotrione at 150 g ha\(^{-1}\),\(^2\) while the same rate was insufficient to control *S. viridis*.\(^1\) Our results agree with previous reports that foliar applications of mesotrione provided excellent control of *A. theophrasti*\(^{1,2,23}\) and *A. retroflexus*.\(^1,2\)

### 3.1.2 Atrazine

By day 1, atrazine-treated plants had significantly lower photosynthesis than untreated plants in all five species studied. This effect persisted for *E. crus-galli*, *A. theophrasti* and *A. retroflexus*, but was not persistent for *Z. mays* or *S. viridis*. In *Z. mays*, the differences in photosynthesis between the atrazine-treated plants and the untreated control plants diminished by day 3. These results agree with those of Shimabukuro\(^24\) who demonstrated the ability of *Z. mays* to detoxify atrazine through metabolic processes. The initial inhibition of photosynthesis by atrazine reveals a susceptible site of action in *Z. mays*, but subsequent metabolism of atrazine results in a return to full photosynthetic capacity.\(^25\) Photosynthesis of *S. viridis* plants treated with atrazine steadily increased after day 3 to levels comparable to plants in the untreated control. Therefore, *S. viridis* may have the ability to metabolize

![Figure 2. Shoot dry mass of *Zea mays, Setaria viridis, Echinochloa crus-galli, Abutilon theophrasti* and *Amaranthus retroflexus* for experiments 1 and 2. Plants were harvested 14 days after treatment with atrazine, mesotrione or atrazine + mesotrione. The y-axis scale varies between species. Individual bars represent the mean of four pots. Error bars represent one standard error of the mean. Upper letters refer to difference within a treatment, between experiments, and lower letters refer to differences between treatments within an experiment.](image-url)
Post-emergence treatments of *Zea mays* L with mesotrione and atrazine

...azarine in a similar, but less efficient manner than *Z mays*. Slower response of plants treated with mesotrione (day 3) compared to those with atrazine (day 1) was expected because mesotrione inhibition of HPPD triggers a series of events that leads to a subsequent effect on photosynthesis, whereas atrazine blocks photosynthetic electron transport directly.

### 3.1.3 Mesotrione plus atrazine

Plants treated with the mesotrione plus atrazine treatment had significantly reduced photosynthesis relative to untreated control plants within 1 day for all five species studied. In contrast to either herbicide applied alone, the combination of mesotrione and atrazine effectively suppressed photosynthesis of all species through day 14 except *Z mays*. Improved weed control with the mesotrione plus atrazine combination is probably a joint consequence of mesotrione damage to carotenoid biosynthesis and an influx of active oxygen species mediated by atrazine. The combination of more displaced photochemical energy and less means of quenching that energy could lead to increased herbicidal activity.

The combination of mesotrione and atrazine did not inhibit *Z mays* photosynthesis differently than atrazine applied alone. Reports of *Z mays* injury from the combination of atrazine and mesotrione in field studies have been inconsistent, with slight increases or decreases in injury that may have been due to environmental factors.14 Our results indicate that the temporary reduction in *Z mays* photosynthesis observed in the combination of atrazine and mesotrione treatment is likely a consequence of atrazine in the mixture.

### 3.2 Dry mass

In general, shoot and root dry masses in experiment 2 were significantly higher than those of experiment 1, a trend likely resulting initial from differences in plant size between experiments at the time of treatment. Shoot and root dry mass of *Z mays* was not significantly affected by the herbicide treatments (Figs 2 and 3). Shoot and root dry mass of *E crus-galli* in each of the herbicide treatments was significantly lower than in control plants. In contrast, shoot and root dry mass of untreated *S viridis* plants was significantly greater than plants of all treatments in experiment 1, whereas only the atrazine plus mesotrione treatment was significantly different from the untreated control in experiment 2. Shoot dry mass of *A retroflexus* in experiment 1 was significantly lower in all of the herbicide treatments than the untreated plants. However, in experiment 2, shoot dry mass of *A retroflexus* in the untreated and mesotrione alone

![Figure 3](image-url)

*Figure 3.* Root dry mass of *Zea mays*, *Setaria viridis*, and *Echinochloa crus-galli* for experiments 1 and 2. Plants were harvested 14 days after treatment with atrazine, mesotrione or atrazine + mesotrione. The y-axis scale varies between species. Individual bars represent the mean of four pots. Error bars represent one standard error of the mean. Upper letters refer to differences within a treatment, between experiments, and lower letters refer to differences between treatments within an experiment.
treatments were similar, yet both were significantly greater than plants treated with atrazine and atrazine plus mesotrione. *Abutilon theophrasti* shoot dry mass did not differ significantly between the herbicide treatments, but shoot dry masses in all treatments were significantly lower than in the untreated plants. Consequently, foliar-applied mesotrione is a more effective control for smaller- (experiment 1) rather than larger-sized (experiment 2) plants of *A. retroflexus*. In contrast, control of *A. theophrasti* with mesotrione was equally effective in both experiments.

### 4 CONCLUSIONS

Mesotrione applied alone controlled all weeds except *A. retroflexus* in experiment 2 and *S. viridis* in experiments 1 and 2, as shown by reductions in photosynthesis. Atrazine alone successfully reduced photosynthesis and dry mass for all weeds except *S. viridis* compared to the untreated plants. The most effective treatment for consistent reductions in photosynthesis and dry mass across all grass and broadleaf weed species was the combination of mesotrione and atrazine. Other than a temporary effect at day 1, *Z. mays* was not affected by any herbicide treatment. In general, reductions in photosynthesis, especially on days 7 and 14, corresponded to reductions in dry mass relative to the untreated plants.

### ACKNOWLEDGEMENTS

The authors thank Justin Williams, Brandon Gordon, Trevor Warburton and Chris Fausett for assisting with plant maintenance and photosynthesis measurements. We also thank Drs JG Carman and DA Johnson for providing insightful suggestions on an earlier version of this manuscript. Appreciation is also extended to Susan Durham for assistance in statistical analysis. Research was funded by the USDA-ARS and the Utah Agricultural Experiment Station. Utah Agricultural Experiment Station Journal Paper No 7543.

### REFERENCES


1084