Chapter VII

Efficacy, absorption, translocation, and metabolism of mesotrione in Canada thistle (*Cirsium arvense*)

Abstract: Field studies were conducted in 2000 to determine if mesotrione alone or in tank-mixtures with low rates of atrazine would control Canada thistle. Mesotrione applied alone postemergence did not adequately control Canada thistle. However, smaller plants in the rosette stage of growth were more susceptible to mesotrione than Canada thistle plants in the bolting stage of growth. Tank-mixtures of mesotrione at 105 g ai/ha with atrazine at 280 g ai/ha improved control of Canada thistle over that with mesotrione alone, especially when Canada thistle plants were bolting. However, in the greenhouse, combinations of mesotrione plus atrazine at 560 g/ha reduced Canada thistle regrowth more than mesotrione alone or mesotrione plus 280 g/ha atrazine. Mesotrione plus atrazine tank-mixtures increased the rate of tissue necrosis compared to the slower development of bleaching symptoms normally associated with mesotrione alone. Laboratory studies were conducted in 2001 to investigate absorption, translocation, and metabolism of ^{14}C-labeled mesotrione. Uptake, translocation, and metabolism of ^{14}C mesotrione in Canada thistle was generally slow and results did not explain the changes in expressed symptoms and increased Canada thistle control associated with mesotrione plus atrazine tank-mixtures. However, higher levels of absorption and translocation and reduced root metabolism of mesotrione in rosette stage plants compared to bolting plants may help explain why Canada thistle is more susceptible to mesotrione in the rosette stage. The changes in symptomology and increased control from mesotrione plus atrazine tank-mixtures were likely due to the interrelationship between the modes of action of atrazine and mesotrione.

Nomenclature: Atrazine; CGA 152005 [1-(4-methoxy-6-methyl-triazin-2-yl)-3-[2-(3,3,3-trifluoropropyl)-phenylsulfonyl]-urea]; clopyralid; dicamba; flumetsulam; mesotrione; primisulfuron; 2,4-D; Canada thistle, [*Cirsium arvense* (L.) Scop.] #[1] CIRAR; corn, *Zea mays* L.

[1] Letters following this symbol are a WSSA-approved computer code for Composite List of Weeds, Revised 1989. Available from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

Additional index words: Triketone herbicides, bleaching herbicides, perennial weeds.

Abbreviations: COC, crop oil concentrate; HAT, hours after treatment; HPPD, p-hydroxyphenylpyruvate dioxygenase; LSS, liquid scintillation spectroscopy; POST, postemergence; PRE, preemergence; PSII, Photosytem II inhibitor; TLC, thin layer chromatography; UAN, urea ammonium nitrate; WAT, weeks after treatment.

Introduction

Canada thistle [*Cirsium arvense* (L.) Scop.] is a broadleaf perennial weed that grows in temperate climates and a broad range of soil types. It is distributed as far west as California, as far east as Maine, and stretches from the northern regions of the United States throughout Canada (Hodgson 1971). Canada thistle reduces crop yields more than any other perennial weed species (Hunter 1996). A population of 20 plants/m^2 can reduce barley (*Hordeum vulgare* L.) yields up to 34% (O'Sullivan et al. 1982). Canada thistle has reduced corn yields 57%, while reducing soybean (*Glycine max* L.) yields as much as 91% (Elakkad and Behrens 1976).

Canada thistle is located throughout the corn-belt, where its high seed production and large root reserves contribute to its continued proliferation. Seeds are wind disseminated and can produce new seedlings within 2 wk after flower opening (Hodgson 1971). Once Canada thistle is established in a new location it can produce roots over 2 m deep and produce aerial shoots from these roots at depths of 77 cm (Klitz 1930). Therefore, soil applied herbicides have little affect on Canada thistle growth from roots. However, a few preemergence (PRE) herbicides registered for weed control in corn and soybean may aid in control of seedling Canada thistle (VanGessel 1999).

Adequate control of Canada thistle requires systemic herbicides that translocate to the roots where root buds must amass toxic levels of the compound (Hunter 1996). Since herbicides are translocated along with natural assimilates to active meristems, stage of growth often affects herbicide movement (Robertson and Kirkwood 1970). Young, actively growing or "bolting" Canada thistle plants direct most of their assimilates to shoot meristematic regions, which may

limit control of underground reproductive structures when herbicides are applied at this time (Hunter 1996). Generally, Canada thistle plants exposed to less than 16 h of sunlight remain in a rosette, a stage where translocation of herbicides to roots is optimal (Hunter and Smith 1972).

Fall applications of systemic herbicides, such as glyphosate or clopyralid, control underground reproductive structures best when Canada thistle is in the rosette stage as compared to the bud stage of growth because assimilates are traveling toward root meristematic regions for storage (Hunter 1996; Miller and Lym 1998). Root reserves are also low in early spring about one month after Canada thistle emergence (Hodgson 1971).

Control of Canada thistle in corn usually requires high rates or multiple applications of dicamba or 2,4-D applied to Canada thistle prior to the bloom stage or when plants are in a fall rosette stage (Hodgson 1971; Carson and Bandeen 1975). Multiple high rate applications of atrazine in combination with tillage can reduce Canada thistle populations over a 2 to 3 yr time period (Parochetti 1974; Carson and Bandeen 1975). Bentazon, a Photosystem II inhibitor like atrazine, also has activity on Canada thistle. Split applications of bentazon have performed better than a single application for control of Canada thistle (Boerboom and Wyse 1988). The acetolactate synthase (ALS, EC 4.1.3.18) inhibiting herbicides nicosulfuron, CGA 152005 [1-(4-methoxy-6-methyl-triazin-2-yl)-3-[2-(3,3,3-trifluoropropyl)-phenylsulfonyl]-urea], halosulfuron, rimsulfuron, and primisulfuron have suppressed Canada thistle in corn (Glenn and Heimer 1994; Sprague et al. 1999). Tank-mixing low rates of 2,4-D or dicamba with primisulfuron or nicosulfuron usually increased Canada thistle control (Glenn and Heimer 1994).

Clopyralid has been reported to control Canada thistle (O'Sullivan and Kossatz 1984; Glenn and Heimer 1994). Previous research has also reported that clopyralid translocates in greater amounts to Canada thistle roots than other herbicides (Devine and Vanden Born 1985; Turnbill and Stephenson 1985). After clopyralid accumulates in Canada thistle roots secondary growth from root buds is reduced (Donald 1988). Furthermore, clopyralid applications made at the rosette stage reduces Canada thistle regrowth in the following year better than clopyralid applications made to bolting stage Canada thistle plants (Miller and Lym 1998).

Few herbicides are available for control of Canada thistle that are effective at various growth stages and that control numerous other weed species economically. Therefore, new herbicides should be evaluated for control of this species.

Mesotrione is the newest member of the triketone family. This herbicide received U.S. EPA registration in 2001 for PRE and postemergence (POST) applications in field corn (Anonymous 2001a). Mesotrione and other triketones function through inhibition of the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD, EC1.13.11.27). HPPD facilitates the conversion of 4-hydroxyphenypyruvic acid to homogentisic acid (Norris et al. 1998; Pallet et al. 1998; Viviani et al. 1998). This enzyme is part of the pathway that converts the amino acid tyrosine to plastoquinone. Plastoquinone is a cofactor for the enzyme phytoene desaturase, a key enzyme in the production of carotenoids. In the absence of carotenoids, plants are unable to protect themselves from photooxidation (Norris et al. 1998).

For optimal mesotrione uptake and weed control, POST applications should include 1% v/v crop oil concentrate (COC) and 2.5% v/v urea ammonium nitrate (UAN) (Wichert and Pastushok 2000). POST mesotrione applications control many annual broadleaf weeds, large crabgrass [*Digitaria sanguinalis* (L.) Scop.], and barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] (Sutton et al. 1999; Beckett and Taylor 2000; Armel et al. 2001). Also, POST mesotrione combinations have increased control of some perennial weed species (Armel et al. 2000; Armel et al. 2000a; Bradley et al. 2000). This activity on perennial species is most likely due to excellent xylem and phloem movement of mesotrione in susceptible plants (Bartlett and Hall 2000; Mitchell et al. 2001). Also, the addition of low rates of atrazine in POST applications enhanced mesotrione efficacy over larger or more difficult to control weeds, including some perennial weeds (Johnson and Young 1999; Armel et al. 2000; Beckett and Taylor 2000; Johnson and Young 2000; Mueller 2000; Armel et al. 2001). However, currently there is no explanation for this increased control from mesotrione plus atrazine tank-mixes.

Mesotrione has activity on the perennial weed horsenettle (*Solanum carolinense* L.) (Jacobi and Brownell, personal communication, 1999). Therefore, mesotrione may be effective on other perennial broadleaf weeds, including Canada thistle. The objective of our field research was to

determine if mesotrione alone or in combinations with low rates of atrazine or clopyralid would control Canada thistle and to compare these treatments with standard herbicide treatments. An additional objective was to investigate if atrazine and COC plus UAN affect the efficacy, uptake, translocation, and metabolism of mesotrione in Canada thistle.

METHODS AND MATERIALS

Field experiment. A study was conducted a two sites (Harrington and Greenwood) in grower fields near Harrington, Delaware in 2000. Methods of soil preparation, planting, and herbicide application were the same at both sites. The soil type was a Sassafras sandy loam (Typic Pedon). Fields were prepared using conventional tillage seedbed preparations and were fertilized according to University of Delaware recommendations (Sims and Gartley 1996). Corn (Pioneer 34B23[2]) was planted on May 15, 2000 at a rate of 66,700 seeds/ha in rows spaced 76 cm apart. After planting, atrazine at 1736 g ai/ha plus *s*-metolachlor at 1344 g ai/ha plus pendimethalin at 1155 g ai/ha was applied PRE to control annual broadleaf weeds and grasses.

Plots were established to receive POST treatments on June 20, 2000. Field plots were 3 m by 6.1 m with a treated area of 2.1 m by 6.1 m; an untreated area 0.9 m wide was maintained between plots. Mesotrione was applied alone at 105 and 210 g/ha POST. Mesotrione at 105 g/ha was also evaluated in POST combinations with low rates of atrazine at 280 g/ha and clopyralid at 140 g/ha. These mesotrione treatments were compared against standard treatments for Canada thistle (Glenn 1999, Hagood 1999, VanGessel 1999, personal communications). Standard comparison treatments were the pre-package mix of primisulfuron at 20 g ai/ha plus CGA 152005 at 20 g ai/ha[3] plus dicamba at 140 g/ha, the pre-package mix of flumetsulam at 39 g ai/ha plus clopyralid at 106 g/ha[4], 2,4-D at 140 g/ha plus dicamba at 280 g/ha, and clopyralid at

[2] Pioneer Hi-Bred International, Inc., 400 Locust Street, Suite 800, Des Moines, IA 50306-3453.

[3] Exceed® herbicide. Syngenta Crop Protection. 410 Swing Road, Greensboro, NC 27419-8300.

[4] Hornet® herbicide. Dow Agrosciences. 9330 Zionville Road, Indianapolis, IN 46268-1054.

280 g/ha. All mesotrione treatments contained an adjuvant system of 1% v/v COC[5] plus 2.5% v/v UAN. Standard treatments contained a recommended adjuvant (Hagood et al. 2000).

Herbicides were applied POST with a propane-powered backpack sprayer calibrated to deliver 220 L/ha at a pressure of 210 kPa from flat fan nozzles[6]. Canada thistle height was variable when POST herbicides were applied and ranged from 12 to 54 cm tall. Canada thistle populations also varied among plots, but each plot generally contained 5 to 30 plants / m^2. Corn height was 30 to 77 cm tall when POST treatments were applied. Canada thistle control was visually assessed 1 and 8 weeks after treatment (WAT).

Greenhouse experiments. A greenhouse study was conducted near Painter, VA to determine optimal rates of mesotrione and atrazine for Canada thistle control. This study was arranged in a 4 by 3 factorial design with mesotrione at 71, 105, 140, and 210 g /ha and atrazine at 0, 280, 560 g ai/ha.

Canada thistle plants were started from roots that were cut into lengths of 2.5 to 5.5 cm. Roots were planted into 9.5 cm by 9.5 cm pots[7] (1 root segment per pot) containing a commercial potting mix[8]. Plants were watered and fertilized[9] as needed to facilitate maximum plant growth and vigor. POST herbicides were applied to Canada thistle 3.5 to 15.5 cm tall. Herbicides were applied using a greenhouse cabinet sprayer at 220 L/ha with a pressure of 210 kPa. A single

[5] Agridex, a mixture of 83% paraffinic mineral oil and 17% polyoxyethylene sorbitan fatty acid ester, Helena Chemical Company, 5100 Poplar Avenue, Memphis, TN 38137.

[6] Teejet 8003 flat fan nozzle. Spraying Systems Company, North Avenue, Wheaton, IL 60188.

[7] T.O. Plastics 4" Fill Pots. Inside dimensions 9.5 cm by 9.5 cm by 8.1 cm. Wetzel, Inc., 1345 Diamond Springs Road, Virginia Beach, VA 23455.

[8] Pro-Mix BX. Premier Horticulture, Inc., Red Hill, PA 18076.

[9] Excel All Purpose 21-5-50. Wetzel, Inc., 1345 Diamond Springs Road, Virginia Beach, VA 23455.

even flow nozzle[10] was placed 30 cm above the highest part of each plant. All treatments contained an adjuvant system of 1% v/v COC and 2.5% v/v UAN.

Visual observations of Canada thistle control were made 4 WAT. Plant heights were measured and shoots were harvested for biomass at 4 and 8 WAT. Shoot biomass was dried to constant moisture content prior to weighing. Plant heights and shoot biomass are represented as a percent reduction in comparison to the untreated check.

Chemicals. Formulated, technical, and radiolabeled samples of mesotrione were provided by Syngenta Crop Protection, Inc. The specific activity of ^{14}C mesotrione was 1230 KBq/µmole and the purity of the radiochemical was 98.5%.

Absorption and translocation studies. Laboratory studies were conducted at The Jealott's Hill International Research Center near Bracknell, Berkshire, UK. Studies examining the absorption, translocation, and metabolism of ^{14}C mesotrione in Canada thistle were developed to determine how atrazine at 560 g/ha and the adjuvant system of 1% v/v COC plus 2.5% v/v UAN enhanced the bio-performance of mesotrione. These studies were arranged in a 2 by 2 by 2 factorial design. Factors included no adjuvant or the adjuvant system of 1% v/v COC plus 2.5% v/v UAN, no atrazine or atrazine at 560 g/ha, and Canada thistle at two stages of growth, a rosette stage (2 to 6 cm, seven-leaf) and a bolting stage (8 to 16 cm, 9- to 11-leaf).

Canada thistle plants for studies with radiolabeled mesotrione were grown from seed and transplanted 1 per pot into a sandy compost soil mix[11]. Seeds were planted at two separate times spaced approximately 4 wk apart so plants would be in both the rosette and bolting stages of growth at herbicide application. Plants were watered and fertilized to facilitate maximum growth and vigor.

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[10] Teejet 8002 EVS flat fan spray tip. Spraying Systems Co., North Avenue, Wheaton, IL 60188.

[11] John Innes Potting Compost (JIP3): 6:4:3 loam:peat:grit, organic matter 4 to 6%. John Innes Manufacturers Association, P.O. Box 8 Harrogate, North Yorkshire, He2 8XB.

When Canada thistle plants reached the appropriate growth stages ^{14}C labeled mesotrione was prepared in combinations with atrazine and COC plus UAN. Approximately 500 KBq of ^{14}C labeled mesotrione (specific activity 1230 KBq/µmole) was spiked into a diluted blank formulation of mesotrione. The blank was diluted 1/300, the dilution factor required to approximate the use ratio between active ingredient and formulants in the actual formulated mesotrione product. Approximately 4 µl of the radiolabeled herbicide solution was applied as 20 by 0.2 µl droplets to the adaxial surface of the newest fully expanded leaf of each Canada thistle plant. Thereby, approximately 4 KBq of radiolabeled mesotrione (specific activity of 123 KBq/µmole) was applied to each plant in the 4 µl of treatment solution.

In the absorption study, mesotrione residues were removed from treated leaves by washing with 5 ml of acidified (pH 1.5) washing solution comprised of a 50:50 ratio of methanol : 0.1M HCl. Residues were removed by washing treated leaves at 2, 6, 24 or 72 hours after treatment (HAT). Washing consisted of five 1ml aliquots of solution released across the treated area of the leaf and collected in a glass scintillation vial. Washes were collected and radioactivity was determined using liquid scintillation spectroscopy (LSS) using 15 ml of scintillation fluid[12] and counted in a liquid scintillation counter[13]. Estimation of radioactivity applied was determined by applying 20 by 0.2 µl droplets of the appropriate treatment solution directly to the inside of a scintillation vial and performing LSS to quantify radioactivity. Percent absorption was determined by differences between estimated radioactivity applied and radioactivity washed off the treated leaf surface at subsequent time points.

Canada thistle plants for translocation studies were treated as described previously in the absorption study. At 2, 6, 24, and 72 HAT plants were carefully washed to remove soil from the roots. These plants were either glued to cards and freeze dried for phosphorimaging or were

[12] Optiphase scintillation fluid. Perkins Elmer Life Sciences, B 1930 Zaventem, Belgium 1+32 2717 7924.

[13] Wallac 1409 DSA liquid scintillation counter. Perkins Elmer Life Sciences, B 1930 Zaventem, Belgium 1+32 2717 7924.

sectioned, placed in cones, combusted using a sample oxidizer[14] and scintillation counted to determine radioactivity distribution throughout the plant over time. Plants for oxidation and scintillation counting were sectioned into treated leaf, roots, and the rest of foliage (including the main stem). Plants for phosphorimaging were stuck to cards, placed in a flower press separated with sheets of blotting paper and freeze dried. Once freeze dried, the cards were covered with 'Mylar' film, placed in a cassette with a phosphor imaging plate, and left to expose for 24 hr in a lead box. The image plates were scanned the next day using a phosphor imager[15] and formatted with accompanying software[16,17].

Metabolism. Canada thistle was treated as described in the absorption and translocation studies. Plants were harvested at 2, 6, 24 and 72 HAT. These plants were sectioned into treated leaf, foliage above the treated leaf, foliage below the treated leaf, and roots. After sectioning, plants were frozen immediately to prevent any further metabolism after sampling. The plant material was then macerated in 50 ml centrifuge tubes using liquid nitrogen and a glass rod and then homogenized in acetone using a probe[18]. After centrifuging at 4000 rpm for 5 minutes, the supernatant was removed and dried down under forced air. The pellets of plant material were dried and combusted to determine the amount of radioactivity bound to the solid plant fragments.

The dried extracts were dissolved in 1ml ethyl acetate so that three 10 µl aliquots could be removed for quantification by LSS. The remaining extract was dried down again and finally made up to 100 µl with ethyl acetate. About 50 µl of this volume was applied to a normal phase

[14] Canberra Packard oximate 80 model 307 sample oxidizer. Canberra Industries, 800 Research Parkway, Meridan, CT 06450.

[15] FujiBas 1500 phosphor imager. Fuji Photo Film (U.K.) Ltd. Fuji Film House, 125 Finchley Road, Swiss Cottage, London NW3 6HY, UK.

[16] Tina 2.09 software. Raytest Isotopenmessgeraete GmbH, Straubenhardt, Germany.

[17] Adobe Photoshop 5.0. Adobe Systems Software Ireland Limited, Unit 3100 Lake Drive, Citywest Business Campus, Saggart D24, Republic of Ireland.

[18] Ultra Turrax Probe. OPTO-LAB di Mantovani Cesare & C. S.n.c. Concordia MO- Italy.

thin layer chromatography (TLC) plate[19] and run in a solvent system of chloroform : methanol : water : formic acid (20:7:1:1 v/v). The samples from different plant parts were run in separate lanes alongside a dilution stock of radiolabeled mesotrione and its metabolites AMBA [4-(methylsulfonyl)-2-nitrobenzoic acid] and MNBA [2-amino-4-(methylsulfonyl)benzoic acid], as references. The radiolabeled bands were visualized using the phosphor imaging system and the relative contributions of each band to the total radioactivity in each lane were expressed as a percentage. In this way, the loss of parent compound and appearance of metabolites were followed over time in different plant parts.

All studies were arranged in a randomized complete block design. Field and greenhouse studies were evaluated with three replications. In the laboratory, the absorption study contained six replications, while the translocation and metabolism studies each contained two replications. All studies were repeated in time.

Crop injury and weed control in field and greenhouse studies were evaluated on a scale from 0 to 100 percent where 0 = no corn injury or Canada thistle control and 100 = corn death or complete Canada thistle control. Analysis of variance (ANOVA) was performed on all studies and data from separate studies were combined and averaged over experiments. Means were then separated using Fisher's Protected LSD test at the $\alpha = 0.05$ significance level. The untreated check was not included in the data analysis.

RESULTS AND DISCUSSION

Field study. Mesotrione symptoms generally appeared as bleached or chlorotic meristematic tissue within 1 WAT. Plants in the rosette stage appeared more susceptible to mesotrione than larger plants in the bolting stage. However, the addition of atrazine to mesotrione improved control of these larger Canada thistle plants. Also, control was more rapid with the atrazine plus mesotrione tank-mixture than with mesotrione applied alone. Mesotrione alone controlled

[19] Silica Gel 60 F254 precoated TLC plates. EM Sciences, 480 Democrat Road, Gibbstown, NJ 08027.

Canada thistle only 59% by 1 WAT, but when atrazine was tank-mixed with mesotrione control increased to 86% (Table 7.1). Also, the symptomology changed with tank-mixtures of mesotrione plus atrazine, as rapid necrosis of older plant tissue masked the typical bleached meristematic tissue associated with mesotrione applied alone.

Mesotrione at 105 g/ha alone controlled Canada thistle 74% by 8 WAT (Table 7.1). However, when mesotrione was applied at 210 g/ha or 105 g/ha tank-mixed with atrazine it controlled Canada thistle 84 to 87%. Even though mesotrione plus atrazine tank-mixtures were effective in controlling Canada thistle, good herbicide coverage was essential for control of all Canada thistle topgrowth. In general, the topgrowth of smaller plants beneath the weed canopy were not adequately controlled from these POST mesotrione plus atrazine applications. Mesotrione plus atrazine and mesotrione applied alone at 210 g/ha controlled Canada thistle similar to flumetsulam plus clopyralid and 2,4-D plus dicamba. However, Canada thistle control was highest with clopyralid at 280 g /ha. Similarly, others have also reported high levels of Canada thistle control with clopyralid (O'Sullivan and Kossatz 1984; Donald 1988).

Greenhouse study. Mesotrione plus atrazine combinations were further examined in the greenhouse to determine rates of these herbicides necessary for optimal control of Canada thistle. Mesotrione at 70 to 210 g/ha controlled Canada thistle 55 to 69% (Table 7.2). Control of Canada thistle by 140 and 210 g/ha mesotrione was increased to 89 and 79%, respectively by tank-mixing with 280 g/ha atrazine and control by all rates of mesotrione was increased to 79 to 94% by tank-mixing with 560 g/ha atrazine. Atrazine alone at 280 or 560 g/ha did not control Canada thistle >31% (data not presented).

Height of Canada thistle plants was reduced 62 to 69% at 4 WAT with mesotrione rates averaged over atrazine rates, and there were no differences between treatments (Table 7.3). Similarly, shoot biomass reductions at 4 WAT were 68 to 74%, but did not differ between mesotrione rates averaged over atrazine rates. Atrazine rates of 0 to 560 g/ha averaged over mesotrione rates reduced shoot height 63 to 70%, but there was no difference between rates. However, when averaged over mesotrione rates, 560 g/ha of atrazine reduced shoot biomass 79%, while atrazine at 0 or 280 g/ha reduced shoot biomass 64 and 68%, respectively.

Height of Canada thistle shoot regrowth was reduced 49 to 82% with mesotrione applied alone at 70 to 210 g/ha by 8 WAT (Table 7.2). Incremental rate increases of mesotrione generally reduced height of shoot regrowth, however this incremental rate increase did not occur with mesotrione rates of 105 and 140 g/ha. The tank-mixtures of 280 and 560 g/ha atrazine with all rates of mesotrione reduced Canada thistle height regrowth 86 to 100% with the exception of mesotrione at 71 g/ha plus atrazine at 280 g/ha, which reduced regrowth height 58%.

Regrowth of Canada thistle shoot biomass was reduced 76 to 91% by mesotrione at 71 to 210 g/ha (Table 7.2). However, tank-mix combinations of mesotrione plus atrazine at either 280 or 560 g/ha reduced regrowth of shoot biomass 98 to 100% except mesotrione at 71 g/ha plus atrazine at 280 g/ha which reduced biomass 79%. Responses of Canada thistle to tank-mix combinations of mesotrione plus atrazine were similar to those in our field studies, in that these combinations were more effective than mesotrione alone.

Absorption. Absorption of ^{14}C mesotrione was slow in Canada thistle. By 2 HAT, only 26 to 44% of applied ^{14}C mesotrione was absorbed by Canada thistle (Table 7.4). However, by 72 HAT, 51 to 77% of applied ^{14}C mesotrione was absorbed by Canada thistle. In comparison, Bartlett and Hall (2000) reported mesotrione absorption of 54 to 85% by 4 HAT in various annual weed species. Mitchell et al. (2001) also reported 55 to 90% absorption of ^{14}C mesotrione in various annual weed species by 24 HAT.

Several factors influenced the absorption of ^{14}C mesotrione in Canada thistle. The adjuvant system of COC plus UAN significantly increased absorption of ^{14}C mesotrione at all time intervals, while the addition of atrazine at 560 g/ha improved ^{14}C mesotrione absorption only at 2 HAT (Table 7.4). There was also a significant difference in absorption between Canada thistle in the rosette and bolting growth stages at all time intervals except 6 HAT. At 2 HAT, Canada thistle in the bolting stage absorbed 37% of ^{14}C mesotrione, while rosette stage plants absorbed only 32% when averaged over atrazine and adjuvant rates (data not presented). However, by 24 HAT plants in the rosette stage absorbed 57% of applied ^{14}C mesotrione, while bolting stage plants absorbed only 48% when averaged over atrazine and adjuvant rates. Similarly, Lym

(1992) reported greater absorption of fluroxypr when applied at an early stage of growth in the perennial weed leafy spurge (*Euphorbia esula* L.).

A significant atrazine by adjuvant interaction for ^{14}C mesotrione absorption occurred at 72 HAT (Table 7.4). When averaged over stage of growth, absorption of ^{14}C mesotrione was 76% when applied in combination with atrazine plus COC plus UAN, but tion was only 70% when ^{14}C mesotrione was applied with just COC plus UAN (data not presented). This significant interaction between atrazine and the adjuvant system can not be explained by these studies.

Translocation. Following absorption, translocation of ^{14}C mesotrione in Canada thistle was low. No more than 14% of the absorbed ^{14}C mesotrione translocated out of the treated leaf by 24 HAT (data not presented). Therefore, only translocation data from 72 HAT is presented. At 72 HAT, the majority of ^{14}C mesotrione remained in the treated leaf. Only 9 to 20% of ^{14}C mesotrione translocated to the rest of the foliage, while 2% or less translocated to the roots (Table 7.5). The stage of growth of Canada thistle impacted the distribution of ^{14}C mesotrione in the treated leaf, rest of foliage, and roots (Table 7.5 and Figure 7.1). There was also a significant atrazine by stage of growth interaction for the level of ^{14}C mesotrione found in the treated leaf and the rest of foliage (Table 7.5). In general, more ^{14}C mesotrione translocated to the rest of foliage of rosette stage Canada thistle in comparison to bolting stage plants when treatments were averaged over atrazine and adjuvant rates (data not presented).

Metabolism. TLC analysis of extracts from ^{14}C mesotrione treated Canada thistle revealed three major bands of radioactivity. The parent mesotrione was identified with an R_f value of 0.7. Two known metabolites, AMBA and MNBA, were identified with corresponding R_f values of 0.4 and 0.6, respectively. Both metabolites have no herbicidal activity (Hall 2001, personal communication). R_f values of mesotrione and its metabolites were confirmed by applying a dilution stock of ^{14}C labeled mesotrione, AMBA, and MNBA in addition to the plant extracts on the TLC plates. Other unidentified metabolites were present in low amounts and were combined for comparison.

Metabolism of ^{14}C mesotrione in Canada thistle was slow and at least 70% of the radioactivity measured in the plant foliage 24 HAT was parent mesotrione (data not presented). Similarly, Mitchell et al. (2001) reported slow metabolism of mesotrione in other broadleaf weeds. Due to this low rate of metabolism, only data from 72 HAT is presented.

Various factors affected the metabolism of mesotrione in the foliage and roots of Canada thistle plants. At 72 HAT, little metabolism occurred in the treated leaf, foliage above the treated leaf, or the foliage below the treated leaf. Generally, parent mesotrione represented 70 to 92% of the radioactivity in these regions of the Canada thistle plant (Tables 7.6 and 7.7). However, various interactions signify some differences in metabolism in these regions. First, metabolism was higher in the treated leaf of rosette stage plants compared to bolting stage plants. Also, metabolism of mesotrione was higher in the foliage above the treated leaf when atrazine was included with ^{14}C mesotrione applications. Finally, there was an adjuvant by atrazine and a growth stage by atrazine interaction for mesotrione metabolism in the foliage below the treated leaf . However, the highest amount of mesotrione metabolism occurred in the roots. At 72 HAT, only 59 to 88% of the radioactivity in the roots was parent mesotrione. Root metabolism of mesotrione was higher in bolting stage Canada thistle plants compared to plants in the rosette stage of growth. These changes in metabolism between different plant parts and growth stages can not be readily explained.

In these studies, tank-mixtures of mesotrione plus atrazine controlled Canada thistle similar to or better than the commercial standards examined except clopyralid. Further, mesotrione had greater activity applied to Canada thistle in the rosette stage of growth than to plants in the bolting stage. Tank-mixtures of low rates of atrazine with mesotrione increased control of Canada thistle, especially those plants in the bolting growth stage. However, symptomology changed with tank-mixtures of mesotrione plus atrazine, as rapid necrosis of older plant tissue masked the typical bleached meristematic tissue associated with mesotrione applied alone.

Uptake, translocation, and metabolism of ^{14}C mesotrione in Canada thistle was generally low and these experiments did not elucidate the changes in symptomology and increased Canada thistle control associated with mesotrione plus atrazine tank-mixtures. However, increased

absorption and translocation and lower root metabolism of mesotrione applied to rosette stage plants compared to bolting stage plants may help explain why Canada thistle is more susceptible to mesotrione in the rosette stage. The changes in symptomology and increased control from mesotrione plus atrazine tank-mixtures is likely due to the interrelationship between the modes of action of atrazine and mesotrione (Figure 7.2). Through inhibition of HPPD, mesotrione blocks production of plastoquinone, a cofactor in the production of the enzyme phytoene desaturase (Norris et al. 1998). However, plastoquinone also serves as an electron shuttle between the Q_B binding niche of the D1 protein and cyt b_6/f complex (Wise and Cook 1998). Atrazine competes with plastoquinone for binding at the Q_B binding niche of the D1 protein (Trebst 1996). Therefore, if mesotrione limits the production of plastoquinone through inhibition of HPPD, it would also limit the competition between plastoquinone and atrazine for binding on the D1 protein, making atrazine a more efficient inhibitor of Photosystem II (PSII). Kim et al. (1999) suggested a similar explanation for synergistic effects with tank-mixtures of the HPPD inhibitor SC 0051 [(2-(2-chloro-4-(methylsulfonyl)benzoyl)-1,3-cyclohexanedione] and the PSII inhibitor diuron. Also, atrazine and other PSII inhibitors increase the level of singlet oxygen species causing lipid peroxidation and a subsequent loss of chlorophyll and carotenoids (Ahrens 1994). Similarly, HPPD inhibitors disrupt the production of α-tocopherol, an important anti-oxidant, which helps neutralize the oxidizing effects of these free radicals species (Hess 1993; Pallett et al. 1998).

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Table 7.1. Mesotrione alone and in combinations with atrazine and clopyralid compared to standard herbicide treatments for postemergence control of Canada thistle in 2000.[ab]

Herbicide treatment	Rate	Control	
		1 WAT	8 WAT
	— g ai/ha —	——— % ———	
Mesotrione[c]	105	59	74
Mesotrione[c]	210	75	84
Mesotrione + atrazine[c]	105 + 280	86	87
Mesotrione + clopyralid[c]	105 + 140	81	89
Primisulfuron + prosulfuron + dicamba[d]	20 + 20 + 140	48	73
Clopyralid	280	77	97
Flumetsulam + clopyralid[d]	39 + 106	55	83
2,4-D + dicamba	140 + 280	54	81
Untreated check[e]		0	0
$LSD_{0.05}$		12	6

[a] Abbreviations: WAT, weeks after treatment.
[b] Means are the average of both 3 replication studies.
[c] Included adjuvant system of 1% v/v crop oil concentrate and 2.5% v/v urea ammonium nitrate.
[d] Included 0.25% v/v non-ionic surfactant.
[e] Untreated check not included in statistical analysis.

Table 7.2. Postemergence mesotrione plus atrazine combinations for control and reductions of Canada thistle shoot regrowth heights and biomass in the greenhouse.[ab]

Herbicide treatment[cd]	Rate	Control 4 WAT	Regrowth height 8 WAT	Regrowth biomass 8 WAT
	— g ai/ha —	—— % ——	———— % reduction ————	
Mesotrione	71	55	49	76
Mesotrione + atrazine	71 + 280	60	58	79
Mesotrione + atrazine	71 + 560	88	96	99
Mesotrione	105	59	63	86
Mesotrione + atrazine	105 + 280	68	86	98
Mesotrione + atrazine	105 + 560	79	96	99
Mesotrione	140	69	74	89
Mesotrione + atrazine	140 + 280	89	100	100
Mesotrione + atrazine	140 + 560	89	100	100
Mesotrione	210	67	82	91
Mesotrione + atrazine	210 + 280	79	97	99
Mesotrione + atrazine	210 + 560	94	100	100
Untreated check		0	0	0
$LSD_{0.05}$		10	13	6

[a] Abbreviations: WAT, weeks after treatment.
[b] Means are the average of both 3 replication studies.
[c] Included adjuvant system of 1% v/v crop oil concentrate and 2.5% v/v urea ammonium nitrate
[d] Untreated check not included in the statistical analysis.

Table 7.3. Postemergence mesotrione and atrazine combinations for height and biomass reductions of Canada thistle in the greenhouse.[ab]

		Canada thistle growth	
		Height	Biomass
Herbicide[cd]	Rate	4 WAT	4 WAT
	— g ai/ha —	——————% reduction ——————	
Mesotrione[e]	71	62	68
	105	64	69
	140	69	74
	210	67	72
	$LSD_{0.05}$	**NS**	**NS**
Atrazine[f]	0	63	64
	280	64	68
	560	70	79
	$LSD_{0.05}$	**NS**	**7**

[a] Abbreviations: WAT, weeks after treatment.

[b] Means are the average of both 3 replication studies.

[c] Included adjuvant system of 1% v/v crop oil concentrate and 2.5% v/v urea ammonium nitrate.

[d] Untreated check not included in the statistical analysis.

[e] Mesotrione rate averaged over atrazine rates.

[f] Atrazine rate averaged over mesotrione rates.

Table 7.4. Influence of atrazine, adjuvant, and growth stage on absorption of foliar applied ^{14}C mesotrione in Canada thistle.[ab]

Treatments[c]			Absorption of ^{14}C mesotrione			
Stage of growth	Atrazine	Adjuvant	2 HAT	6 HAT	24 HAT	72 HAT
			%			
Rosette	none	none	26	35	45	67
	none	yes	33	50	64	77
	yes	none	28	33	49	60
	yes	yes	42	58	70	77
Bolting	none	none	32	40	39	55
	none	yes	37	53	55	63
	yes	none	35	40	41	51
	yes	yes	44	55	57	76
$LSD_{0.05}$			8	8	9	10
Adjuvant system vs. no adjuvant system			P=0.0001	P=0.0001	P=0.0001	P=0.0001
Atrazine vs. no atrazine			P=0.0119	NS	NS	NS
Rosette vs. bolting stage of growth			P=0.0155	NS	P=0.0001	P=0.0003
Adjuvant/atrazine interaction			NS	NS	NS	P=0.0204

[a] Abbreviations: HAT, hours after treatment.

[b] Means are the average of both 6 replication studies.

[c] Rosette stage represents plants 2 to 6 cm or 7 leaf, while bolting stage plants were 8 to 16 cm or 9 to 11 leaf. When included in treatments atrazine was applied at 560 g ai/ha, while 1% v/v crop oil concentrate and 2.5% v/v urea ammonium nitrate was the selected adjuvant system.

Table 7.5. Influence of atrazine, adjuvant, and growth stage on absorption and translocation of foliar applied ^{14}C mesotrione in Canada thistle at 72 hours after treatment.[a]

Treatments[b]			Translocation of ^{14}C mesotrione		
Stage of growth	Atrazine	Adjuvant	Treated leaf	Rest of foliage	Roots
			%		
Rosette	none	none	78	19	2
	none	yes	78	20	2
	yes	none	79	19	2
	yes	yes	84	15	1
Bolting	none	none	90	9	1
	none	yes	88	11	1
	yes	none	84	15	1
	yes	yes	86	13	1
$LSD_{0.05}$			7	6	NS
Adjuvant system vs. no adjuvant system			NS	NS	NS
Atrazine vs. no atrazine			NS	NS	NS
Rosette vs. bolting stage of growth			P=0.0001	P=0.0002	P=0.0159
Atrazine/growth stage interaction			P=0.0160	P=0.0381	NS

[a] Means are the average of both 2 replication studies..

[b] Rosette stage represents plants 2 to 6 cm or 7 leaf, while bolting stage plants were 8 to 16 cm or 9 to 11 leaf. When included in treatments atrazine was applied at 560 g ai/ha, while 1% v/v crop oil concentrate and 2.5% v/v urea ammonium nitrate was the selected adjuvant system.

Table 7.6. Influence of atrazine, adjuvant, and growth stage on metabolism of foliar applied ^{14}C mesotrione in the treated leaf and the foliage above the treated leaf of Canada thistle at 72 hours after treatment.[a]

Treatments[c]			Percentage of parent ^{14}C mesotrione and metabolites							
			Treated leaf				Foliage above treated leaf			
Stage of growth	Atrazine	Adjuvant	R_f = 0.7	R_f = 0.6	R_f = 0.4	other	R_f = 0.7	R_f = 0.6	R_f = 0.4	other
			%							
Rosette	none	none	87	4	3	7	92	2	2	4
	none	yes	76	4	7	13	90	4	7	3
	yes	none	74	5	4	16	80	8	2	11
	yes	yes	73	4	13	12	83	3	13	5
Bolting	none	none	84	5	2	13	88	5	1	8
	none	yes	89	4	3	5	86	6	2	9
	yes	none	85	5	2	8	70	4	3	25
	yes	yes	89	3	2	6	88	2	8	8
$LSD_{0.05}$			12	NS	9	NS	19	5	NS	15
Adjuvant system vs. no adjuvant system			NS	NS	NS	NS	NS	NS	P=0.0198	NS
Atrazine vs. no atrazine			NS	NS	NS	NS	P=0.0479	NS	NS	NS
Rosette vs. bolting stage of growth			P=0.0037	NS	P=0.0265	NS	NS	NS	NS	NS
Adjuvant/atrazine interaction			NS	NS	NS	NS	NS	P=0.0451	NS	NS
Growth stage/atrazine interaction			NS	NS	NS	NS	NS	P=0.0292	NS	NS
Growth stage/adjuvant interaction			NS	NS	NS	NS	NS	NS	NS	NS

[a] Means are the average of both 2 replication studies.

[b] Rosette stage represents plants 2 to 6 cm or 7 leaf, while bolting stage plants were 8 to 16 cm or 9 to 11 leaf. When included in treatments atrazine was applied at 560 g ai/ha, while 1% v/v crop oil concentrate and 2.5% v/v urea ammonium nitrate was the selected adjuvant system.

[c] R_f = 0.7 represents parent mesotrione; R_f = 0.6 represents the metabolite AMBA; R_f = 0.4 represents the metabolite MNBA; other represents other metabolites.

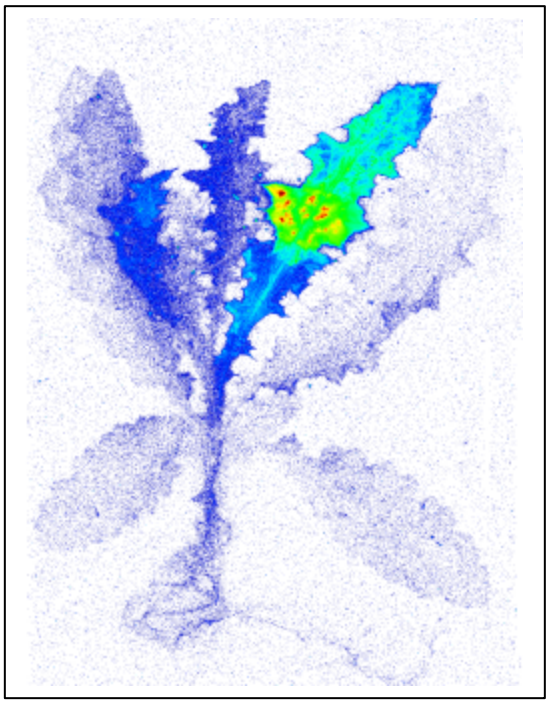
Table 7.7. Influence of atrazine, adjuvant, and growth stage on metabolism of foliar applied ^{14}C mesotrione in foliage below the treated leaf and the roots of Canada thistle at 72 hours after treatment.[a]

Treatments[c]			Percentage of parent ^{14}C mesotrione and metabolites							
			Foliage below the treated leaf				Roots			
Stage of growth	Atrazine	Adjuvant	R_f = 0.7	R_f = 0.6	R_f = 0.4	other	R_f = 0.7	R_f = 0.6	R_f = 0.4	other
			%							
Rosette	none	none	93	2	4	2	83	3	5	10
	none	yes	92	3	1	5	79	5	6	9
	yes	none	84	3	8	5	88	3	4	8
	yes	yes	78	2	10	11	67	2	23	11
Bolting	none	none	72	7	9	12	67	6	17	11
	none	yes	85	5	3	8	65	7	11	17
	yes	none	80	7	6	8	59	6	20	15
	yes	yes	75	9	3	13	70	5	12	15
$LSD_{0.05}$			14	6	NS	NS	26	NS	NS	NS
Adjuvant system vs. no adjuvant system			NS	NS	NS	NS	NS	NS	NS	NS
Atrazine vs. no atrazine			P=0.0118	NS	NS	NS	NS	P=0.0430	NS	NS
Rosette vs. bolting stage of growth			P=0.0010	P=0.0001	NS	P=0.0344	P=0.0007	P=0.0001	NS	P=0.0179
Adjuvant/atrazine interaction			P=0.0141	NS	NS	NS	NS	NS	NS	NS
Growth stage/atrazine interaction			P=0.0260	NS	P=0.0402	NS	NS	NS	NS	NS
Growth stage/adjuvant interaction			NS	NS	NS	NS	NS	NS	P=0.0214	NS

[a] Means are the average of both 2 replication studies.

[b] Rosette stage represents plants 2 to 6 cm or 7 leaf, while bolting stage plants were 8 to 16 cm or 9 to 11 leaf. When included in treatments atrazine was applied at 560 g ai/ha, while 1% v/v crop oil concentrate and 2.5% v/v urea ammonium nitrate was the selected adjuvant system.

[c] R_f = 0.7 represents parent mesotrione; R_f = 0.6 represents the metabolite AMBA; R_f = 0.4 represents the metabolite MNBA; other represents other metabolites.



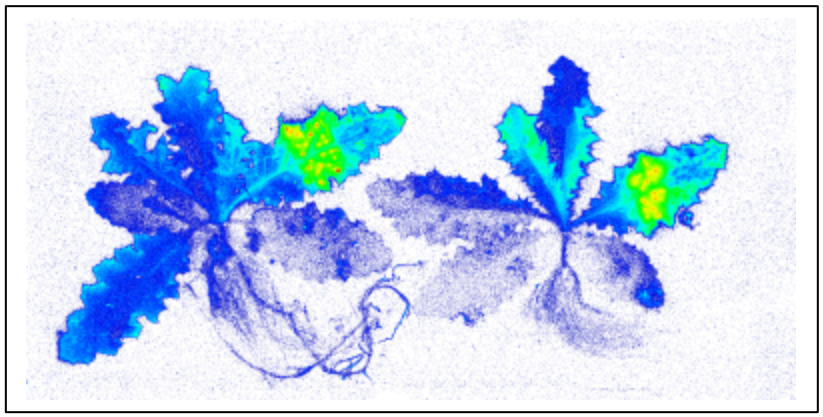


Figure 7.1: Movement of ^{14}C mesotrione with COC plus UAN in bolting stage Canada thistle (left) and rosette stage Canada thistle (above) at 72 hours after treatment.

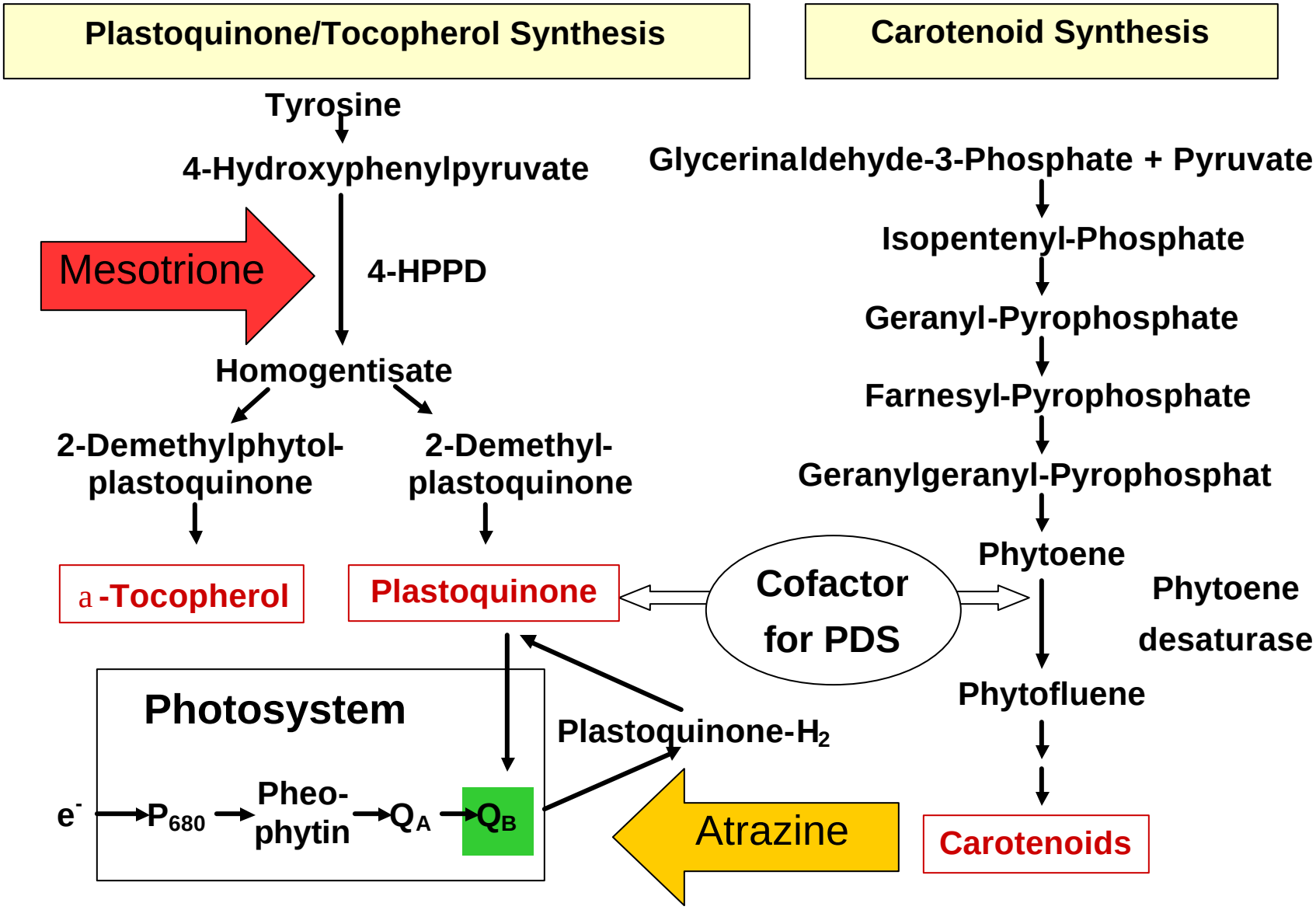


Figure 7.2. The postulated interrelationship between the HPPD inhibitor mesotrione and the PSII inhibitor atrazine. (Figure prepared by Martin Schulte, Syngenta Crop Protection, Inc.)