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Ruizzo, Michael A.

A MECHANISM OF QUIZALOFOP-ETHYL SELECTIVITY IN MONOCOTYLEDONOUS AND DICOTYLEDONOUS SPECIES

The Ohio State University

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A MECHANISM OF QUIZALOFOP-ETHYL SELECTIVITY IN
MONOCOTYLEDONOUS AND DICOTYLEDONOUS SPECIES

DISSERTATION

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

By

Michael A. Ruizzo, B.S. M.S.

* * * * *

The Ohio State University

1986

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INTRODUCTION

A major segment of current herbicide/plant science research is now being directed towards the development and implementation of various degrees of selectivity. The term selectivity refers to the fact that, under a given set of conditions, certain species of plants (weeds) are killed or seriously injured, whereas other species of plants (crops) are not injured.

Quizalofop-ethyl is one of a number of experimental systemic postemergence herbicides which express great activity on graminaceous plants. The degree of selectivity now being implemented with the development of these compounds has not been defined in terms of the primary biochemical or biophysical interference imposed that leads to plant lethality (mechanism of action). The role of mechanism of action research on herbicides is to enable researchers to design more effective and safer herbicides. Such studies provide information on how these compounds affect plants at the molecular level which ultimately determine their selectivity.

For a herbicide to interfere with the major metabolic plant systems at the cellular and molecular levels (chloroplast-associated reactions, mitochondrial-mediated responses, nucleic acid metabolism and protein synthesis, and
membrane interactions) it must reach the cell following application to the plant or to the soil. Anatomical, morphological, biochemical, and physiological factors operate to control herbicidal entry into and distribution within the plant. Internally, additional actions such as metabolic alterations, sorption to inactive sites, and complex formation reduce the availability of the herbicide for reaction at sites through which phytotoxicity is expressed. It is conceivable that an extremely small percentage of herbicide applied actually becomes available for internal reaction with growth controlling processes which further emphasizes the need to identify and understand the mechanisms responsible for selectivity.

A large part of our present knowledge concerning the nature and regulation of processes such as electron transport, photophosphorylation, and photosynthesis derives from experiments with isolated chloroplasts. The pigments and enzymes that catalyze photosynthesis are compartmentalized within the cell in chloroplasts. The chloroplast can therefore be considered a partially autonomous cellular organelle, which is the sole location for all molecular events involved in radiant energy capture. Effects on ATP synthesis (photophosphorylation) in the chloroplast system must be investigated as much of the underlying herbicidal effects are involved with the free energy of the cell. ATP therefore creates phosphorylated species of high free energy, which can participate in reactions that could not otherwise take place in plants (ie. growth and development).
Inhibitors, many of which are herbicides, have played, and will continue to play, an important role in the physiology, biophysics, and biochemistry of the aforementioned processes. The scope of this research concentrated on investigating a primary mechanism of action of quizalofop-ethyl through its effect(s) on photosynthetic processes in plants. Efforts involved determinations of quizalofop-ethyl toxicity, translocation, and degradation in plant species. With this information, an understanding of the nature of activity of this and related herbicides may lead to modifications of molecules to alter their penetrability, translocation, toxicity, degradation, and ultimately increase their selective effectiveness.
CHAPTER I

Adjuvant Types Affect Cucumber (Cucumis sativus)

Sensitivity to Quizalofop-Ethyl Under Field and Greenhouse Conditions

ABSTRACT. Two cucumber cultivars [Cucumis sativus (L.) 'Calypso' and 'Carolina'] susceptibility to the ethyl ester of quizalofop [(±) ethyl-2-[4-[(6-chloro-2-quinoxyaliny1)oxy]phen oxy]propionic acid] were investigated under field and greenhouse conditions. Yield of the two cultivars was significantly reduced under field conditions with a single or repeat application of the ethyl ester of quizalofop at 0.14 or 0.28 kg ai/ha. Under greenhouse conditions, quizalofop-ethyl significantly suppressed cucumber plant fresh weight with or without the presence of an adjuvant. Enhancement of herbicide activity was directly related to concentration of adjuvant. At a higher rate of quizalofop-ethyl, the addition of the lower concentration of adjuvant increased the herbicidal effect (growth suppression). Visual injury was also dependent on the rate of quizalofop-ethyl and the concentration of added adjuvant. At a lower herbicide concentration, increasing adjuvant concentration enhanced visual phytotoxicity while at a higher quizalofop-ethyl concentration, greater phytotoxicity was observed only at the lower adjuvant concentration.
INTRODUCTION

Quizalofop-ethyl is a systemic postemergence herbicide that selectively controls both annual and perennial grasses in nongraminaceous crops (21). Quizalofop controlled late season grass without crop injury in bell pepper (Capsicum annum L.) (1). Kurtz (17) reported greater than 95% control of bermudagrass [Cynodon dactylon (L.) Pers. #1 CYNDA] in cotton (Gossypium hirsutum L.) with quizalofop-ethyl at 0.14 and 0.28 kg ai/ha. Kells\textsuperscript{2} reported excellent control of quackgrass [Agropyron repens (L.) Beauv. #AGRRE] at various growth stages in soybean [Glycine max (L.) Merr.] with 0.56 and 1.12 kg ai/ha rates of quizalofop-ethyl. Herbicide induced injury was reported to be 38% and 40% in the 1980 and 1981 soybean trials, respectively. The injury did not significantly reduce soybean yields. Dekker and Anderson (7) reported optimum quackgrass control with quizalofop-ethyl when quackgrass was in the 4 to 5 leaf stage. A number of annual grass weeds were reported to show extensive

---

\textsuperscript{1}Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Science 32, Suppl. 2. Available from WSSA, 309 West Clark St., Champaign, IL 61820.

susceptibility to quizalofop-ethyl at various stages of growth (2, 5, 8, 13). Johnson and Hopen (15) reported reduced tomato yields with postemergence grass herbicides in combination with oil or surfactant. Yield of Calypso cucumbers that received single applications of 0.28 kg/ha and repeat applications of 0.28 or 0.56 kg/ha of fluazifop was not different from cultivated checks (20). A single application at 0.56 kg/ha significantly reduced yields at one of two locations.

The presence of adjuvants has been shown to influence the phytotoxicity of herbicides (3, 9, 19). Chen and Penner (4) found the presence of a crop oil concentrate in the spray mixture increased the efficacy of low dosages of sethoxydim [2-[(1-ethoxymino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one] and acifluorfen [5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid] on barnyardgrass [Echinochloa crus-galli (L.) P. Beauv. #ECHCG]. At higher herbicide rates the concentration of crop oil concentrate was not critical for weed control. Yet, other studies reported a decrease in sethoxydim/adjuvant activity when herbicide rate exceeds 0.1 kg/ha (3, 5, 10, 11). Adjuvants are significant components of nearly all commercial herbicide formulations and are often present at concentrations equaling those of the herbicides themselves (12, 14).

Currently, there are no postemergence grass herbicides available for use in cucurbits. There is also limited information concerning the selectivity mechanism of many of the postemergence graminicides on broadleaf plants. It is the intent of this
research to investigate quizalofop-ethyl's 'broadleaf activity' through evaluation of cucumber phytotoxicity due to herbicide and adjuvant rate.

MATERIALS AND METHODS

Field Studies. Experiments were conducted at the vegetable crops branch of the Ohio Agricultural Research and Development Center at Fremont, Ohio between 1983 and 1985. Soil was a sandy loam with three percent organic matter. Cucumber cultivars were seeded in single rows 9.1 m long on 0.9 m centers. Treatments consisted of quizalofop-ethyl at rates of 0, 0.035, 0.07, 0.14, and 0.28 kg ai/ha applied with either a crop oil concentrate (1% v/v) consisting of 83% (v/v) paraffin oil and 17% (v/v) polyoxyethylene sorbitan fatty acid esters3, a nonionic surfactant containing alkylarylpolyoxyethylene glycols, free fatty acids, and isopropanol4 (0.25% v/v), or no adjuvant. Treatments were applied with a CO2 backpack sprayer calibrated to deliver 275 L/ha at 207 kPa.

In 1983 and 1985 applications were made when the cucumbers had approximately 4 true leaves (about June 7). Also in 1983 a

3Ortho Crop Oil Concentrate; Chevron Chemical Co., Richmond, CA.

4X-77 Spreader; Chemistry confidential. Chevron Chemical Co., Richmond, CA.
second application was made two weeks following the initial treatment. One application was made in 1984, at the 8 to 12 true leaf stage (June 13), with the addition of adjuvants to measure their effect(s) on quizalofop-ethyl performance. Crop injury ratings were made using a visual rating scale of 1 (complete crop kill) to 10 (no crop injury). Evaluations were made two weeks after treatment. Cucumber harvest was made at normal crop maturity and consisted of multiple harvests. Yield was recorded as fruit weight/row and data were converted to percent of weed free controls. Cultural practices were performed according to current recommendations. Experimental design was a randomized complete block with four replications/treatment.

Greenhouse Studies. Calypso cucumber seed was planted in 10.2 cm diameter pots containing a soil-less medium and placed on a greenhouse bench. Environmental conditions were a day/night temperature of 30°/18° C, 70 ± 10% relative humidity, and a 14h photoperiod (mean photosynthetic photon flux density, PPFD, of 400 to 600 umol m⁻² s⁻¹ of natural light at top of plant canopy). Plants were irrigated daily with 200 ppm nitrogen. Treatments were applied when plants were in the 2 to 3 true leaf stage.

Quizalofop-ethyl was applied at rates of 0, 0.07, and 0.14 kg ai/ha with either a crop oil concentrate at 0.5% (0.5X) or 1.0% (1X) (v/v), a nonionic surfactant (previously described) at 0.13% (0.5X) or 0.25% (1X) (v/v), or no adjuvant. Treatments were applied with a CO₂ pressurized greenhouse type conveyor sprayer calibrated to deliver 480 L/ha at 207 kPa. The media surface was covered with
perlite prior to spraying to prevent media introduced effects. The perlite was removed following spray application. Experimental design was a randomized complete block with four replications/treatment. Plot size was a single plant pot/replication. Treatment values represent the mean of 4 replicates repeated 3 times [n=12; experimental means were averaged when experimental error was homogeneous according to Bartlett's test for homogeneity (22)].

Crop injury was visually evaluated 14 days after treatment on a percent injury scale from 0 (no effect) to 100 (complete kill). Plant foliage was harvested by cutting the stems at the media surface and recording their fresh weights. Root tissue was visually inspected for morphological and physiological abnormalities at the time of harvest.

RESULTS AND DISCUSSION

Field Studies. In 1983, quizalop-ethyl at 0.035 and 0.28 kg ai/ha reduced cucumber yields 18% and 97%, respectively (Figure 1). Crop injury ratings paralleled yield reductions (Figure 2). Visual injury was first observed 10 to 14 days following application as irregular chlorotic areas on treated tissue which became necrotic. This agrees with injury symptoms in soybean described by Kells². Broadleaf symptoms are not unlike those reported for grass plants (13), except with cucumber, injury symptoms are only present on treated foliage. A second crop injury rating was conducted two weeks following a second application of quizalofop-ethyl which
Figure 1. Cucumber yield as a function of quizalofop-ethyl rate in 1983 through 1985 field experiments. Yield is represented as percent of weed free controls. Bars represent the 95% confidence interval of that mean.
Figure 1.
Figure 2. Cucumber phytotoxicity as a function of quizalofop-ethyl rate in 1983 through 1985 field experiments. Crop phytotoxicity scale: 10 = no crop injury, 1 = complete kill. Bars represent the 95% confidence interval of that mean.
Figure 2.

Graph showing the effect of quinaldine-ethyle rate (μg/μl) on cucumber phytotoxicity.
substantiated the initial findings. Again, injury symptoms were only present on treated foliage. New growth from surviving plants appeared normal. At sublethal rates of quizalofop-ethyl, the time period of growth suppression could significantly reduce the yield capacity of this crop.

There is evidence that the addition of adjuvant to quizalofop-ethyl increased visual damage and reduced yields compared with quizalofop-ethyl applications without adjuvants (Tables 1 and 2). The contrast of crop oil concentrate versus nonionic surfactant was not significant therefore, adjuvant represents combined effects. Adjuvant controls were non-phytotoxic in the 1984 and 1985 trials. In 1984, yield was reduced approximately 20% with quizalofop-ethyl alone and 30% with quizalofop-ethyl plus adjuvant at a 0.28 kg ai/ha rate. The addition of an adjuvant intensifies the herbicidal effect of quizalofop-ethyl as with many other herbicides (3, 9, 15, 19).

Crop injury in 1984 was not as severe as injury ratings reported in 1983 (Figures 1 and 2). It is believed that environmental conditions and plant growth stage at the time of application may have had a significant effect on cucumber's susceptibility to quizalofop-ethyl. The growing season conditions for 1983 contained an extensive period of above normal temperatures associated with periods of drought. Past research has shown environment to effect herbicide performance (16, 18). McWhorter (18) reported increased activity of metriflufen-methyl [2-[4-(4-trifluoromethylphenoxy)phenoxy]propanoic acid] with
Table 1. Effect of quizalofop-ethyl on cucumber yield under field conditions.

<table>
<thead>
<tr>
<th>Quizalofop-ethyl rate (kg ai/ha)</th>
<th>Yield&lt;sup&gt;ab&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>control</td>
<td>--</td>
</tr>
<tr>
<td>0.035</td>
<td>--</td>
</tr>
<tr>
<td>0.07</td>
<td>--</td>
</tr>
<tr>
<td>0.14</td>
<td>--</td>
</tr>
<tr>
<td>0.28</td>
<td>--</td>
</tr>
</tbody>
</table>

<sup>a</sup> Yield values expressed as percent of weed free controls.

<sup>b</sup> Means within a column followed by a (*) are significantly different from the control in that column, based on a single degree of freedom analysis at the 5% level.

<sup>c</sup> All 1983 herbicide treatments contained crop oil concentrate 1% (v/v).
Table 2. Effect of quizalofop-ethyl on cucumber expressed as visual phytotoxicity.

<table>
<thead>
<tr>
<th>Quizalofop-ethyl rate (kg ai/ha)</th>
<th>1983 no adjuvant</th>
<th>1983 adjuvant</th>
<th>1984 no adjuvant</th>
<th>1984 adjuvant</th>
<th>1985 no adjuvant</th>
<th>1985 adjuvant</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>0.035</td>
<td>--</td>
<td>8.8*</td>
<td>8.5*</td>
<td>9.1*</td>
<td>8.8*</td>
<td>8.7*</td>
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<td>0.07</td>
<td>--</td>
<td>9.0*</td>
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<td>--</td>
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<td>6.8*</td>
<td>3.5*</td>
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<tr>
<td>0.28</td>
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<td>8.8*</td>
<td>5.5*</td>
<td>1.9*</td>
<td>7.7*</td>
<td>3.8*</td>
</tr>
</tbody>
</table>

*a* Phytotoxicity: 10 = no crop injury, 1 = complete kill.

*b* Means with a column followed by a (*) are significantly different from the control in that column, based on a single degree of freedom analysis at the 5% level.

*c* All 1983 herbicide treatments contained crop oil concentrate 1% (v/v).
increasing temperatures on johnsongrass [Sorghum halepense (L.) Pers.# SORHA] and soybean. Kells et al. (16) report that greater quackgrass control was observed at 30° than at 20° C and that moisture stress significantly reduced quackgrass control with fluazifop-butyl.

In 1985, quizalofop-ethyl plus adjuvant reduced cucumber yield approximately 24% and 85% at the 0.035 and 0.28 kg ai/ha rates, respectively (Table 1). Quizalofop-ethyl at 0.035 and 0.28kg ai/ha, with no adjuvant, caused a 23% and 72% decline in cucumber yield, respectively. In 1985, the addition of an adjuvant increased visual crop injury an average of 30% at the 0.28 kg ai/ha rate of quizalofop-ethyl (Table 2). Visual injury symptoms expressed were median between those observed in 1983 and 1984 (Figure 2).

Greenhouse Studies. Under the conditions of this study, cucumber plants were susceptible to quizalofop-ethyl with or without an adjuvant (Figures 3 and 4). Adjuvant controls were non-phytotoxic and the interaction of adjuvant by herbicide was also not significant (appendix A). Plant fresh weight was reduced 10% with quizalofop-ethyl (no adjuvant) at a rate of 0.14 kg ai/ha (Figure 3). The addition of adjuvant did enhance quizalofop-ethyl activity up to a critical concentration of adjuvant. The 0.5X adjuvant concentration caused a significantly greater decline in plant fresh weight when compared to the 1X adjuvant in 0.14 kg ai/ha quizalofop-ethyl combinations (Figure 3). At the 0.07 kg ai/ha rate, of quizalofop-ethyl adjuvant combinations, changing adjuvant
Figure 3. Effect of quizalofop-ethyl on cucumber plant fresh weight as a function of adjuvant under greenhouse conditions. Symbols represent: herbicide plus no adjuvant (HA0), herbicide plus 0.5X adjuvant (HA0.5), and herbicide plus 1X adjuvant (HA1.0). Bars represent the 95% confidence interval of that mean expressed as the percent of control.
Figure 3.
concentration did not significantly effect fresh weight, but adjuvant at 0.5X and 1X significantly increased quizalofop-ethyl injury in comparison to quizalofop-ethyl alone. Root tissue was unaffected at the time of evaluation.

Surfactant effects can range from no effect at low concentrations to toxicity at high levels (19). Jansen et al. (14) observed a marked suppression of herbicidal activity as a function of surfactant concentration. Various studies have also reported a decline in herbicide activity when herbicide rate was increased versus adjuvant concentration (3, 5, 10, 11). The relationship between herbicide and adjuvant is at best only partly resolved.

Initially, quizalofop-ethyl plus the 1X concentration of adjuvant caused significant phytotoxicity to cucumber at a herbicide rate of 0.07 kg ai/ha (Figure 4). When quizalofop-ethyl concentration was increased to 0.14 kg ai/ha phytotoxicity decreased and was no longer significantly different from the quizalofop-ethyl (no adjuvant) treatment. Expression of cucumber phytotoxicity increased as quizalofop-ethyl concentration increased at the 0.5X concentration of adjuvant (Figure 4). At a 0.14 kg ai/ha rate of quizalofop-ethyl plus 0.5X adjuvant the greatest degree of cucumber phytotoxicity was expressed at approximately 40% of the control. It can be speculated, that the reduction in quizalofop-ethyl phytotoxicity at the higher rate of adjuvant lies in the relationship of many factors such as the dose response relationship of herbicide and adjuvant, the plant species, as well as cuticular penetration (6).
Figure 4. Effect of quizalofop-ethyl on cucumber expressed as visual phytotoxicity as a function of adjuvant under greenhouse conditions. Symbols represent: herbicide plus no adjuvant (HA0), and herbicide plus 0.5X adjuvant (HA0.5), and herbicide plus 1X adjuvant (HA1.0). Bars represent the 95% confidence interval of that mean expressed on a percent phytotoxicity scale: 0 = no effect, 100 = complete kill.
CUCUMBER PHYTOTOXICITY (%)
Results of these studies indicate quizalofop-ethyl does express activity on cucumber. Generally, yield is reduced under field conditions with as little as a single application of quizalofop-ethyl at 0.14 kg ai/ha to cucumber plants in the 4 true leaf stage. At a later growth stage tolerance to quizalofop-ethyl is increased. Under greenhouse conditions, quizalofop-ethyl suppresses cucumber growth with greater phytotoxicity found in the presence of a crop oil concentrate or nonionic surfactant. At a higher rate of quizalofop-ethyl, the addition of the lower concentration of adjuvant increased the herbicidal effect (growth suppression). Visual phytotoxicity was also dependent on the rate of quizalofop-ethyl and the concentration of added adjuvant. At a lower herbicide concentration, increasing adjuvant concentration enhanced visual phytotoxicity while at a higher quizalofop-ethyl concentration, greater phytotoxicity was observed only at the lower adjuvant concentration. Injury symptoms in the greenhouse appeared as a chlorosis/necrosis of treated tissue confirming field studies. The interrelationship of herbicide and adjuvant in these studies, indicates that concentration of adjuvant is important in determining quizalofop-ethyl toxicity. This effect on toxicity may or may not be present in graminaceous species which could help determine quizalofop-ethyl's selective action as well as aid in management of herbicide usage.
LITERATURE CITED


CHAPTER II

BEHAVIOR OF QUIZALOFOP-ETHYL IN A SUSCEPTIBLE
BROADLEAF CROP TO DETERMINE SELECTIVITY

ABSTRACT. A bioassay was conducted to determine the effects of quizalofop-ethyl [(+)-ethyl-2-[4-[(6-chloro-2-quinoxyalinyl) oxy] phenoxy] propionic acid] on growth of cucumber [Cucumis sativus (L.) 'Calypso']. Microliter droplet application of quizalofop-ethyl at a 10^{-3} M concentration, inhibited the relative growth rate (RGR) and net assimilation rate (NAR) of the treated cucumber leaf 45% and 52%, respectively. Within two days of herbicide application chlorosis/necrosis developed. Expression of herbicidal injury was localized on the treated leaf with no visible symptoms observed on adjacent leaves.

In another experiment, radiolabeled (^{14}C) quizalofop-ethyl was applied to leaves of cucumber and corn [Zea mays (L.) 'Gold Cup'] to compare translocation patterns between two susceptible plant species and relate this information to the observed selectivity of the herbicide. Cucumber autoradiographs showed minimal translocation of ^{14}C-quizalofop-ethyl 192 hours after treatment (HAT). In contrast, corn autoradiographs showed both apoplastic and symplastic transport of quizalofop-ethyl 3 and 24 HAT. Quantification of ^{14}C in cucumber revealed 96% of
absorbed $^{14}$C was confined to the treated leaf after 192h of exposure. The lack of $^{14}$C-quizalofop-ethyl transport is consistent with localized herbicidal effects expressed in the bioassay. These experiments suggest that translocation differences exist between susceptible species of dicotyledonous and monocotyledonous plants. The observed differences in translocation may contribute to the selective action of quizalofop-ethyl, but may not be the basis for selectivity.
INTRODUCTION

The ethyl ester of quizalofop is currently being developed for annual and perennial grass control in broadleaf crops (16). Field and greenhouse research indicate that quizalofop-ethyl is injurious to cucumber plants with rates as low as 0.035 kg ai/ha (Chapter I). Developing symptoms on cucumber plants concur with results of field and greenhouse studies with quizalofop-ethyl on grass plants (12). Treated leaves of susceptible grass plants showed a complete suppression of growth within 24 hours of foliar application of the herbicide. This was followed by yellowing and necrotic symptoms throughout the leaf system six days after treatment (12).

Absorption and translocation of post-emergence grass herbicides has been reported in a variety of tolerant and susceptible plant species (1, 15, 18). In an uptake, translocation, and metabolism study with $^{14}$C-fluazifop-butyl [(+)-butyl-2-[4-5{(5-(trifluoromethyl)-2-pyridinyl)oxy}phenoxy] propanoic acid], Velovitch compared activity in foxtail millet [Setaria italica

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(L.) P. Beauv. to common cocklebur [Xanthium pensylvanicum (Wallr.) #XANST]. Minimal translocation of $^{14}$C occurred in the foxtail millet as 75% of the applied $^{14}$C fluazifop-butyl remained in the treated leaf after 5 days. Translocation of $^{14}$C-fluazifop-butyl did occur in common cocklebur as concentrations of $^{14}$C in the treated leaf decreased over time with a concomitant increase of $^{14}$C in other plant tissues. Kells et al. (15) reported that differential absorption and translocation of $^{14}$C-fluazifop-butyl did not contribute to the selectivity of fluazifop-butyl in tolerant soybean [Glycine max (L.) Merr.] and susceptible quackgrass [Agropyron repens (L.) Beauv. #AGRRE]. Autoradiographs of treated plants indicated basipetal translocation in both species after 6h with foliarly applied $^{14}$C-fluazifop-butyl. Buhler et al. (2) also report that differential absorption of $^{14}$C-haloxyfop-methyl [methyl 2-[4-[(3-chloro-5- (trifluoromethyl) -2-pyridinyl) oxy] phenoxy] propanoate] was not a selectivity factor between tolerant soybean and susceptible grass species, but translocation was greater in the tolerant soybean.

Quizalofop-ethyl contains the oxyphenoxypyanoate moiety found in many other investigated grass specific herbicides (1, 8, 9, 23). Transport of these herbicides is reported to be directly correlated to the de-esterification capacity of the treated plant.

2Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Science 32, Suppl. 2. Available from WSSA, 309 West Clark St., Champaign, IL 61820.
which accounts for the transport of these compounds as their free acid to their site(s) of action (9, 13, 18). Whole plant autoradiographic studies with various grass plants, revealed that the transport of $^{14}C$-benzoylprop-ethyl from a treated leaf, to be predominantly acropetal with limited basipetal movement (13). A similar application with $^{14}C$-benzoylprop showed a greater basipetal movement, approximately 5 times that of benzoylprop-ethyl, especially in dicotyledonous species. Therefore, $^{14}C$-benzoylprop exhibits greater symplastic mobility. Hendley et al. (9) reported similar results with esters of $^{14}C$-pyridinyloxyphenoxypropionate herbicides applied to quackgrass leaves.

Wilhm et al. (23) report that $^{14}C$-quizalofop-ethyl was not as readily absorbed by quackgrass as $^{14}C$-haloxyfop. The $^{14}C$ detected in the leaf wash was considerably higher for quizalofop-ethyl than haloxyfop. Translocation appeared to be limited to the phloem of treated quackgrass with small amounts of $^{14}C$ in rhizomes and adjacent quackgrass shoots (23). The researchers proposed, since quackgrass absorbed more haloxyfop than quizalofop-ethyl under conditions giving similar quackgrass control, that indicates greater activity of the quizalofop-ethyl molecule. Ikai et al. (12) reported a 20% increase in penetration of $^{14}C$-quizalofop-ethyl over a six day period. But, the amount of translocated radioactivity from treated leaves was minimal.

There is currently no information available as to the absorption and translocation scheme of quizalofop-ethyl in
broadleaf plants, especially those exhibiting sensitivity. The proposed site of action of quizalofop-ethyl in grass plants is meristematic tissues. Therefore, translocation of the active form of quizalofop-ethyl to its proposed site(s) of action would be critical for herbicidal expression. Differences in the absorption and translocation patterns between a susceptible broadleaf and susceptible grass species would provide evidence of selectivity based on translocation patterns and aid in determining a mechanism(s) of action at the proposed site. The objectives of this research were to compare: a) absorption and translocation of $^{14}$C-quizalofop-ethyl in a susceptible broadleaf (cucumber) to a susceptible grass species and b) to relate this information to the observed selectivity of the herbicide.

MATERIALS AND METHODS

General Conditions. All studies were conducted in a growth chamber with the following conditions: 14h photoperiod, photosynthetic photon flux density (PPFD) of 210 umol m$^{-2}$ s$^{-1}$ at canopy surface, and 70% ± 1% relative humidity. Temperatures were 25° ± 1° C for cucumber and 28° ± 1° C for corn during the light period and 22° ± 1° C during the dark period.

Calypso cucumber seeds were germinated in vermiculite under the above conditions. Five day old seedlings were transferred to 400 ml amber bottles (one plant/per bottle) containing a 35% strength nutrient solution (21). Gold Cup corn seeds were planted in 15 cm diameter plastic pots filled with vermiculite. After seedlings
emerged pots were thinned to 1 corn plant per pot. Corn plants were surface irrigated as needed to maintain media moisture. Corn plants were fertilized with nutrient solution 3 times per week.

Bioassay. A bioassay was conducted to determine quizalofop-ethyl effects on cucumber growth and determine optimum treatment conditions to elicit the desired herbicidal activity with $^{14}$C-quizalofop-ethyl. A treatment solution was prepared by combining technical grade herbicide with acetone, glycerol, distilled water, and oxysorbic (20 POE) (polyoxyethylene sorbitan monolaurate) surfactant (91.8:4:4:0.2 v/v/v/v). Agitation of the mixture produced a stable emulsion. Thin layer chromatograms (TLC) of technical quizalofop-ethyl produced no degradation products and purity was greater than 95%.

Quizalofop-ethyl was applied to cucumber plants in the 2 to 3 true leaf stage. Three 10 ul droplets of herbicide at 0, $10^{-6}$, $10^{-5}$, $10^{-4}$, and $10^{-3}$ M were applied to the adaxial surface of the first true leaf of 14 day old cucumber plants. The timing of application was based on the physiological status of the cucumber plant and treatment leaf (11).

Plant tissue was harvested 10 days after treatment, prior to the onset of flower bud formation. Plants were sectioned into leaves (including cotyledons), shoot, and root. Total and treated leaf area were recorded. Plant sections were dried at $70^\circ$ C for 48h and weights recorded. Untreated plants were also harvested at

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$^3$Information provided by Biochemicals Dept. of E.I. duPont de Nemours and Co., Inc., Wilmington, DE.
the onset of the experiment to determine initial leaf area and dry weights. Relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) were calculated on a whole plant or treated leaf basis (7).

Radioassay. Radiolabeled $^{14}$C-quizalofop-ethyl was received as a solid (uniformly labeled phenyl ring; spec. ac. 8.13 mCi/mmol). The herbicide was dissolved in acetone and a radiolabel purity determination was performed using thin layer chromatography (TLC). A 2 µl droplet of $^{14}$C-quizalofop-ethyl (140,304 dpm) was spotted on a 5 by 20 cm thin layer (250 µm) silica gel plate and developed for 10 cm in toluene/acetone/methanol/glacial acetic acid/distilled water (40:25:15:10:10 v/v/v/v/v). Radioactivity on the thin layer plate was located and quantified using an automatic thin layer radiochromatogram scanner. Radiolabel purity of $^{14}$C-quizalofop-ethyl was greater than 99% (appendix B).

A stable treatment emulsion was prepared with $^{14}$C-quizalofop-ethyl as previously described. The final herbicide concentration in the $^{14}$C treatment solution was 0.6 mM. This is within the rate range delivering the desired herbicidal response (see results of bioassay). Cucumber plants were treated with $^{14}$C-quizalofop-ethyl when in the 2 to 3 true leaf stage. Three 10 µl droplets (0.05 uCi/10 µl; 0.6 mM) were applied as described in the bioassay. Corn plants were treated

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4 Silica gel plate. Sigma Chemical Co., St. Louis, MO.

with $^{14}$C-quizalofop-ethyl when in the 3 leaf stage. One 10 ul
droplet (0.05 uCi/10ul; 0.6mM) was applied midleaf along the
midvein of the adaxial surface of the second emerged leaf.
Treatment leaves of cucumber and corn were positioned horizontally
prior to treatment to prevent surface movement of applied
$^{14}$C-quizalofop-ethyl.
Absorption and Translocation. Cucumber plants treated as described
above, were harvested at 3, 12, 48, and 192 hours after treatment
(HAT) and sectioned into 5 parts: treated leaf, apical leaves,
cotyledons, shoot, and root. Plants were immediately frozen in
liquid nitrogen, lyophilized, and stored at -60$^\circ$C. To remove
unabsorbed $^{14}$C-quizalofop-ethyl, the treated leaf was washed
twice by immersion in 20 ml 10% (v/v) aqueous acetone for 30s$^{-1}$
(5). Quizalofop-ethyl is highly soluble in acetone. A 5 ml
aliquot of each leaf wash was added to 15 ml of INSTA GEL$^6$ liquid
scintillation cocktail for radioassay as gels. The total
radioactivity remaining in the plant was considered to be absorbed
herbicide. For each plant the percentage of the total recovered
$^{14}$C by each plant part (including leaf wash) was determined.
The amount of radioactivity in each plant part was quantified
by combustion$^7$ and liquid scintillation spectrometry$^8$. Five

$^6$INSTA-GEL, a liquid scintillation cocktail for aqueous and
nonaqueous samples. Packard Instruments, Downers Grove, IL.

$^7$Packard TriCarb Model 306. Packard Instruments, Downers
Grove, IL.

$^8$Beckman LS 6800. Beckman Instruments, Inc. Fullerton, CA.
ml aliquots of nutrient solution were mixed with 15 ml scintillation cocktail for radioassay of $^{14}C$. Counts were accumulated to two sigma error to provide reliable measurements of low levels of radioactivity (22). Counts per minute were corrected for combustion efficiency, background, and dilution. Quench correction was achieved by internal standardization and resulting data were converted to disintegrations per minute. All data were converted to percent of total $^{14}C$ recovered or percent of applied $^{14}C$ for each plant.

One plant of corn and cucumber was processed for autoradiography at the end of each treatment period (3). Treatment periods for corn were 3 and 24 hours. Autoradiographs were developed for 7 days.

Statistical Analysis. All experiments were designed as a randomized complete block with 4 single plant replications. In the growth bioassay, treatment means represent the mean of two experiments. Statistical analysis of treatment means was performed using analysis of variance (single degree of freedom F test) and orthogonal polynomial fitting (19).

RESULTS AND DISCUSSION

Bioassay. Quizalofop-ethyl did not significantly alter the RGR of cucumber plants when compared to control plants (Figure 5). When examining quizalofop-ethyl effects on the treated leaf only, the $10^{-5}$ M to $10^{-3}$ M herbicide concentration range caused a 19% to 45% decline in RGR (Figure 5). The $10^{-6}$ M herbicide
Figure 5. Effect of quizalofop-ethyl on the relative growth rate (RGR) of cucumber. Symbols represent: intact plant (INT PLT) and treated leaf (TRT LF). Bars represent the 95% confidence interval of that treatment mean.
Figure 5.
concentration did not reduce the treated leaf's growth rate.

Cucumber plant NAR declined with increasing quizalofop-ethyl concentration. A slight, but significant decrease in NAR of 13% was observed at the $10^{-3}$ M quizalofop-ethyl treatment (Figure 6). As with RGR, the NAR of the treated leaf was more extensively inhibited in comparison to whole plant NAR. Treated leaf NAR declined 52% at the $10^{-3}$ M quizalofop-ethyl treatment when compared to the control NAR (Figure 6). Ten days after treatment, there was no significant difference in LAR of treated and untreated cucumber plants on a whole plant or treated leaf basis.

The data suggest that the effect of quizalofop-ethyl on cucumber RGR and NAR is localized to the region of application. All quizalofop-ethyl treatments caused chlorosis/necrosis of cucumber leaf tissue within 2 days. Under field conditions injury symptoms would not be visible until 10 days after treatment. Injury symptoms were more pronounced at the higher herbicide concentrations. Effects of quizalofop-ethyl on a whole plant basis are limited but, are greatly pronounced when considering only treated tissue.

Suppression of growth related functions appears to be a consistent effect of quizalofop-ethyl in susceptible plants (12). The degree of this effect appears to differ dependent on plant species and/or site of herbicidal activity.

Radioassay. Initial uptake of $^{14}$C-quizalofop-ethyl (3h) was rapid with more than 49% of applied $^{14}$C quantified in the intact cucumber plant (Table 3). Continued uptake and redistribution
Figure 6. Effect of quizalofop-ethyl on the net assimilation rate (NAR) of cucumber. Symbols represent: intact plant (INT PLT) and treated leaf (TRT LF). Bars represent the 95% confidence interval of that treatment mean.
Figure 6.
Table 3. Distribution of percent of applied \( ^{14} \text{C}-\text{quizalofop-ethyl} \) in cucumber plants over time.

<table>
<thead>
<tr>
<th>Hours After Treatment</th>
<th>Assayed ( ^{14} \text{C} )</th>
<th>( \bar{X} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>49.5</td>
<td>53.6</td>
</tr>
<tr>
<td>12</td>
<td>38.1</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>56.0</td>
<td></td>
</tr>
<tr>
<td>192</td>
<td>70.8</td>
<td></td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Assayed ( ^{14} \text{C} )</th>
<th>( ^{14} \text{C} ) not accounted for at each exposure period.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact plant</td>
<td>( ^{14} \text{C} )</td>
</tr>
<tr>
<td>Leaf wash</td>
<td>( ^{14} \text{C} )</td>
</tr>
<tr>
<td>Unrecovered(^{b})</td>
<td>( ^{14} \text{C} )</td>
</tr>
</tbody>
</table>

\(^{a}\) Means across columns were not significantly different \((P = 0.05)\)

\(^{b}\) Unrecovered = \( ^{14} \text{C} \) not accounted for at each exposure period.
up to 192h, showed no significant changes on an intact plant basis. Radiolabeled quizalofop-ethyl recovered in the leaf wash averaged approximately 40% of applied $^{14}$C over time with no significant change in amount of radioactivity present (Table 3). Three to 12h following application of $^{14}$C-quizalofop-ethyl to a susceptible broadleaf, cucumber, absorption was complete. This is similar to $^{14}$C-quizalofop-ethyl treatment to susceptible quackgrass (23). After 96h 55% of the $^{14}$C recovered was still present in the quackgrass leaf wash. Uptake by cucumber and quackgrass differed from barnyardgrass [Echinochloa crus-galli (L.) Beauv. #ECHCG] in that barnyardgrass continued to absorb $^{14}$C-quizalofop-ethyl over a six day period (12).

The distribution of $^{14}$C-quizalofop-ethyl absorbed was confined to the treated leaf during all exposure periods with no apparent translocation to any other plant part (Table 4). When averaged over all exposure times, the treated leaf contained 96% and 51% of absorbed and applied $^{14}$C-quizalofop-ethyl, respectively (Table 5). Though translocation of $^{14}$C-quizalofop-ethyl was reported in susceptible grasses, only small amounts of $^{14}$C were detected in plant parts other than the treated leaf (12, 21). The lack of transport of $^{14}$C-quizalofop-ethyl is consistent with the location of herbicidal effects on growth of cucumber plants expressed in the bioassay. The activity was confined to the treated leaf.

Plants such as cucumber, soybean, wheat, and corn contain complex systems of ester hydrolases (14). These enzymes were found
Table 4. Translocation of foliarily applied $^{14}$C-quizalofop-ethyl in cucumber 3, 12, 48, and 192 hours after treatment.

<table>
<thead>
<tr>
<th>Plant Portion</th>
<th>Hours After Treatment$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Apical leaves</td>
<td>2.5</td>
</tr>
<tr>
<td>Treated leaf</td>
<td>92.8*</td>
</tr>
<tr>
<td>Cotyledons</td>
<td>0.4</td>
</tr>
<tr>
<td>Shoot</td>
<td>3.9</td>
</tr>
<tr>
<td>Root</td>
<td>0.4</td>
</tr>
</tbody>
</table>

$^a$ Means within a column followed by (*) are significantly different ($P = 0.05$).
Table 5. Translocation of foliarily applied $^{14}$C-quizalofop-ethyl in cucumber averaged over all exposure times$^a$.

<table>
<thead>
<tr>
<th>Plant Portion</th>
<th>% of applied</th>
<th>% of recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical leaves</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Treated leaf</td>
<td>51.5*</td>
<td>96.5*</td>
</tr>
<tr>
<td>Cotyledons</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Shoot</td>
<td>0.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Root</td>
<td>0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

$^a$ Means within a column followed by (*) are significantly different ($P = 0.05$).
to differ between species and even in different parts of the same plant (17). Information concerning the in vitro action of plant esterases on ester containing herbicides (8, 9, 13, 20) must be considered in determining a basis for selectivity of quizalofop-ethyl between plant species. Factors affecting de-esterification in vitro could help explain changes in in vivo herbicide movement and efficacy (10). The lack of transport of quizalofop-ethyl in cucumber may be related to the lack of de-esterification of this herbicide which could limit or localize its herbicidal activity to treated tissue.

Autoradiographs of cucumber plants agreed with quantitative results for translocation and distribution of $^{14}$C-quizalofop-ethyl (Figure 7). In cucumber, translocation of $^{14}$C was minimal and apparently confined to the apoplast. This was determined qualitatively from autoradiographs showing the characteristic wedge shaped pattern of $^{14}$C-distribution spreading towards the outer margin of treated leaves (3). Autoradiographs of the 3h and 192h exposure periods express this pattern very clearly (Figure 7). Autoradiographs of cucumber for all exposure times were similar therefore, the initial (3h) and final (192h) exposure time periods were presented.

In contrast, corn autoradiographs showed both apoplastic and symplastic transport of $^{14}$C-quizalofop-ethyl. Detection of radiolabel transported from the treated area increased in the distal portions of the leaf at 3h (Figure 8). After 24h, $^{14}$C-quizalofop-ethyl has begun to move from the treated leaf to
FIGURE 7. Translocation and distribution of $^{14}$C-quizalofop-ethyl in cucumber. Plant and corresponding autoradiograph following harvest 3h (A) and 192h (B) after treatment. Plant left and autoradiograph right. The treated leaf is marked with an arrow.
FIGURE 8. Translocation and distribution of $^{14}$C-quizalofop-ethyl in corn. Plant and corresponding autoradiograph following harvest 3h (C) and 24h (D) after treatment. Plant left and autoradiograph right. The treated leaf is marked with an arrow.
the stem and acropetally into a newly developing corn leaf. Basipetal transport and accumulation in meristematic tissues reflect a source: sink photosynthate relationship providing evidence of symplastic transport of $^{14}$C-quizalofop-ethyl in corn. This agrees with transport in grasses with quizalofop-ethyl (12, 23), as well as other postemergence grass herbicides (4, 8). It is apparent, that not unlike other grass specific herbicides, differences in plant species susceptibility to quizalofop-ethyl may lie in the translocation differences at the cellular level (1) as well as the reaction of these cells to the herbicide at specific sites. The observed differences in translocation may contribute to the selective action of quizalofop-ethyl, but may not be the basis for selectivity.
LITERATURE CITED


CHAPTER III

Inhibition of Chloroplast-Mediated
Reactions by Quizalofop-Ethyl

ABSTRACT. A mechanism of action of quizalofop-ethyl [(+)
ethyl-2-[4-[(6-chloro-2-quinoxyaliny) oxy] phenoxy] propionic
acid] herbicide was determined as a basis for selective action
between monocotyledonous and dicotyledonous plants.
Quizalofop-ethyl inhibited electron transport in both cucumber
(Cucumis sativus L.) and corn (Zea mays L.) chloroplasts.
Half-maximal (I50) inhibition of ATP synthesis was achieved with
a 50 uM concentration of quizalofop-ethyl in coupled corn
chloroplasts. Coupled cucumber chloroplast ATP synthesis was not
inhibited at herbicide concentrations up to 100 uM. Corn
chloroplast fractions contained greater quantities of bound
14C-quizalofop-ethyl than cucumber chloroplast fractions
following incubation which is consistent with the inhibition of ATP
synthesis. Thin layer radiochromatograms of 14C labeled
quizalofop-ethyl show no metabolism of parent herbicide incubated
in light and dark chloroplast-mediated reactions. Results suggest
that quizalofop-ethyl herbicide may be classified as an energy
transfer inhibitor in photosynthetic reactions with activity
dependent on plant species.
INTRODUCTION

Photosynthetic inhibition is a specific site of herbicide action (6, 7, 9, 16, 18, 22). The chloroplast has been targeted for herbicide development in that the majority of available commercial herbicides act on the chloroplast (5). Many herbicides are active on photo-induced electron transport with inhibition at a site closely associated with photosystem II (PS II) (10, 16) and at a point intermediate between the two photosystems (22, 23).

The chloroplast mediated conversion of light energy into ATP (photophosphorylation) is also a site of herbicide action. Inhibitors of photophosphorylation act on the CF_{1}-CF_{0} complex (1, 12, 13, 15). Nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether) herbicide has been shown to inhibit photophosphorylation at near 10 \mu M concentrations (13). Higher concentrations of nitrofen (> 10^{-5} M) yield a DCMU-type inhibition of photosynthetic electron flow and uncoupling of photophosphorylation. The inhibition of photophosphorylation was correlated to growth reduction of a cell-free algal system (13). Trifluralin (\(\alpha\alpha\alpha\)-trifluoro-2,6-dinitro-N, N-dipropyl-p-toluidine) and diallate [S-(2,3-dichloroallyl) diisopropylthiocarbamate] herbicides have also been shown to exhibit activity on chloroplast-mediated reactions (18). Both herbicides inhibit electron transport, ATP synthesis, and cytochrome f reduction at physiological concentrations in isolated spinach chloroplasts.
Preliminary studies on the mode of action of quizalofop-ethyl, a new postemergence grass killing herbicide (19), on a broadleaf plant have demonstrated the following: suppression of growth of treated tissue, chlorosis followed by necrotic symptoms, and a significant decline in gross photosynthetic capacity of treated plants estimated by effects on net assimilation rate. Results are in agreement with current findings with grass plants (8). Once an inhibitor (herbicide) reaches its active site(s) an interference is imposed to key metabolic reactions which may lead to a sequence of events that ultimately cause lethality. Determining the type and extent of interference reveals a mechanism of action of the particular compound. Research concerning the biochemical mechanism of action of this herbicide has just begun (8). Investigations have not been focused on photosynthetic inhibition nor on the differences between monocotyledonous and dicotyledonous species. Evidence is presented with respect to quizalofop-ethyl induced inhibition of chloroplast-mediated reactions in monocotyledonous and dicotyledonous plants. We will demonstrate that quizalofop-ethyl is a potent inhibitor of photophosphorylation dependent on plant species which may be a mechanism for this herbicide's selective action.

MATERIALS AND METHODS

Plant Material. *Cucumis sativus* L. cv Calypso (cucumber) and *Zea mays* L. cv Gold Cup (corn) seeds were germinated in vermiculite. Five day old cucumber seedlings were transferred to a 35% nutrient
culture (24). Corn plants were fertilized with nutrient solution three times per week. Plants were grown in a growth chamber with the following conditions: 14 h photoperiod, PPFD of 210 umol m$^{-2}$ s$^{-1}$ at canopy surface, and 70% relative humidity. Day night temperature regime was 25/22°C and 28/22°C for cucumber and corn, respectively.

Reagents. Quizalofop-ethyl and its radiolabeled isotope were provided by E.I. duPont de Nemours and Co., Inc. Gramicidin and NH$_4$Cl were obtained from Sigma. ATP standards and luciferin/luciferase monitoring reagents were obtained from LKB-Wallac. All other reagents were purchased from Sigma and were of analytical grade.

Mechanical Isolation of Chloroplasts. Fifty grams of 7 day old cucumber cotyledon tissue were homogenized in a Waring blender for two 5s bursts. The isolation medium (100 ml) contained: 300 mM sorbitol, 3 mM MgCl$_2$, 0.5 mM EDTA, and 30 mM Na-Tricine (pH 7.8). The homogenate was filtered through 16 layers of cheesecloth and centrifuged at 2500g for 1.5 min. The resulting chloroplast pellet was osmotically shocked by twice washing in 10 ml distilled water and centrifugation (2500g) for 1.5 and 3 min. Chloroplasts were resuspended in 5 ml of resuspension medium containing: 200 mM sorbitol, 10 mM MgCl$_2$, 0.5 mM EDTA, BSA 5 mg/ml, and 5 mM HEPES (pH 7.4). Ten grams of corn leaf tissue were homogenized for 6s in isolation medium containing: 350 mM sorbitol, 1 mM MgCl$_2$, 5 mM EDTA, BSA 2 mg/ml, and 25 mM HEPES (pH 7.8). The homogenate was filtered through two layers of Miracloth and centrifuged at 6000g
for 20s. The chloroplast pellet was washed in 10 ml of 
resuspension medium containing: 300 mM sorbitol, 5 mM EDTA, BSA 1 
mg/ml, and 25 mM HEPES (pH 8.0) and centrifuged at 1500g for 2 min. 
Pellets were resuspended in 5 ml of resuspension medium. All 
isolations were conducted at 0 to 4° C. Broken chloroplast 
preparations were used immediately following isolation. 
Chlorophyll concentration was determined according to Arnon (2). 
Oxygen Evolution. Effects of quizalofop-ethyl on light induced 
ferricyanide-dependent O2 evolution were measured 
polarographically with a YSI model 53 O2 monitor (Yellow Springs) 
using a Clark electrode. Reaction temperature was regulated by a 
water bath at 25° C (cucumber) or 30° C (corn). 
Quizalofop-ethyl, being insoluble in water, was dissolved in 
acetone at time of treatment. Acetone concentration did not exceed 
0.2% of reaction medium and had no deleterious effects on 
chloroplast-mediated reactions in these experiments. Assay 
concentrations of quizalofop-ethyl were 25, 50, 75, and 100 μM. 
Light sources were two 375-w photoflood lamps. PPFD, at the 
reaction chamber surface, was 1500 to 1800 umol m-2 s-1 at 400 
to 700 nm. Cucumber or corn chloroplasts (30 μg Chl/ml) were 
added to a reaction medium (14) containing 3 mM ferricyanide to 
measure photosynthetic electron flow by the Hill reaction (H2O → 
ferricyanide). Chloroplasts and substrate/inhibitors (all at pH 
7.6) were added in the dark 1 min prior to illumination. Light 
driven reactions continued for 3 min. Coupled and uncoupled 
electron transport were subjected to treatment with
quizalofop-ethyl. Uncoupling reagents were gramicidin (10 uM) and NH₄Cl (10 mM). Data points represent the mean of two O₂ evolution runs/experiment with experiments repeated four times.

Photophosphorylation Assay. Quizalofop-ethyl activity on photophosphorylation was determined from illuminated chloroplasts under conditions described for O₂ evolution. Photosynthetic O₂ evolution rates were linear throughout the photophosphorylation controls, 4 to 5 min, indicating steady state conditions. The reaction was terminated and ATP extracted by addition of 0.6 M TCA, homogenizing in a Polytron mixer (Brinkman Instruments), and centrifugation at 14,500g for 10 min. TCA was removed by partitioning the ATP extract against three volumes of cold diethyl ether (0 to 4°C). Remaining ether was evaporated by bubbling N₂ through the extract. ATP extracts were adjusted to pH 7.0 with 1 N NaOH and immediately stored at -60°C. ATP content was assayed by the luciferin/luciferase method (4). Internal standards were introduced in all measurements. Each data point represents the mean of three samples done in triplicate. Values represent ATP levels above dark controls. Each experiment was carried out twice.

Chloroplast Binding/Metabolism Assay. Herbicide binding assays used the same suspension medium used for electron transport and photophosphorylation measurements, except BSA was omitted. Reaction conditions for cucumber and corn chloroplast suspensions were as previously described. Metabolism of uniformly ring labeled ¹⁴C-quizalofop-ethyl (spec. act. 8.13 mCi/m mole) in a chloroplast suspension (30 ug Chl/ml) of cucumber or corn, was measured to
determine if inhibitor activity in chloroplast-mediated reactions is due to parent compound or metabolite(s). An aliquot (10 ul) of \(^{14}\text{C}\)-quizalofop-ethyl (2 x \(10^5\) cpm, 100 uM) was added to the assay (5 ml total volume) of chloroplast ferricyanide-dependent \(O_2\) evolution. Dark incubation and reaction times were 1 and 3 min, respectively. Radiolabeled quizalofop-ethyl was extracted by partitioning the chloroplast suspension against two 5 ml volumes of toluene. Separated toluene was evaporated by \(N_2\) bubbling at 60° C. The dried remainder was redissolved in acetone (500 ul) and sampled (5 ul) for liquid scintillation spectrometry. The reaction medium was centrifuged (5000 g; 3 min) and 0.5 ml aliquots of clear supernatant were removed for counting. The amount of bound inhibitor was calculated from the difference between total radioactivity added to the chloroplast suspension and the combined amounts of free inhibitor found in the extract (nonaqueous phase) plus that found in the supernatant (aqueous phase). The procedure was a modification of that used by Pfister et al. (17). Five microliter aliquots of extracted \(^{14}\text{C}\) (nonaqueous phase) were spotted on thin layer silica plates for metabolism determinations. The effect of light on herbicide degradation was also measured with suspension minus chloroplasts in light and dark. The solvent system used for TLC was toluene/acetone/methanol/glacial acetic acid/distilled water (40:25:15:10:15 by volume ratio).

Radioactivity present on thin layer chromatograms was located and quantified using a Berthold LB276 TLC Scanner (Beta Analytical). Aliquots were co-chromatographed and scans of extractions included:
\[ ^{14} \text{C-quizalofop-ethyl standard (treatment solution)}, \]
\[ ^{14} \text{C-herbicide (light, no chloroplasts)}, \]  
\[ ^{14} \text{C-herbicide (dark, no chloroplasts)}, \]  
\[ ^{14} \text{C-herbicide (light plus chloroplasts)}, \]  
\[ ^{14} \text{C-herbicide (dark plus chloroplasts)}, \]  
\[ \text{and a (light plus chloroplasts) control. Counts per minute were corrected for extraction efficiency, background, and dilution. Quench correction was achieved by internal standardization and resulting data were converted to disintegrations per minute.} \]

RESULTS

Quizalofop-ethyl herbicide effectively inhibited both coupled and uncoupled electron flow from water to ferricyanide in cucumber and corn broken chloroplast suspensions (Figures 9 and 10).

Though concentration of herbicide in the chloroplast suspensions reached 100 \( \mu \text{M} \), half-maximal \((I_{50})\) inhibition was not achieved. Quizalofop-ethyl has a low water solubility \((3 \times 10^{-5} \text{ g/100 ml at } 20^\circ \text{C})\) which limited the inhibitor concentration to a maximum of 100 \( \mu \text{M} \) in the reaction medium. There was no difference in herbicide inhibition of coupled electron flow in both plant species, with electron transport rates approximately 70\% of control values at a 75 \( \mu \text{M} \) concentration of quizalofop-ethyl (Figure 9).

When electron flow was uncoupled, a difference in chloroplast susceptibility was observed between plant species (Figure 10). The cucumber chloroplast suspension exhibited greater sensitivity to quizalofop-ethyl in the 50 to 75 \( \mu \text{M} \) concentration range.

Inhibition of uncoupled electron flow in corn chloroplast
Figure 9. Influence of quizalofop-ethyl concentration on coupled photosynthetic electron transport.

Ferricyanide-dependent O₂ evolution, coupled system, control rates averaged 110 umol O₂/mg Chl/h. Data points represent treatment means ± SE.
Figure 9.
Figure 10. Influence of quizalofop-ethyl concentration on uncoupled photosynthetic electron transport. Ferricyanide-dependent O$_2$ evolution, uncoupled system, control rates averaged 380 umol O$_2$/mg Chl/h. Data points represent treatment means ± SE.
Figure 10.
suspensions was limited by the solubility of quizalofop-ethyl in
that inhibition continued to increase with increasing herbicide
concentration up to the 100 uM maximum. The difference in
sensitivity was diminished at the 100 uM concentration, where
cucumber and corn chloroplast suspensions exhibited uncoupled
electron flow rates 65 to 70% of controls (Figure 10).

Noncyclic photophosphorylation was very sensitive to
quizalofop-ethyl herbicide, with inhibition dependent on plant
species. Photophosphorylation in cucumber chloroplasts expressed
tolerance to quizalofop-ethyl up to a 100 uM concentration (Figure
11). In comparison, corn chloroplast suspension ATP formation was
very sensitive to quizalofop-ethyl. Herbicide in the 50 to 100 uM
concentration range inhibited photophosphorylation 50 to 75%,
respectively, with half-maximal inhibition (I_{50}) achieved at a 50
uM concentration (Figure 11).

The amount of chloroplast bound $^{14}$C-quizalofop-ethyl was
determined in cucumber and corn chloroplast suspensions (30 ug
Chl/ml) under photosynthetic (light) and non-photosynthetic (dark)
conditions. Toluene extracted $^{14}$C-quizalofop-ethyl (nonaqueous
phase) present in cucumber chloroplast suspensions was greater than
the nonaqueous phase of corn chloroplast suspensions in both light
and dark (Table 6). Exposure of chloroplast suspensions to light
did not greatly decrease the amount of $^{14}$C-quizalofop-ethyl
extracted within a plant species. When comparing species, the
radiolabeled herbicide present in the corn chloroplast (aqueous)
reaction medium was almost 4 and 6 times, in light and dark,
Figure 11. Influence of quizalofop-ethyl concentration on photophosphorylation associated with noncyclic electron transport. Data points represent treatment means ± SE.
Figure 11.
Table 6. Partitioning of $^{14}$C-quizalofop-ethyl following ferricyanide-dependent noncyclic electron transport in isolated chloroplasts.

<table>
<thead>
<tr>
<th>Source</th>
<th>Conditions</th>
<th>Nonaqueous</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>dpm x 10^{-3}</td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>light</td>
<td>121.8 ± 6.8</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>dark</td>
<td>133.4 ± 3.9</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>cucumber</td>
<td>light</td>
<td>112.7 ± 2.7</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>dark</td>
<td>120.3 ± 6.3</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>corn</td>
<td>light</td>
<td>92.3 ± 13.9</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>dark</td>
<td>82.4 ± 3.8</td>
<td>7.3 ± 0.3</td>
</tr>
</tbody>
</table>

\textsuperscript{a} $^{14}$C-quizalofop-ethyl added to a final concentration of 100 uM.

\textsuperscript{b} Final chlorophyll (Chl) content 150 ug in 5 ml reaction medium.
respectively, that found in the cucumber suspension aqueous phase (Table 6). This translates to significantly more 14C-quizalofop-ethyl bound to corn versus cucumber chloroplast fractions (Table 7). Averaged over light and dark treatments, approximately 23% more 14C-herbicide was bound in the corn chloroplast suspensions. Degradation of 14C-quizalofop-ethyl in light, with no chloroplasts present, appears to be negligible and not a factor in the binding assay (Table 6).

To determine if inhibition of chloroplast-mediated-reactions was due to parent compound and/or metabolite(s) thin layer chromatography (TLC) was performed on extracted (nonaqueous) 14C-quizalofop-ethyl from the binding assay. Recoveries of extracted 14C were greater than 95% for all assays. Examination of the chromatograms of all extracts showed that, under all assay conditions, the parent ester constituted the major component (Figures 12 and 13). No metabolites were detected. When co-chromatographed with 14C-quizalofop-ethyl standard the extract Rf values were consistently the same for cucumber and corn chloroplast suspensions. Results suggest that quizalofop-ethyl induced inhibition of chloroplast-mediated reactions is due to the parent ester of the herbicide.

**DISCUSSION**

Evidence is presented on the effects of quizalofop-ethyl herbicide on electron transport, photophosphorylation, chloroplast binding and metabolism in monocotyledonous and dicotyledonous plant
Table 7. Calculated chloroplast bound $^{14}\text{C}$-quizalofop-ethyl following ferricyanide-dependent noncyclic electron transport.

<table>
<thead>
<tr>
<th>Chl Source</th>
<th>Conditions $^{ab}$</th>
<th>Bound-$^{14}$C $\text{dpm x 10}^{-3}$</th>
<th>% Total Applied</th>
</tr>
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<tbody>
<tr>
<td>cucumber</td>
<td>light</td>
<td>86.2</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td>dark</td>
<td>78.5</td>
<td>39.3</td>
</tr>
<tr>
<td>corn</td>
<td>light</td>
<td>127.8</td>
<td>56.8</td>
</tr>
<tr>
<td></td>
<td>dark</td>
<td>135.3</td>
<td>60.1</td>
</tr>
</tbody>
</table>

$^{a}1^{4}$C-quizalofop-ethyl added to a final concentration of 100 uM.

$^{b}$Final chlorophyll (Chl) content 150 ug in 5 ml reaction medium.
Figure 12. Thin layer radiochromatographs of extracted $^{14}$C-quizalofop-ethyl from cucumber chloroplast suspensions following ferricyanide-dependent noncyclic electron transport. A: $^{14}$C-quizalofop-ethyl standard, B: $^{14}$C-herbicide/light/no Chl, C: $^{14}$C-herbicide/dark/no Chl, D: $^{14}$C-herbicide/light/Chl, E: $^{14}$C-herbicide/dark/Chl, F: light/Chl control.
Figure 13. Thin layer radiochromatographs of extracted $^{14}$C-
quizalofop-ethyl from corn chloroplast suspensions
following ferricyanide-dependent noncyclic electron
transport. A: $^{14}$C-quizalofop-ethyl standard, B: $^{14}$C-herbicide/light/no Chl, C: $^{14}$C-herbicide/
dark/no Chl, D: $^{14}$C-herbicide/ light/Chl, E: $^{14}$C-herbicide/dark/Chl, F: light/Chl control.
species. Recent research by Ikai et al. (8), on the site of action of quizalofop-ethyl, indicated inhibition of the biosynthesis of protein, RNA, and lipid in grass plants. Electrolyte leakage was also observed from cells incubated with $10^{-5}$ M quizalofop-ethyl for 14h. Though their results did not show the primary target of quizalofop-ethyl, they suggest the herbicides effect on cell membranes is critical for determination of a site of action. This research concentrated on quizalofop-ethyl induced photosynthetic inhibition. Quizalofop-ethyl appears to only weakly inhibit noncyclic electron transport ($H_2O \rightarrow$ ferricyanide) acting similarly in both cucumber and corn chloroplast suspensions. Uncoupling electron flow did not greatly alter quizalofop-ethyl inhibition as was found with the herbicide nitrofen in isolated spinach chloroplasts (13). The results of the electron transport studies suggest that activity of quizalofop-ethyl on photosynthesis is not directly attributable to inhibition of the Hill reaction ($H_2O \rightarrow$ ferricyanide), but may be exerted at an alternate photosynthetic site acting in conjunction with electron transport.

Electron transport dependent phosphorylation can be inhibited by uncoupling phosphorylation from electron transport, inhibiting electron transport, or by interfering with a terminal step of ATP synthesis (16, 25). Though the effects on electron transport were minimal, quizalofop-ethyl reduced rates of ATP synthesis up to 75% in corn chloroplast suspensions. The difference in chloroplast species susceptibility to quizalofop-ethyl, observed with inhibition of photophosphorylation, can be attributed to many
factors such as: function of the chloroplast coupling factor complex, light induced pH changes and endogenous adenylate levels (15, 20, 21). No attempt was made to determine the cause of this difference in susceptibility at this stage of the research. The data described provides evidence that quizalofop-ethyl herbicide may functions as an energy transfer inhibitor in grass species. Though the degree of electron transport inhibition is less than most compounds found in this classification, inhibition of electron transport is directly dependent on the conditions of photophosphorylation. Energy transfer inhibitors generally exhibit slight or no inhibition of basal (non-phosphorylating) electron transport (15). The non-herbicides DCCD (N,N'-dicyclohexylcarbodiimide) and phlorizin (4,4',6'-trihydroxy-2'-glucosidodihydrochalcone), inhibit photophosphorylation as well as coupled electron transport in chloroplasts (1, 16). DCCD was also found to inhibit uncoupled electron flow. These compounds inhibit photophosphorylation by interacting with the membrane bound ATPase in chloroplasts. This CF$_1$-CF$_0$ coupling factor is thought to catalyze the terminal steps in photophosphorylation (3). These results indicate that quizalofop-ethyl probably has a direct effect on photophosphorylation in chloroplasts in addition to its effects on electron transport. The mechanism of quizalofop-ethyl inhibition of cucumber growth is yet to be determined in light of our present results, but effects are localized and concur with findings of physiological effects on grass plants (8).
The greater amount of $^{14}$C-quizalofop-ethyl bound to corn versus cucumber chloroplast fractions may contribute to the inhibitory effects shown in photophosphorylation. If the equilibrium of free to bound inhibitor is dependent on the concentration of inhibitor, amount of chloroplasts, and the affinity of the inhibitor to a binding site (11), then differences in corn and cucumber binding of $^{14}$C-quizalofop-ethyl, at a constant inhibitor and Chl concentration, may be due to inherent or quizalofop-ethyl induced changes at the binding site of these species. Thylakoids of susceptible and resistant chloroplasts of two weed biotypes have shown a marked difference in the membranes affinity to bind atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] or diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] herbicides (17).

Metabolism of $^{14}$C-quizalofop-ethyl chloroplast-mediated reactions, was not a factor in susceptibility to this herbicide in our studies. It is apparent that the inhibitory action of quizalofop-ethyl is due to its parent form. Quizalofop-ethyl appears to exhibit multiple activity in terms of a mechanism of action. The observed differences in electron transport, photophosphorylation, and herbicide:chloroplast binding in these experiments, have all contributed in determining the activity of this herbicide in plants. These results, in conjunction with others (8), should provide a basis for the elucidation of this and similar compounds mechanisms of action resulting in a better understanding of their selectivity.
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GENERAL SUMMARY AND CONCLUSIONS

Results of field and greenhouse studies investigating quizalofop-ethyl's 'broadleaf activity' confirm this herbicide's toxicity to cucumber. Generally, yield is reduced under field conditions with as little as a single application of quizalofop-ethyl at 0.14 kg ai/ha to cucumber plants in the four true leaf stage. At a later growth stage tolerance to quizalofop-ethyl is increased. Under greenhouse conditions, quizalofop-ethyl suppresses cucumber growth with greater phytotoxicity expressed in the presence of a crop oil concentrate or nonionic surfactant (adjuvants). The relationship between herbicide and adjuvant is at best only partly resolved. This was evident with quizalofop-ethyl adjuvant combinations. Cucumber sensitivity to quizalofop-ethyl was reduced at the higher concentration of adjuvant and herbicide in quizalofop-ethyl adjuvant combinations. It can be speculated that the decline in toxicity lies in the interrelationship of many factors such as the dose response relationship of herbicide and adjuvant, the plant species, as well as physical application factors. Injury symptoms in field and greenhouse studies appeared as a chlorosis/necrosis of treated tissue. The use of quizalofop-ethyl in a weed control scheme for cucumbers, would adversely affect crop yield with degree 83
dependent on the crop's stage of growth at time of application. The herbicide effect was augmented by the addition of adjuvant up to a critical concentration of adjuvant.

In cucumber growth bioassays, quizalofop-ethyl caused a reduction in relative growth rate (RGR) and net assimilation rate (NAR) when examining treated tissue only. Effects on a whole plant basis were limiting. The data suggest that the effect of quizalofop-ethyl on growth suppression was localized to the region of application. This would indirectly indicate a lack of transport of quizalofop-ethyl, or its active herbicidal component, from the site of application.

Radioassays indicated that 3 to 12h following foliar application of $^{14}$C-quizalofop-ethyl to cucumber plants, absorption was complete. The distribution of absorbed $^{14}$C was confined to the treated leaf during all exposure periods (3 to 192h) with no apparent translocation to any other plant part. The lack of transport of $^{14}$C-quizalofop-ethyl was consistent with the location of herbicidal effects on growth of cucumber plants expressed in the bioassay. The activity was confined to the treated leaf.

Autoradiographs comparing $^{14}$C-quizalofop-ethyl transport in cucumber versus corn plants revealed differences dependent on plant species. In cucumber, translocation of $^{14}$C was minimal and confined to the apoplast even after 192h of exposure. In contrast, corn autoradiographs show both apoplastic and symplastic transport of $^{14}$C-quizalofop-ethyl from 3 to 24h. It is apparent, that
differences in species susceptibility to quizalofop-ethyl may lie in translocation differences at the cellular level as well as the reaction of these cells to the herbicide at specific sites. Results from the radioassays suggest that the similar activity of quizalofop-ethyl observed in susceptible species is not dependent on transport of this herbicide. Although translocation of quizalofop-ethyl may account for different quantities of herbicide at the active site, it is the activity expressed at that site that determines selectivity.

There has been evidence of a selective mechanism in ester containing herbicides, where de-esterification by plant esterases in vitro could help explain changes in in vivo herbicide movement and efficacy. Therefore, the lack of transport of quizalofop-ethyl in cucumber may be related to the lack of de-esterification of this herbicide which could limit or localize herbicidal activity to treated tissue. This is not to say that cucumber does not contain the same ester hydrolases as found in corn, but that these enzyme complexes may be present in different amounts within the plants and even within their plant parts. Morphological and anatomical differences between cucumber and corn may not allow contact and therefore function of cucumber esterase complexes to de-esterify quizalofop-ethyl to a mobile form (possibly it's de-esterified acid).

Evidence was presented on the effects of quizalofop-ethyl on electron transport, photophosphorylation, chloroplast binding and metabolism in chloroplast suspensions of monocotyledonous and
dicotyledonous plant species. Quizalofop-ethyl appears to only weakly inhibit noncyclic electron transport (H₂O → ferricyanide) acting similarly in both cucumber and corn chloroplast suspensions.

Uncoupling of electron flow did not greatly alter quizalofop-ethyl inhibition. The results of the electron transport studies suggest that activity of quizalofop-ethyl on photosynthesis is not directly attributable to inhibition of the Hill reaction (H₂O → ferricyanide), but may be exerted at an alternate photosynthetic site acting in conjunction with electron transport. Though the effects on electron transport were minimal, quizalofop-ethyl reduced rates of ATP synthesis (photophosphorylation) up to 75% in corn chloroplast suspensions. Cucumber chloroplasts photophosphorylation was not significantly inhibited by quizalofop-ethyl. These observed differences suggest a mechanism of action of quizalofop-ethyl which can serve as a basis for selective action between monocotyledonous and dicotyledonous plant species. The inhibitory actions of quizalofop-ethyl demonstrated in this research may classify this compound as an energy transfer inhibitor. Energy transfer inhibitors act directly on phosphorylation and may or may not affect electron transport.

The amount of chloroplast bound ¹⁴C-quizalofop-ethyl was determined in cucumber and corn chloroplast suspensions. Corn chloroplast fractions were calculated to contain greater quantities of bound ¹⁴C-quizalofop-ethyl than cucumber chloroplast fractions.
following incubation which was consistent with the observed inhibition of ATP synthesis.

Metabolism of $^{14}$C-quizalofop-ethyl in chloroplast-mediated reactions was not a factor in susceptibility to this herbicide in this study. It is apparent that the inhibitory action of quizalofop-ethyl was due to its parent form.

Quizalofop-ethyl appears to exhibit multiple activity in terms of a mechanism of action. The interactions of quizalofop-ethyl and adjuvant under field and greenhouse conditions, reductions in relative growth rate (RGR) and net assimilation rate (NAR), translocation differences dependent on plant species, and differences in effects on electron transport, photophosphorylation, and herbicide:chloroplast binding in these experiments, have all contributed in determining the activity of this herbicide in monocotyledonous and dicotyledonous plant species. These results should provide a basis for the elucidation of this and similar compounds mechanisms of action resulting in a better understanding of their selectivity.
LIST OF REFERENCES


Table 8. ANOVA: effects of quizalofop-ethyl and adjuvant type on cucumber; data analyzed as visual phytotoxicity.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
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<td>2</td>
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<td>0.34</td>
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<td>herbicide rate</td>
<td>2</td>
<td>13009.3</td>
<td>15.23**</td>
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<td>adjuvant type</td>
<td>1</td>
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<tr>
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<td>2</td>
<td>5938.9</td>
<td>6.95**</td>
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<sup>a</sup> Significant at P = 0.01
Table 9. ANOVA: effects of quizalofop-ethyl and adjuvant type on cucumber; data analyzed as percent of controls.

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<th>Source</th>
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<th>F Value&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>11.12**</td>
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</tbody>
</table>

<sup>a</sup> Significant at P = 0.01
Appendix B. Data relative to Chapter 2.

Figure 14. Radiochromatogram of $^{14}$C-quizalofop-ethyl (140,304 dpm $R_f = 0.74$); radiolabel purity scan.
APPENDIX C. DATA RELATIVE TO CHAPTER 3.

Figure 15. Influence of quizalofop-ethyl concentration on coupled photosynthetic electron transport in isolated cucumber chloroplasts. Spectrophotometric determination of ferricyanide reduction at 420 nm. Control rates averaged 250 umol ferricyanide reduced/mg Chl/h. Data points represent treatment means.
Figure 15.
Figure 16. Influence of quizalofop-ethyl concentration on uncoupled photosynthetic electron transport in isolated cucumber chloroplasts. Spectrophotometric determination of ferricyanide reduction at 420 nm. Control rates averaged 765 umol ferricyanide reduced/mg Chl/h (uncoupling agent = 6 mM CH₃NH₂). Data points represent treatment means.
Figure 16.
Figure 17. Potassium ferricyanide calibration curve for spectrophotometric determination of electron transport in isolated cucumber chloroplasts (absorbance at 420 nm).
Figure 17.