Field and Greenhouse Bioassays to Determine Rotational Crop Response to Mesotrione Residues

by

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ABSTRACT

FIELD AND GREENHOUSE BIOASSAYS TO DETERMINE ROTATIONAL CROP RESPONSE TO MESOTRIONE RESIDUES

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Field and greenhouse bioassay experiments were conducted to evaluate the effects of mesotrione soil residues on injury and yield of soybean, green bean, pea, cucumber, sugar beet and lettuce. There was a significant difference of mesotrione carryover between studies which can be explained by differences in soil pH and moisture. The conventional and the simulated field residue carryover studies successfully measured mesotrione persistence and rotational crop sensitivity. The simulated residue carryover study provided a more rigorous test of rotational crop sensitivity to mesotrione residues than the conventional residue carryover study, especially at higher doses for the more sensitive crops. The greenhouse bioassay was a simple and sensitive tool in detecting small amounts of herbicides present in the soil. Dose-response curves developed for sugar beet and green bean indicate similarities between results from the greenhouse and from the field mesotrione residue carryover study. Under similar environmental and soil conditions to those observed in these studies and using similar dose applications, sugar beet, green bean and cucumber injury and yield reductions are likely when these crops are grown in soils containing mesotrione residues.

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1.0 INTRODUCTION

Herbicide persistence is an important consideration in crop production since residues can potentially injure sensitive crops grown in rotation. Damage to high-value vegetable crops can result in substantial economic loss. Residues can cause great variability in plant growth and quality and in worse-case scenarios, can cause complete crop loss and significant economic cost to growers. The length of time a herbicide remains persistent in the soil depends on the herbicide (method of degradation, water solubility and rate of application) and the site characteristics (soil type, rainfall and temperature). The benefits of applying a persistent herbicide include season-long weed control and reduced pesticide applications.

Mesotrione is a triketone herbicide registered and marketed by Syngenta under the name Callisto® for annual broadleaf weed control in field, seed and sweet corn, cranberry and blueberry. This herbicide has many desirable features for use in integrated weed management programs in these crops. It provides broad spectrum control of major broadleaf weeds and some grasses as well as providing residual control of later germinating weeds. Mesotrione has a favourable environmental profile. The herbicide inhibits the essential plant enzyme HPPD (p-hydroxphenyl pyruvate dioxgenase), which is found in the plant's chloroplasts. The HPPD enzyme produces pigments that protect plant cells from photodegradation. In susceptible plants, mesotrione causes visual symptoms of bleaching that eventually leads to necrosis and plant death. These symptoms appear three to five days after a post-emergent application with weed death occurring within two to three weeks.

Mesotrione is a very effective herbicide for weed control, however, due to its residual activity, it can cause injury to sensitive crops grown in rotation, one year after application. The current label has recropping intervals of three months for winter wheat,

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ten months for spring wheat, and eleven months for alfalfa, potato, soybeans, transplanted tomato and white beans. The label does not include recropping intervals for most vegetable crops and suggests performing a field bioassay before planting potentially sensitive crops.

There is a need for a simple method for detecting mesotrione residues prior to planting sensitive crops to assist with crop rotation planning and to prevent crop failures and economic losses. Bioassays are often used to determine the presence of phytotoxic levels of herbicide residues in the soil. Conventional field bioassays are relatively easy to perform compared to more expensive and time consuming chemical assays, however, field bioassays require at least two growing seasons. Developing a field bioassay that requires only one year of field research to determine the carryover potential of herbicide residues to sensitive crops in rotation could be useful. A greenhouse bioassay is also useful because, not only can it be used to determine if chemical residues are present in the soil at high enough concentration to adversely affect crop growth, but it is simple, economical and less time consuming than field bioassays, with results available within two to three weeks.

The purpose of this research was to evaluate the relative sensitivity of six crops to mesotrione residues. The specific objectives were to:

1. Reduce a two-year field residue study to a one-year study by growing sensitive crops in soil treated with reduced doses of mesotrione.

2. Develop a greenhouse bioassay to detect mesotrione residues in the field and compare plant response (injury, plant dry weight reduction) observed in the greenhouse with plant response (injury, plant dry weight and yield reduction) observed in the field.

3. Rank sugar beet, lettuce, cucumber, green bean, pea and soybean based on their relative sensitivities to mesotrione residues.

Mesotrione is being utilized more by corn growers because of its effective control of broadleaf weeds, its flexible weed control timing and tank-mixing options to provide better grass control. However, growers that rotate corn with potentially sensitive vegetable crops may hesitate to use mesotrione due to its residual characteristics. The data from this research will provide growers with in-depth information on the sensitivity to mesotrione residues of six crops grown in rotation. A one-year "simulated" residue carryover study could replace the conventional two-year residue study when there is a concern with herbicide carryover damage. This will save time and money for industry and research institutions and it could also have broader implications for other herbicides that have long residual characteristics.

2.0 LITERATURE REVIEW

2.1 Mesotrione

2.1.1   Classification

The herbicidal activity of triketones was first discovered in 1982 by Zeneca Ag Products (now Syngenta), while studying leptospermone, a natural product found in the bottlebrush plant, *Callistemon* spp. (Michaely and Kratz 1986; Michaely and Kratz 1988). This compound showed corn tolerance when applied PRE and POST and provided broad-spectrum control of grass and broadleaf weeds (Lee et al. 1997).

Mesotrione [2-(4-methylsulfonyl-2-nitrobenzoyl)-1,3-cyclohexanedione] is a triketone (benzoyl-1,3-cyclohexanediones), bleaching herbicide, belonging to the chemical family group 27. Triketones act by competitively inhibiting the enzyme *p*-hydroxyphenylpyruvate dioxygenase (HPPD) (Prisbylla et al. 1993). Mesotrione is classified as a weak acid with a dissociation constant (pKa) of 3.12 at 20°C (Mitchell et al. 2001). Mesotrione has a favourable environmental profile. Degradation in the soil is by microbial breakdown with ultimate metabolism to CO_2. The half-life of mesotrione in soils (with pH 4.6 to 7.7 and organic matter 0.6 to 3.6%) ranges from 2 to 14 days with a mean of 9 days. Adsorption coefficients range from 1 to 5 and the K_{OC} (soil organic carbon sorption coefficient) ranges from 14 to 390 (Wichert et al. 1999). Foliar uptake of mesotrione by weed species is rapid with 40 to 70% of the chemical being absorbed within one hour of application (Wichert et al. 1999). Mesotrione is readily absorbed by shoots, roots, stems and leaves (Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) 2008) and translocates both acropetally and basipetally (Wichert et al. 1999). Selectivity in corn is due to rapid metabolism (Wichert et al. 1999), and slower uptake relative to that of susceptible weed species (Mitchell et al. 2001). Mesotrione is

stable to hydrolysis at pH 4 to pH 9 at temperatures between 25°C and 50°C (Pest Management Regulatory Agency 2005). The chemical and physical properties of mesotrione are listed in Table 2.1.

Triketones are expected to have very little weed resistance issues. Studies that examined over 680,000 ethyl methanesulfonate-mutagenized Arabidopsis *thaliana* seeds showed no resistance to mesotrione (Wichert et al. 1999). However in 2009, tall waterhemp (*Amaranthus tuberculatus*) biotypes collected from seed corn field located in Iowa and Illinois, were identified as being resistant to the HPPD-inhibiting herbicides, mesotrione and tembotrione (Hausman et al. 2011; McMullan and Green 2011).

2.1.2 Mode of Action

In plants HPPD is responsible for catalyzing the conversion of p-hydroxyphenyl pyruvate (4-HPP) to homogentisate (HGA) (Crouch et al. 1997). HGA is a key precursor of α-tocopherol and plastoquinone (PQ) which is an essential cofactor for phytoene desaturase. Phytoene desaturase is a key enzyme involved in the carotenoid biosynthesis pathway (Mayer et al. 1990; Mayer et al. 1992; Norris et al. 1995). Carotenoids located in plant photosynthetic tissues act as accessory pigments that protect chlorophyll and plant cell membranes by quenching free radicals, singlet oxygen and other reactive species (Siefermannharms 1987). Thus by inhibiting HPPD, the herbicides deplete levels of plastoquinone, resulting in the depletion of carotenoids. This, in effect, leads to bleaching symptoms followed by necrosis of the meristematic tissue within three to five days of application and throughout the entire plant in approximately two weeks (Wichert et al. 1999).

2.1.3 Use Patterns

Mesotrione is a preemergence and postemergence herbicide that controls broadleaf weeds and some grass weeds in corn (*Zea mays*). It was first registered in Canada by Syngenta Crop Protection under the trade name of Callisto® in 2004. The current Canadian label for mesotrione approves use in field, seed and sweet corn, cranberry and blueberry.

Callisto® is a suspension concentrate with the active ingredient mesotrione present at 480 grams per litre. Preemergence doses for field corn, production seed corn and sweet corn are 0.30 L/ha (140 g ai ha^{-1}), early postemergence doses for field corn only are 0.30 L/ha (140 g ai ha^{-1}), while late postemergence doses for field corn are 0.21 L/ha (100 g ai ha^{-1}) mixed with atrazine (280 g ai ha^{-1}) and a non-ionic surfactant (0.2% v/v). The current Canadian label has the following rotation restrictions; winter wheat, 3 months; spring wheat, 10 months; soybeans, white beans, alfalfa, transplanted tomatoes and potatoes, 11 months after application. Mesotrione can be tank mixed with a range of herbicides including s-metalochlor (Dual™ Magnum®), s-metalochlor + atrazine (Primextra®), atrazine (AAtrex®), nicosulfuron/rimsulfuron (Ultim®), nicosulfuron (Accent®) and glyphosate (Roundup®) (Syngenta Crop Protection Canada, Inc. 2011).

Mesotrione controls many major weeds that interfere with corn production such as *Digitaria, Echinochloa, Abutilon, Amaranthus, Ambrosia, Chenopodium, Polygonum,* and *Solanum* (Sutton et al. 1999). More specifically it controls triazine-resistant weeds like redroot pigweed (*Amaranthus retroflexus*) and common lambsquarters (*Chenopodium album*) (Wichert et al. 1999).

2.2 Factors Influencing Residue Persistence

Following application of a herbicide, the process of redistribution and degradation begins. The many pathways the applied herbicide may follow include volatization, photolysis, chemical/microbial degradation, leaching, soil adsorption, and plant uptake. The target for the herbicide is usually the crop or weed, however, less than 1% of a foliar application, and less than 5% of a soil application will reach the target site (Clay 1993). Most of the remaining herbicide will enter the soil body and the interaction between the chemical and soil begins (Wauchope et al. 2002).

Herbicides are considered residual if they provide season- long weed control due to the persistence of biologically active residues in the soil. "Carry-over" occurs when herbicide residues remain in the soil at phytotoxic levels until planting of the next crop. Persistence defines the length of time a pesticide remains active in the soil (Helling 2005). Herbicide persistence is measured by the field dissipation half-life (DT_{50}) where DT_{50} is the time it takes the herbicide residue to decline in soil or water by 50% (Helling 2005). Typically herbicide dissipation follows first order kinetics where the rate at which the herbicide decays is proportional to its concentration in the soil at any given point in time. The following equation explains this type of dissipation:

$C = C_0 exp[-k_d t]$

Where C_0 is the initial concentration in the soil, C is the concentration in the soil after *t* days, and k_d is the dissipation rate coefficient (Helling 2005).

Persistence is primarily determined by adsorption (Wauchope et al. 2002). Helling (2005) describes adsorption as the accumulation of herbicide in the soil solution- soil colloid interface, or the soil-air interface. The soil sorption coefficient (K_d) is the ratio of the amount of a certain herbicide adsorbed to soil divided by the concentration of herbicide remaining in aqueous solution at or near equilibrium (Weber et al. 2000). A K_d

value higher than 100 indicates a herbicide that is strongly adsorbed and will not be mobile in soil and also unavailable for microbial degradation (Wauchope et al. 2002). Any chemical that is not adsorbed to the soil will transform, transport or be available for plant uptake (Helling 2005).

Transformation

Transformation of a herbicide in the soil occurs either by photochemical, chemical and or microbial degradation (Guth 1980). Microbial degradation by bacteria, fungi and algae, accounts for almost 90% of all environmental degradation (Seiber 2002). Factors such as soil temperature, aeration, moisture, pH, organic matter and nutrients influence microbial degradation rates (Helling 2005). Generally transformation leads to metabolites that are less toxic or less mobile and persistent than the parent material. Exceptions do occur in which there is an activation of the transformed material that leads to products that actually enhance toxicity, stability (persistence), mobility, and lipohilicity. One notable example is the metabolites of DDT (Dichloro-Diphenyl-Trichloroethane), DDE (Dichlorodiphenyldichloroethylene) and DDD (Dichlorodiphenyldichloroethane) which causes thinning of bird's eggshells. These compounds can persist for years in soil and water and have toxic effects on wildlife (Seiber 2002).

Transportation

Once in the soil, chemicals may be transported either by leaching, runoff or volatilization. Leaching occurs when chemicals move from the surface of the soil layer to and through the soil vadose zone and eventually to groundwater. Rainfall or irrigation causes this downward movement of the chemical in the soil (Helling 2005; Seiber 2002). Leaching capability is based on the herbicide (water solubility and sorption coefficient) (Seiber 2002) and the characteristics of the soil (Helling 2005).

Runoff is used to describe the transport of herbicides in solution, suspension or while adsorbed to particulates. Like leaching, runoff is affected by rainfall. The degree to which the herbicide will be transported is influenced by herbicide application timing, intensity and duration of rainfall, soil moisture, soil texture, surface crusting, compaction, topography, chemical formulation and cultural practices (Helling 2005).

Volatilization or vapourization is another method by which a herbicide can be transported after application but before it is incorporated in the soil. Most chemicals have low vapour pressure and thus volatilization accounts for very little chemical redistribution. It is most likely to occur when the application is made to moist soils succeeded by a long period of drying. Adsorption of the chemical to the soil will greatly decrease volatilization of a chemical (Helling 2005).

Adsorption and degradation in soils are important factors in determining the persistence of the herbicide (Helling 2005). These two factors are dynamic in that they are influenced by other factors such as soil type, use patterns and weather conditions (Hurle and Walker 1980).

2.2.1 Soil Characteristics

pH

Soil pH affects persistence of herbicides by affecting adsorption. It is dependent on the ionization of the chemical. For instance, chemicals may protonate at a low pH (weakly basic amines) or become anions at neutral or alkaline conditions (weak acids). The soil surface may also be affected by the soil pH, causing variation in binding ability of certain herbicides (Helling 2005). Soil pH also can have an effect on the makeup of soil microorganisms which will influence degradation of the chemical (Hurle and Walker 1980).

Organic matter

Herbicide adsorption to soil has been largely associated with the organic matter (OM) or organic carbon (OC) content (Helling 2005). Microbial activity is generally greater in soil with higher organic matter thus one might expect that there would be more degradation in high organic matter soils (Hurle and Walker 1980). However, herbicide dissipation is generally slower in soils with high organic matter because the herbicide will adsorb to the soil colloids (Clay 1993; Hurle and Walker 1980) resulting in less degradation (Hurle and Walker 1980). Goring and Hamaker (1972) suggests that an increase in organic matter only increases the rate of degradation in mineral soil up to a limiting value at which point the rate loss would be slowed. Clay (1993) suggests that with weakly adsorbed herbicides, there is no consistency of the effect of organic matter on persistence. Soils high in organic matter have little leaching. This results in more herbicide present in the surface of the soil, making it more available for adsorption or desorption. Also on the soil surface there is more microbe activity because of higher aeration, moisture, temperature and nutrient levels, making it more likely to dissipate (Clay 1993).

Soil Texture

The size and texture of the soil particles can have an effect on persistence, but can be hard to differentiate from the associated effects of soil organic matter, moisture content and aeration (Clay 1993). Herbicides persist longer in light sandy soils when compared to silt and clay soils (Hurle and Walker 1980). Imazaquin adsorbed significantly more to soils higher in clay and organic matter than the silt loams (Basham et al. 1987). Also cultural practices differ between soil types and this could have an impact on persistence. For example residues are less with lower aggregate density, cultivation and addition of organic matter (Clay 1993).

2.2.2 Environmental Conditions

Weather affects herbicide persistence mainly through its effect on soil moisture and temperature. Higher soil moisture is associated with increased rates of biological processes and hence increased rates of herbicide degradation (Helling 2005; Hurle and Walker 1980). However, saturated soils can actually inhibit microbe growth (Helling 2005). Higher soil temperatures, like moisture, increases degradation processes (by effect of increased microbe activity). However, volatilization also increases with temperature. A 2.2-fold increase in herbicide dissipation rate per 10°C increase is an estimated effect (Helling 2005). Temperature may be more important than moisture content as Clay (1993) suggest that even in temperate regions, herbicide residues levels were still found to be high when moisture levels were low.

2.2.3 Herbicide Use Patterns

Applying herbicides at higher doses can increase persistence but not necessarily affect DT_{50} values (Helling 2005). Repeated application of the same herbicide can also affect persistence by encouraging adaptation by soil microorganisms resulting in more rapid degradation than in newly treated soil (Clay 1993). This acceleration of breakdown reduces the herbicide's effectiveness as a control product (Clay 1993). Fryer et al. (1980) found that there was no residue accumulation when repeated applications of linuron, simazine and tri-allate were made to field plots. Additionally, soil treated with two annual applications of MCPA for seven years "retained an enhanced ability to degrade new additions even after 178 weeks" (Fryer et al. 1980).

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Soil Depth

Variation in dissipation rates of atrazine at different soil depths has been identified (Harris et al. 1969). Despite variable results, it was concluded that persistence of the herbicide at lower depths was greater than those in the plow layer. Chlorsulfuron and metsulfuron-methyl degradation rates decreased with increased depth in the soil and this was attributed to a decrease in microbial biomass in the subsoil (Walker et al. 1989).

2.2.4   Herbicide Properties

The water solubility, soil adsorption, vapour pressure and the chemical's susceptibility to be altered by chemical or microbial degradation also affects its persistence. Chemicals that have low water solubility are more likely to adsorb to soil colloids, be present in dry soil, are less likely to leach and thus more likely to persist (Hager and Refsell 2008). Granular herbicides can be more persistent than other formulations, especially with volatile compounds but do depend on the weather conditions after application (Hurle and Walker 1980).

2.3 Mesotrione in the soil

Mesotrione is a weak acid with dissociation constant (pK_a) of 3.12 (Mitchell et al. 2001). Chaabane et al. (2008) and Dyson et al. (2002) found that mesotrione adsorption was primarily affected by soil pH and to a lesser extent, by percent organic matter (OM) and that this was correlated with mesotrione degradation. Mesotrione dissociates from the molecular to anionic form as pH rises. At pHs below 3.1, mesotrione is in a molecular state, making it more likely to adsorb to soil colloids and less likely to degrade. At pHs above 3.1 it is an anionic state (Burnside et al. 1969) making it less likely to adsorb to soil colloids and more likely to degrade (Dyson et al. 2002). A certain amount

of the molecular form of the compound will be present in soil when the soil pH is several

pH units above the pK_a (Nicholls and Evans 1991).

Rouchaud et al. (2000) studied mesotrione dissipation on four different sites in

Belgium and found that there was 90% dissipation of mesotrione in the 0 – 10 cm soil

surface layer in 3.6 months in sandy soil and 4.7 months in sandy loam, loam and clay

soils. At the end of the growing season, there was 6% (sandy soil) to 10% (loam soil) of

the original applied dose left in the 0 – 10 cm soil surface layer. There was no

mesotrione residue detected in the 15 – 20 cm soil layer. In terms of movement through

the soil, mesotrione was present in the 0-2 and 2-4 cm soil surface layers in loam soil

two months following application (150 g ai ha^{-1}). It then moved downward by uniform

diffusion to the 10-15 cm soil layer. Movement of mesotrione in a sandy loam soil was

similar. There were no significant residues detected in the 15-20 cm soil layer during the

cropping period or after corn was harvested (Rouchaud et al. 2000) . On these soils, the

half-lives of mesotrione were determined in the 0-10 cm surface soil layer: 50 days in a

loam soil (pH 6.4, OM 1.45%), 41 days in a sandy loam and a clay loam soil (pH

6.9/7.2, OM 1.94%/1.95%) and 34 days in a loamy sand soil (pH 6.8, OM 1.43%).

Dyson et al. (2002) examined the adsorption and degradation of mesotrione in 15

soils from Europe and the USA (samples taken from field and then treated with

mesotrione in the lab). Degradation was defined by fitting the log version of simple first-

order kinetics to the decline of mesotrione residue levels. Among the various soil types,

the half-lives varied from 4.5 (clay loam, pH 7.1, OM 3.3%) to 32 (silt loam, pH 5.0, OM

2.0%) days. On these same soils, soil adsorption coefficients (K_d values) ranged from

0.13 to 5.0 L/kg. Wichert et al. (1999) determined that the half-life of mesotrione in soils

ranged from 2 to 14 days with a mean of 9 days. The soil studied ranged in pH from 4.6

to 7.7 and organic matter of 0.6 to 3.6% with K_d values ranging from 1 to 5 (Wichert et al. 1999).

Mesotrione is less persistent in clay, but is more persistent in heavy sandy loam and is most persistent in sandy and sandy loam soils. Weather conditions (rainfall and temperature within the first weeks of application) soil texture and application dose play a major role in mesotrione persistence (Maeghe et al. 2002). Results from years with heavy rainfall (especially within two months of application) suggest that there was more degradation of mesotrione and thus less crop yield loss than in years with low rainfall (Maeghe et al. 2002). This study also suggested that heavy rainfall that occurs more than two months after application do not have an influence on mesotrione persistence.

Degradation of mesotrione in the soil

Mesotrione aerobically biotransforms in soil to MNBA (4-(methylsulfonyl)-2-nitrobenzoic acid) and AMBA (2-amino-4-(methylsulfonyl)benzoic acid) as minor products (<10% of applied mesotrione) (Pest Management Regulatory Agency 2005). Degradation in soil is by microbial breakdown with ultimate metabolism to CO_2. Durand et al. (2006a) identified and isolated a *Bacillus sp*. from cloud water (also common in soil) that demonstrates a rapid transformation of mesotrione. The metabolites were AMBA in significant, but low concentrations. No MNBA metabolites were found (Durand et al. 2006a). Further experiments by Durand et al. (2006b) identified five metabolites including AMBA while the other four have never been described before. The four new metabolites corresponded to a reduction of the nitro function of mesotrione. MNBA was also not identified in this study (Durand et al. 2006b). A more recent study by Batisson et al. (2009) also supported the findings by Durand et al. (2006b). They were able to isolate two adapted strains of mesotrione degrading species, *Bacillus* sp and *Arthrobacter* sp. Only the *Bacillus* sp, strain was capable of completely biotransforming

mesotrione. Bonnet et al. (2008) assessed the toxicity of mesotrione to two microorganisms (*Tetrahymena pyriformis* and *Vibrio fisheri*) that are commonly used in evaluating ecotoxicology. They found that AMBA was more toxic than mesotrione itself while MNBA was less toxic than the parent molecule.

2.4 Phytotoxicity and Rotational Crops

Persistent herbicides, even in very small amounts, can have serious phytotoxic effects on sensitive crops grown in rotation (Cobucci et al. 1998; Greenland 2003; Johnson and Talbert 1993; O'Sullivan et al. 1998; O'Sullivan et al. 1999; Renner and Powell 1991). The half-life of a herbicide in the soil provides an indication of how long the herbicide will persist in the soil, but it is variable in that it depends on soil conditions and hence degradation processes. Vegetable and legume crops have shown variable levels of sensitivity to mesotrione one year after application (Felix et al. 2007; Robinson 2008; Soltani et al. 2007; Torma et al. 2004). Felix et al. (2007) found that direct seeded crops such as snap beans, cucumber and red clover, emerged and grew poorly in soils treated in the previous year with 2X and 4X applications of mesotrione. Significant yield reductions were reported for all of these crops. Transplanted cabbage response to mesotrione residues varied with locations (Felix et al. 2007). At one site, there was no yield reduction across all treatments, however, at the second site, plants remained stunted and had so little head formation that the crop was not harvested. Transplanted bell peppers and processing tomato had significant injury at both sites and this was reflected in reduced yields. The transplanted crops showed injury later than the direct seeded crops and this was attributed to recovery time for transplant shock. While the transplants are recovering from shock there is likely little absorption or plant uptake of the herbicide residues (Felix et al. 2007). Differences in crop response between

locations was attributed to differences in precipitation from herbicide application to planting of the vegetable crop, and also to differences in soil clay content, organic matter and pH (Felix et al. 2007).

Soltani et al. (2007) examined the effect of mesotrione residues on four types of dry beans; cranberry, kidney, black and white beans. These crops were grown one and two years after pre emergent and post emergent applications of mesotrione to field corn. Residues caused little injury to black and white beans, but caused significant injury to cranberry and kidney beans one year after application. Injury to cranberry and kidney beans increased with increasing dose and POST application caused more injury than PRE on all bean types. Cranberry bean was most sensitive to mesotrione residues, followed by kidney, black and white beans. None of the treatments caused injury to any of the bean types two years after application (Soltani et al. 2007).

One year after the application of mesotrione to corn, there was no yield reduction in winter canola, winter wheat, spring barley, pea, sugar beet or sunflower with applications ranging from 168 to 336 g ai ha^{-1}. The only crop that showed sensitivity was lettuce where 100% of the plants died at the high dose (Torma et al. 2004).

Mesotrione is generally not applied alone, rather it is applied with other chemicals such as atrazine to control a wider range of broadleaf weeds (Abendroth et al. 2006; Armel et al. 2003; Bollman et al. 2006; Creech et al. 2004; Johnson et al. 2002; Sutton et al. 2002; Whaley et al. 2006; Whaley et al. 2009). The carryover effects of combining different herbicides has been studied (Geisel et al. 2008; Moyer and Hamman 2001; Robinson 2008; Soltani et al. 2005). Robinson (2008) reported significant injury to broccoli, carrot, cucumber and onion one year after mesotrione application. Injury was increased for all these crops when atrazine was tank mixed with mesotrione and this resulted in a reduction in plant dry weight and yield. Potato was the only crop

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recommended for planting the year following application of mesotrione plus atrazine. For all the other crops a two-year recropping interval was suggested (Robinson 2008).

2.5 Methods of Detecting Herbicide Residues

Liquid chromatography, gas chromatography, mass spectrometry, capillary electrophoresis and immunoassays are all analytical methods used for detecting herbicide residues in the soil. Additionally, various bioassay methods have been developed to detect residues of herbicides in the soil.

A soil bioassay is used as a quantitative procedure to determine the presence and/or concentration of a specific chemical in a soil sample (Sunderland et al. 1991). Streibig (1988) defined a bioassay as "an experiment for estimating the potency of a herbicide by analysis of the reaction that follows its application to living organisms". A bioassay uses susceptible plants to determine if the chemical is present in high enough concentrations to inhibit germination or growth (O'Sullivan 2005; Santelmann 1997). The plant species or organism chosen for the bioassay should be sensitive to extremely small amounts of the chemical and respond in a very clear and in a measurable manner. Evaluation of the test organism response varies and depends on the herbicide and its effects on the test organism. For example, germination tests are used for herbicides that inhibit germination of sensitive species. Other assessment options include fresh and dry weights of the shoot or root or both, lengths of leaf or of the plant, visual injury ratings, photosynthetic activity, and chlorophyll content (Horowitz 1976).   A bioassay can be performed in the field or in a controlled environment (Hager and Refsell 2008). In the field, potentially sensitive plants are grown in the treated soil. With a controlled environment bioassay, treated soil is sown with a test plant and observations are made on plant injury, germination inhibition and/or shoot and root growth.

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Soil bioassays have been used to detect different herbicide residues, in particular sulfonylureas (Eliason et al. 2004; Geisel et al. 2008; Hernandez-Sevillano et al. 2001; Stork and Hannah 1996; Sunderland et al. 1991). O'Sullivan (2005) developed a grower-friendly greenhouse bioassay to determine the effect of imazethapyr residues in soil on vegetable crops. There are fewer published details about using a bioassay to detect mesotrione. Maeghe et al. (2002) used a greenhouse bioassay to detect mesotrione, sulcotrione and isoxaflutole residues in different soil types. Sugar beet and red clover were regarded as extremely sensitive to these herbicides and were selected as the test species for their bioassay. The authors concluded results varied by year due to variation in weather conditions. Additionally it was reported that persistence was determined by soil texture; persistence was least with clay soil and greatest with sandy and sandy loam soil types (Maeghe et al. 2002).

Analytical methods require extracting the chemical from the soil, using specialized equipment. Levels detected can be parts per million (ppm) or parts per billion (ppb) (Hager and Refsell 2008). However, chemical extraction is typically costly and complicated to perform. Additionally a soil bioassay actually measures the phytotoxic portion, or the amount of the herbicide available for biological activity, whereas chemical extraction measures the total amount of herbicide present. According to Appleby (1985), if the question is "how much potential exists for herbicidal effects on plants?" and not "how much herbicide residue is present in the soil?" then the choice should be a bioassay. Soil bioassay methods are also able to detect very small amounts of herbicide in the soil (Santelmann 1997). Since plant growth is directly affected by environmental conditions (temperature, moisture, humidity), variation can occur in test plant response if these factors are not controlled (Santelmann 1997).

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Analytical methods for detecting mesotrione in water, crops, and soil have been developed (Alferness and Wiebe 2002; Mastichiadis et al. 2003; Stoob et al. 2005). Stoob et al. (2005) developed a fully automated online solid phase-extraction-liquid chromatography-tandem mass spectrometry to quantify antibiotics and pesticides (including mesotrione) in natural waters. A low-cost fluoroimmunoassay was developed by Mastichiadis et al. (2003) to determine mesotrione in water and Alferness and Wiebe (2002) used liquid chromatography with fluorescence detection to detect mesotrione residues and metabolites in crops, soil and water.

2.6 Dose-Response Curves

Dose-response curves are often used to "quantify plant sensitivity to a herbicide" (Seefeldt et al. 1995) and can be developed from herbicide bioassays. Usually the biological response is explained by nonlinear regression models, which has an upper limit, defined by a very low herbicide dose, a lower limit, defined by a very high or lethal herbicide dose, and the midpoint between these two points, ED_{50} (50% effective dose) (Streibig and Kudsk 1993). The shape of a dose-response curve is sigmodial (Knezevic et al. 2007; Seefeldt et al. 1995) and the most common model is the log-logistic model with three or four parameters (Knezevic et al. 2007). The mathematical expression for a four parameter log-logistic model relating the response y to the dose x is:

$$y = C + \frac{D - C}{1 + \exp[b(\log(x) - \log(I_{50}))]}$$

Where C = lower limit, D = upper limit, b = slope, and I_{50} = dose giving 50% response (Knezevic et al. 2007; Seefeldt et al. 1995). Depending on the experiment, GR_{50} (50% growth reduction), LD_{50} (50% lethal dose), or ED_{50} (50% effective dose) can be also be

used instead of I_{50}. I_{50} is the most accurate estimate of plant sensitivity to an herbicide, thus it is the term most commonly used in weed science research (Seefeldt et al. 1995).

Herbicide dose selection is critical in determining the shape and fit of the curve. Seefeldt (1995) suggests selecting a wide range of doses that cover a range of responses (from no effects to complete plant death). In order to do this, it is more efficient to have doses spaced on a logarithmic scale rather than on a linear scale. Four doses is the minimum amount in order to fit the log-logistic curve, but five to seven is better. It is important that the doses selected do not cause responses clustered around the upper or lower limits. Doses near the I_{50}, the range in which the greatest plant response occur, should allow for shifts in the curve from one experiment to another (Seefeldt et al. 1995). Assuming that the dose-response is sufficiently described by the logistic curve and that the I_{50} value is a proper measurement of the potency of the herbicide, dose-response curves can be compared by using the I_{50} values (Nielsen et al. 2004) . Figure 2.1 illustrates an example of a logistic dose-response curve.

2.7 Simulated Carryover of Herbicides

There are a few studies that have examined the carryover effects of herbicides by "simulating" an actual carryover study. Barnes and Lavy (1991) evaluated the effects of recently applied residual levels (simulated residue carryover) and "actual" residue carryover of imazaquin and norflurazon on the growth and yield of corn, cotton, grain, sorghum, rice and wheat. Crops responses were compared between the two types of studies by using "closely matched" herbicide concentrations. The herbicide concentrations were determined by growth chamber bioassays. For example corn and cotton yield taken from actual carryover plots that received imazaquin at 0.28, or 0.56 kg ai ha^{-1} in 1988 were compared with yield taken from simulated carryover plots that

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received 0.016 or 0.30 kg ai ha^{-1} in 1989. The authors' conclusions were that the higher concentrations of herbicides in the simulated study have a more of an effect on sensitive crops than "similar" concentration of herbicides in the actual carryover study. It was suggested that this effect occurred because of differences in the herbicide binding mechanism with the soil. In the actual carryover study, the herbicide has a longer period to bind to the soil and thus resulting in reduced activity compared to the simulated study (Barnes and Lavy 1991).
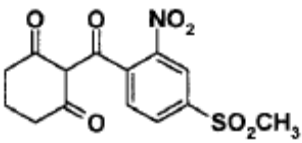
Rabaey and Harvey (1997) examined sweet corn hybrid sensitivity to imazethapyr residues by conducting a greenhouse tolerance trial, a simulated carryover field trial and a conventional carryover field trial. In the greenhouse, imazethapyr was sprayed at low concentrations (0 to 0.04 ppm) to soil and from this study, 11 corn hybrids were selected based on a range of sensitivity, to be evaluated in the simulated field studies. In the simulated study, imazethapyr was applied at 0, 11, 22, and 44 g ai ha^{-1}, 15 days prior to corn planting. In the conventional carryover study, imazethapyr was applied at 0, 70, and 140 ha^{-1} PPI and soybeans were planted, grown to maturity and harvested. In the following year sweet corn was planted into the plots that were treated the previous year. It was determined by correlation, that plant height reduction in the greenhouse was a "reliable indicator of hybrid sensitivity to simulated imazethapyr" in the field. The authors also report that the simulated carryover study provided more rigorous test of imazethapyr hybrid sensitivity than the conventional carryover study (Rabaey and Harvey 1997). Other studies have evaluated the effects of reduced rates of herbicides on crops such as cotton and rice to simulate an actual carryover study (Grey et al. 2005; Zhang et al. 2002). These studies did not conduct a conventional herbicide carryover study so a comparison could not be made to the simulated herbicide carryover study.

2.8 Research Hypothesis

The purpose of this research is to evaluate the relative sensitivity of sugar beet, lettuce, cucumber, green bean, pea and soybean to mesotrione residues. Mesotrione residues can cause significant variability in plant growth and quality and can cause entire crop losses in extreme cases. Thus there is a need for a simple method for detecting mesotrione residues prior to planting sensitive crops to assist with crop rotation planning.

1. Null Hypothesis: A one-year field study of growing sensitive crops in soil treated with reduced doses of mesotrione will not have similar results compared to a two-year field residue study of growing sensitive crops in soil treated with labeled rates of mesotrione the year previous on corn.

2. Null Hypothesis: A greenhouse bioassay used to detect mesotrione residues in the field cannot be used to compare plant response (injury, plant dry weight reduction) observed in the greenhouse with plant response (injury, plant dry weight and yield reduction) observed in the field.

3. Null Hypothesis: There will be no difference between the relative sensitivities of sugar beet, lettuce, cucumber, green bean, pea and soybean to mesotrione residues.

Table 2.1. Physical and chemical properties of mesotrione.

Structure:	
Chemical name:	2-(4-mesyl-2-nitrobenzoyl)-3-hydroxycyclohex-2-enone or 2-(4-methylsulfonyl-2-nitrobenzoyl)-1,3-cyclohexanedione
Common name:	Mesotrione
Chemical formula:	$C_{14}H_{13}O_7NS$
Physical state:	Opaque solid
Molecular weight:	339.32 g/mol
Melting point:	165 °C
Dissociation constant:	3.12 (pKa@20 °C)
Vapour pressure:	4.27×10^{-8} mm Hg (20 °C)
Water solubility:	2.2 g/l @ pH 4.8 (20 °C) 15 g/l @ pH 6.9 (20 °C) 22 g/l @ pH 9 (20 °C)
Information from Wichert et al. (1999).	

The figure shows plant biomass (% of Untreated) on the y-axis ranging from 0 to 100, plotted against Log(dose) on the x-axis. The curve starts near 100 at the Upper limit (D), decreases through a sigmoidal shape, and levels off at the Lower limit (C). The midpoint is marked as Log(I_{50}).
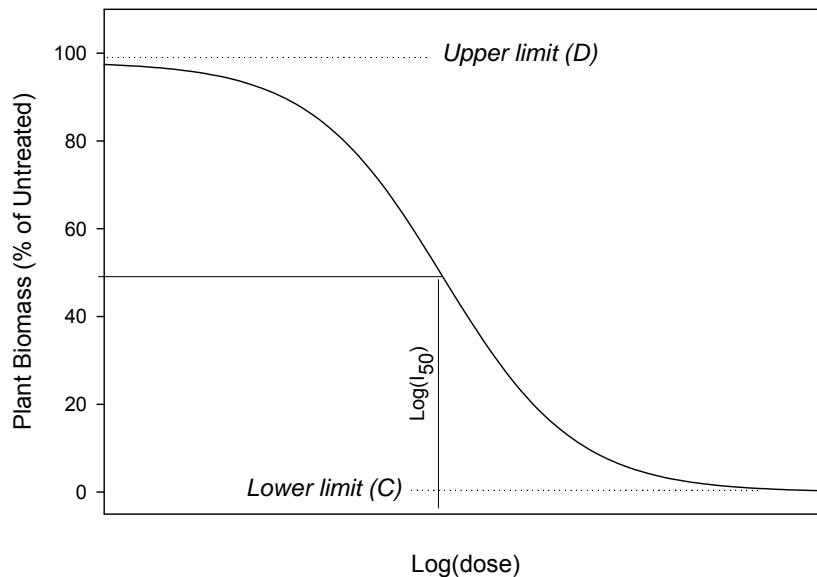
Figure 2.1.   The logistic dose-response curve. The figure shows plant

biomass as a percent of the untreated control plotted

against the log(dose). The four parameters used are the

upper limit (D), the lower limit (C), the concentration

resulting in 50% response (I_{50}) and the slope (b). Graph

derived from Streibig and Kudsk (1993).

3.0 CROP RESPONSE TO CARRYOVER OF MESOTRIONE RESIDUES IN THE FIELD (CONVENTIONAL RESIDUE CARRYOVER AND SIMULATED RESIDUE CARRYOVER STUDY)

3.1 Abstract

Two residue field studies were conducted from 2005 to 2007 at the Simcoe Research Station, Simcoe, Ontario to evaluate the effects of mesotrione soil residues on injury, plant dry weight and yield of sugar beet, cucumber, pea, green bean and soybean and to verify the potential of reducing a two-year field residue study (conventional residue carryover) to a one-year field study (simulated residue carryover study) by growing these crops in soil treated with reduced doses of mesotrione applied in the same year. There was a significant difference of mesotrione carryover between 2006 and 2007 and differences between years can be explained by differences in soil pH and soil moisture. The conventional and the simulated residue carryover studies successfully measured mesotrione persistence and rotational crop sensitivity. Both studies showed that sugar beet was the most sensitive crop with injury, plant dry weight reductions and yield loss due to mesotrione residues as high as 100%. Green bean was the next most sensitive crop to mesotrione residues followed by pea, cucumber, and soybean. The simulated residue carryover study provided a more rigorous test of rotational crop sensitivity to mesotrione residues than the conventional residue carryover study, especially at higher doses for the more sensitive crops. For the other crops, responses to mesotrione residues were similar between the conventional and simulated residue carryover studies.

Nomenclature: Mesotrione, 2-(4-methylsulfonyl-2-nitrobenzoyl)-1,3-cyclohexanedione; soybean, *Glycine max* Merr. L.; green bean, *Phaseolus vulgaris* L; pea, *Pisum sativum* L.; cucumber, *Cucumis sativus* L.; sugar beet, *Beta vulgaris* L., leaf lettuce, *Lactuca sativa* L.

Key words: Mesotrione, conventional residue carryover, simulated residue carryover, mesotrione residues, rotational crops.

3.2 Introduction

Herbicide persistence is an important consideration in crop production since residues can potentially injure sensitive crops grown in rotation. Damage to high-value vegetable crops can result in substantial economic loss. Vegetable crops have shown variable levels of sensitivity to residues one year after herbicide application (Felix et al. 2007; Robinson 2008; Soltani et al. 2007; Torma et al. 2004). In Ontario, 72% of the province's vegetable crops are grown in southern Ontario (Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) 2011) and these vegetable growers often rotate their crops with field corn. One benefit of a field corn – vegetable crop rotation is that growers have many more options for managing weeds, particularly those that are difficult to control in vegetables. However, growers need to consider the potential of herbicide persistence and the effect it may have on vegetable crops grown in the rotation, one or two years after application to corn.

Mesotrione is a triketone herbicide registered for pre and postemergence broadleaf weed control in corn (*Zea mays*). It was first registered in Canada by Syngenta Crop Protection under the trade name of Callisto® in 2004. The current Canadian label for mesotrione approves preemergence use in field, seed and sweet corn, cranberry and blueberry and postemergence use in field corn, cranberry and blueberry (Syngenta Crop

Protection Canada, Inc. 2011). Mesotrione acts by competitively inhibiting the enzyme *p*-hydroxyphenylpyruvate dioxygenase (HPPD) (Prisbylla et al. 1993). In plants HPPD is responsible for catalyzing the conversion of p-hydroxyphenyl pyruvate (4-HPP) to homogentisate (HGA). By inhibiting HPPD, the herbicide depletes levels of plastoquinone, resulting in the depletion of carotenoids. This, in effect, leads to bleaching symptoms followed by necrosis of the meristematic tissue within three to five days of application and throughout the entire plant in approximately two weeks (Wichert et al. 1999).

Mesotrione provides control of redroot pigweed (*Amaranthus retroflexus* L.), common lambsquarters (*Chenopodium album* L.), velvetleaf (*Abutilon theoprasti* Medicus), Eastern black nightshade (*Solanum ptycanthum* Dun.), barnyard grass (*Echinochloa crusgalli* (L.) Beauv.), wild mustard (*Sinapis arvensis* L.), ragweed species (*Ambrosia* spp.) and crabgrass species (*Digitaria* spp.) (Sutton et al. 1999; Syngenta Crop Protection Canada, Inc. 2011; Wichert et al. 1999). It also provides residual control of later germinating weeds (Wichert et al. 1999).

Mesotrione is an effective herbicide for weed control, however, due to its residual activity, it can cause injury to sensitive crops grown in the rotation, one year after application. The current label has recropping intervals of three months for winter wheat, ten months for spring wheat, and eleven months for alfalfa, potato, soybean, transplanted tomato and white bean (Syngenta Crop Protection Canada, Inc. 2011). The label does not include recropping intervals for most vegetable crops and suggests performing a field bioassay before planting potentially sensitive crops.

Several studies have shown that mesotrione does persist one year after application and can harm sensitive crops grown in rotation (Felix et al. 2007; Robinson 2008). A two-year carryover study is useful in indicating crop sensitivity, however, a field bioassay

that requires only one year of field research to determine the carryover potential of herbicide residues to sensitive crops in the rotation would be useful. Some studies have examined the carryover effects of herbicides by "simulating" a conventional two year carryover study (Barnes and Lavy 1991; Grey et al. 2005; Rabaey and Harvey 1997; Wiatrak et al. July, 2009; Wiatrak et al. July, 2009; Zhang et al. 2002). However, there have been no simulated residue carryover studies involving mesotrione and vegetables crops.

The specific objectives of this research were to (1) evaluate the effects of soil residues of mesotrione on visual injury, plant dry weight and yield of sugar beet, lettuce, cucumber, pea, green bean and soybean grown in rotation with corn, (2) rank these crops based on their relative sensitivities to mesotrione residues, (3) verify the potential of reducing a two-year field residue study to a one-year field study by growing sensitive crops in soil treated with reduced doses of mesotrione, applied in the same year and (4) provide a comparison between the bioactivity of newly-applied mesotrione at reduced doses and mesotrione applied at labeled doses one year earlier.

3.3 Materials and Methods

3.3.1 Conventional Residue Carryover Study

Two field experiments were conducted over a three-year period (2005 to 2007) at the University of Guelph, Simcoe Research Station, Simcoe, Ontario, Canada. The location of the experiments was selected based on soil characteristics that are typical for growing vegetable crops in rotation with corn and that would provide a worse-case scenario for mesotrione residue carryover (sandy soil texture, low soil organic matter). The soil was a Wattford (glaciolacustrine sands), fine sandy loam (sand 70%, silt 25%; clay 5%), CEC 6.1, pH 6.0; OM 1.7%, (2005) and CEC 5.4, pH 6.7, OM 1.6% (2006). Monthly (April to September) precipitation and temperatures for 2005 to 2007 are presented in Table 3.1.

Each experiment was established as a randomized complete block design with four replications. Two passes with a disk and field cultivator were made and 300 kg ha^{-1} of 34-0-0 fertilizer was applied and incorporated prior to the initiation of each experiment. Field corn (N3030BT LL, Syngenta Seeds, NK brand) was planted on May 18, 2005 and May 15, 2006, using a John Deere Max Emerge Plus- VacuMeter precision planter in plots that were 4.5 m wide and 10 m long. Six rows of corn per plot were seeded at a depth of 2.5 cm, rows spaced 75 cm apart, giving a plant population of approximately 53,000 plants ha^{-1}. Mesotrione was applied preemergence to corn after planting on May 19, 2005 and May 19, 2006 at 0, 70, 140 (1X), 280, 420 and 560 g ai ha^{-1}. The labeled preemergence dose of mesotrione on field corn in Canada is 140 g ai ha^{-1}. Each replication also included six untreated plots for subsequent use in the simulated residue carryover study. Spray applications were made using a CO_2 pressurized back-pack sprayer delivering 200 L ha^{-1} of spray solution at 200 kPa pressure, using 8002 flat-fan nozzles (Teejet 8002 SS tip, Spraying Systems Co., Wheaton, IL 60188). The corn was mowed at maturity and soil incorporated. In the following spring the entire experiment area was lightly tilled with a Lilliston Rolling Cultivator. Soil was sampled from each field plot prior to planting on May 16, 2006 and May 17, 2007, at a 0-15 cm depth. The soil samples were analyzed at the University of Guelph, Laboratory Services, for mesotrione residues, using liquid chromatography–tandem mass spectrometry (LC-MS/MS). The experimental design was a randomized complete block design with a split-plot arrangement and four replications with herbicide treatment as main plot and crop as subplot. Soybean (*Glycine max* (L.) Merr. 'S15-B1'), green bean (*Phaseolus vulgaris* L. 'Matador'), pea (*Pisum sativum* L. 'Durango'), cucumber (*Cucumis sativus* L. 'Vlaspik'), sugar beet (*Beta vulgaris* L. 'Crystal 963') and leaf lettuce (*Lactuca sativa* L. 'Lasting Green M.I.') were seeded on May 29, 2006 and May 28, 2007, using an Almaco® cone seeder mounted on a John Deere planter. One row of each crop was direct seeded into

each plot using a between-row spacing of 75 cm. Sugar beet, lettuce and cucumber were planted at a depth of 1.25 cm and pea, green bean and soybean were planted at a depth of 2.5 cm. To ensure uniform seed germination, the entire experiment area was irrigated with 2.5 cm of water, one day after planting. Despite the irrigation, there was poor germination of lettuce in both years and for this reason lettuce crop ratings and measurements could not be completed. Plots were maintained weed-free season long with cultivation and hand-hoeing as required.

Visual crop injury ratings were made on each crop in both years using a 0 to 100% scale, where 0 = no effect and 100 = crop death. Injury ratings were taken at 21 and 39 days after planting (DAP). Above ground plant samples of each crop were taken from 1.0 m of the plot area at 40 DAP for fresh and dry weights. For the dry weights, samples were placed in a paper bag and dried at 70°C. All crops were hand harvested at maturity, by removing the center 3.0 m of each crop row, using methods appropriate for each crop. For sugar beet, the roots were dug using spades; the tops were cut off using a knife and then the roots were counted and weighed. Sugar beets were harvested on October 25, 2006 and October 22, 2007. Cucumbers were harvested on July 21, 2006 and July 20, 2007, by pulling plants and removing all cucumbers 1.0 cm in diameter or larger. Cucumbers were graded based on sizes defined by the Ontario Processing Vegetable Growers and the Farm Products Marketing Act (Appendix) and counted and weighed. Green beans and peas were harvested on July 27, 2006, August 10, 2007 and July 21, 2006, July 25, 2007, respectively, by removing the pods from each plant. All pods were counted and weighed. Soybeans were harvested on October 26, 2006 and October 25, 2007, by pulling plants by hand and then putting them through an ALMACO® stationary thrasher which removed the beans from the stalks and the pods. The beans were then weighed. For all crops, plant counts were recorded.

3.3.2 Simulated Residue Carryover Study

Field experiments were conducted in 2006 and 2007 at the University of Guelph, Simcoe Research Station, Simcoe, Ontario, Canada. The experiments were established as a randomized complete block design with four replications. To avoid differences due to soil type, this study was randomized within the conventional residue carryover study. The experiment area was cultivated, fertilized and planted with field corn as previously described for the conventional residue carryover study in 3.2.1. Plots designated for the simulated residue carryover study were left untreated, other than cultivation for weed control. Mesotrione was applied before crop planting on May 19, 2006 and May 18, 2007 at 0, 7, 14 (10% of 1X), 28, 42 and 56 g ai ha^{-1}. Seeding dates, planting and harvest procedures, crop cultivars, spray applications and crop ratings and measurements were performed using the same equipment and methods (including plot dimensions) as previously described in 3.2.1. Soil characteristics are described in 3.2.1.

3.3.3 Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using the MIXED procedure of SAS statistical software (Ver. 9.2, SAS Inst., Cary, NC). The variances were partitioned into the fixed effects of mesotrione dose and into the random effects of year and replicated block-by-year interaction. The assumptions of the variance analysis were tested ensuring that the residuals were random, homogeneous, with a normal distribution about a mean of zero using residual plots and the Shapiro-Wilk normality test. To meet assumptions of the variance analysis, fresh weight of cucumber and percent injury at 21 DAP for soybean from the conventional residue carryover study and for the simulated residue carryover study, % injury at 39 DAP, and dry weights of soybean, % injury at 39 DAP of pea, cucumber yields from the 2006 study were

subjected to a log transformation. Means were back-transformed to the original scale for presentation of results. When the effect of year and the interaction with mesotrione dose was not significant after analysis, the data was pooled by year. Means were separated using Fisher's protected LSD. The Type I error was set at 0.05 for all statistical comparisons. The SAS MIXED procedure is shown in the Appendix.

3.4 Results and Discussion

3.4.1 Sugar beet

In both years of both studies (conventional and simulated residue carryover), there was significant variability in sugar beet plant growth within some treated plots. At intermediate levels of mesotrione not all plants in the plots were killed and those that survived grew very large, due to lack of competition. For this reason, it was necessary to separate marketable yields from total yields. In this study, a marketable sugar beet plant was one that had a root weight of 0.5 to 2 kg. Only marketable yields are presented.

There was a significant interaction between year and mesotrione dose for several of the ratings for both studies, therefore, all data for 2006 and 2007 are discussed and reported separately. In the conventional residue carryover study in 2006 and 2007, mesotrione injury was evident on sugar beet 21 days after planting (DAP) and changed little in severity by 39 DAP (Table 3.2). The same trends were evident in the simulated residue carryover study (Table 3.3). In both residue carryover studies, injury was reduced in 2007 compared to 2006, particularly at the low mesotrione application doses. Injury was more severe in the simulated residue carryover study (Table 3.3). Injury symptoms included a brief initial bleaching followed by chlorosis and necrosis of the tissue. Injury symptoms, in general, increased with increasing mesotrione dose. Mesotrione injury ranged from 42 to 100% in 2006 and from 6 to 89% in 2007, 39 DAP,

in the conventional residue carryover study (Table 3.2). In 2006, injury in the simulated residue carryover study at 39 DAP ranged from 58 to 98% at mesotrione doses of 7 to 56 g ai ha^{-1} (Table 3.3). In 2007, injury was less severe, ranging from 5 to 100%.

Plant dry weights were reduced as mesotrione dose increased. As with plant injury, reductions in 2006 were more severe than in 2007. In the conventional residue carryover study in 2007 there was no reduction in plant dry weight at mesotrione application doses of 140 g ai ha^{-1} or lower (Table 3.2). Plant dry weight reductions were more severe for the simulated carryover residue study, especially in 2006 (Table 3.3). Plant dry weights were reduced by almost 100% for all doses of mesotrione in 2006. In 2007, the 7 g ai ha^{-1} application dose had only 26% plant dry weight reduction. In all studies and in both years, yield reduction followed a similar pattern to plant injury and plant dry weight reductions. Yield reductions were higher in 2006 than in 2007 for the conventional residue carryover study (Table 3.2). In 2007, mesotrione injury resulted in yield reductions of only 16% at the 140 g ai ha^{-1} (1X) application dose and approximately 100% at the 2X to 4X application doses, when compared to the untreated control. In 2006, yield reductions for the simulated residue carryover study ranged from 66 to 100%. In 2007, mesotrione at 7 g ai ha^{-1} caused no yield reduction (Table 3.3). At 14 to 56 g ai ha^{-1} yields were reduced from 71 to 100%.

The response of sugar beet in terms of injury, plant dry weight and marketable yield reduction, were more severe in the simulated residue carryover study than in the conventional residue carryover study. In 2007, the impact of mesotrione residues in sugar beet were less pronounced than in 2006 for both conventional and simulated studies. These results do not agree with the findings of Torma et al. (2004) who did not observe any yield reduction in sugar beet with applications of mesotrione the previous year at doses ranging from 168 to 336 g ai ha^{-1}. The study by Torma et al. (2004) was

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conducted on soil with a higher soil pH (7.1) and higher organic matter (3.1%) which
could explain the difference in sugar beet sensitivity.

3.4.2   Cucumber

There was a significant interaction between year and mesotrione dose for most of
the ratings on cucumber for the simulated residue carryover studies only, thus all data
for 2006 and 2007 simulated carryover studies are discussed and reported separately.
Cucumber was less sensitive to mesotrione residues than sugar beet. Mesotrione injury
in cucumber was evident 21 DAP and generally declined in severity by 39 DAP. Injury
symptoms included bleaching, chlorosis and necrosis of the tissue. Injury symptoms
tended to increase with mesotrione dose. In the conventional residue carryover study,
injury in cucumber ranged from 10 to 33%, 39 DAP (Table 3.4). This was similar to injury
observed in the simulated carryover study in 2006, which ranged from 14 to 40%. In
2007, injury was less and ranged from 0 to 29% (Table 3.5). Mesotrione resulted in a
47% plant dry weight reduction only at 56 g ai ha^{-1}, 40 DAP in the 2007 simulated
residue carryover study. Mesotrione reduced plant dry weight from 2 to 28% (Table 3.4)
and from 0 to 37% (Table 3.5) and yield reduction ranged from 3 to 33% and 0 to 38%,
in the conventional and in the 2006 simulated residue carryover studies, respectively,
when compared to the untreated control. In the 2007 simulated residue carryover study
there was no yield reduction due to mesotrione (Table 3.5). Cucumber response, in
terms of plant injury, dry plant weight reduction and yield was similar for the conventional
and simulated residue carryover studies.

These results agree with the findings of Felix et al. (2007) who observed 19 to 20%
and 19 to 35% injury 42 DAP from mesotrione applied pre emergence at 210 and 420
g ai ha^{-1} at two different sites, respectively. Felix et al. (2007) reported significantly

reduced cucumber yields at one location. Robinson (2008) observed similar injury in

cucumber but at lower mesotrione doses. He reported 18 and 19% injury one year after

applying mesotrione at 140 and 280 g ai ha^{-1}, respectively. This injury resulted in a 19

and 17% reduction of cucumber dry weight at mesotrione doses of 140 and 280 g ai

ha^{-1}. The biggest difference between studies, however, was that Robinson (2008)

reported a 62 and 70% yield loss in cucumber for these doses, respectively. Robinson

(2008) hypothesized that the high organic matter of the soil (4.5% and 5.7% at two sites)

where the trials were conducted could explain the difference in levels of yield reduction

between his study and the study by Felix et al. (2007).

3.4.3 Green bean

There was a significant interaction between year and mesotrione dose for several of

the ratings on green bean in both studies thus all data for 2006 and 2007 studies are

presented and reported separately. Mesotrione injury in green bean was evident 21 DAP

and increased slightly in severity by 39 DAP, in both years of the conventional residue

carryover study (Table 3.6) and in the 2006 simulated residue carryover study (Table

3.7). However, in the 2007 simulated residue carryover study, injury decreased slightly

by 39 DAP (Table 3.7). Injury in green bean was less in 2007 than in 2006, in the

conventional residue carryover study (Table 3.6). In the 2006 conventional residue

carryover study, injury in green bean at 39 DAP ranged from 30 to 81%. In 2007, injury

at 39 DAP ranged from 18 to 59% at the 1X to 4X mesotrione application doses. Injury

in the simulated residue carryover study in both years was similar to the level of injury

observed in the conventional residue carryover study. Injury at 39 DAP ranged from 26

to 88% and from 5 to 78% at 7 to 56 g ai ha^{-1} in 2006 and 2007, respectively (Table 3.7).

Injury symptoms in green bean consisted mainly of chlorosis followed by necrosis of the

tissue and plant stunting and became more obvious over time. In general, injury

symptoms increased with increasing mesotrione dose.

In the 2006 conventional residue carryover study, injury resulted in a significant

reduction in plant dry weight, when compared to the untreated control. There was a 58,

80 and 91% plant dry weight reduction when mesotrione was applied the previous year

at 2X, 3X and 4X application doses, respectively (Table 3.6). Yields were reduced by 64

to 95% at the same mesotrione application doses. In 2007, plant dry weight was reduced

by 47 and 37% only at the 3X and 4X application doses, respectively, however, there

was no reduction in yields when compared to the untreated control (Table 3.6). Similar

results were observed by Felix et al. (2007) who observed 13 to 96% injury at 42 DAP

with previously applied mesotrione of 420 g ai ha^{-1}. The variable injury between locations

also resulted in very little yield reduction in one location but 99% yield reduction in the

other location (Felix et al. 2007).

With green bean, the simulated residue carryover study provided a more rigorous

test of sensitivity to mesotrione residues. In the simulated residue carryover study, injury

resulted in significant plant dry weight reductions, ranging from 52% to 88% and 55% to

73% at the mesotrione doses of 14 to 56 g ai ha^{-1} for 2006 and 2007, respectively, when

compared to the untreated control (Table 3.7). Yields were also significantly reduced in

the simulated residue carryover study, especially in 2006. Yield reduction in 2006 ranged

from 35 to 97% at mesotrione application doses of 7 to 56 g ai ha^{-1}. In 2007, yield

reduction ranged from 23 to 58% at 28 to 56 g ai ha^{-1} (Table 3.7).

3.4.4 Pea

There was no significant interaction between year and mesotrione dose for pea in

both studies thus all data for 2006 and 2007 studies were combined. Mesotrione injury

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on pea, for the conventional and simulated residue carryover studies, was evident 21 DAP and decreased in severity by 39 DAP. Injury symptoms in pea were characterized by bleaching, chlorosis and necrosis of the tissue. Symptoms mostly increased with increasing mesotrione dose. In the conventional residue carryover study, injury in pea at 21 DAP ranged from 1 to 49% at previously applied mesotrione doses of 0.5 to 4X application doses, respectively, but by 39 DAP this injury had decreased to 4 to 29% for the same mesotrione application doses (Table 3.8). In general, the simulated residue carryover study provided a more rigorous test of pea sensitivity to mesotrione residues, especially at higher doses. Injuries were only slightly higher in the simulated residue carryover study. Injury at 21 DAP ranged from 1 to 73% at mesotrione application doses of 7 to 56 g ai ha^{-1}, but at 39 DAP, injury ranged from 1 to 40% for the same application doses (Table 3.8). Plant dry weight reduction ranged from 6 to 25% and 16 to 51% in the conventional and simulated residue carryover studies at 280 to 560 and 28 to 56 g ai ha^{-1} of mesotrione, respectively, 40 DAP. In the conventional residue carryover study, injury did not result in any significant pea yield reductions, when compared to the untreated control, however, in the simulated carryover study, injury did result in a significant pea yield reduction of 39 and 47% at 42 and 56 g ai ha^{-1} of mesotrione. The conventional residue carryover results are similar to Torma et al. (2004) who observed no injury or yield reduction in pea when applying mesotrione one year previous at doses ranging from 168 to 336 g ai ha^{-1}.

3.4.5   Soybean

There was no significant interaction between year and mesotrione dose for soybean in both studies thus all data for 2006 and 2007 studies are combined. Mesotrione injury on soybean was evident 21 DAP and increased slightly in severity by 39 DAP in the

conventional residue carryover study but decreased in the simulated residue carryover study (Table 3.9). Injury symptoms included chlorosis and necrosis of the tissue but did not cause plant stunting. Plants that had initial injury due to mesotrione seemed to compensate and grow taller than those plants that did not show any mesotrione injury. Injury symptoms generally increased in severity with increasing mesotrione dose. In the conventional residue carryover study, injury to soybean at 39 DAP was 13, 19 and 26% at mesotrione doses of 280, 420 and 560 g ai ha^{-1}, applied the previous year, respectively (Table 3.9). Injury in the simulated residue carryover study at 39 DAP was 10%, 12% and 10% at mesotrione doses of 28, 42 and 56 g ai ha^{-1}, respectively (Table 3.9). The simulated and conventional residue carryover studies provided an equal test of sensitivity of soybean to mesotrione residues. For both studies, injury did not result in reduction in plant dry weight or yields at maturity when compared to the untreated control. Field observations show that soybean with carryover injury from mesotrione typically exhibits bleaching of the tips and margins of new growth on the plant but usually there is no yield reduction. These results agree with the recommendations of the current Canadian label for mesotrione which lists a re-cropping interval of 11 months for soybean (Syngenta Crop Protection Canada, Inc. 2011).

3.4.6 Chemical analysis of mesotrione carryover

The amount of mesotrione carryover from the previous year was measured by chemical analysis, as described in 3.3.1. Table 3.10 verifies that there was less mesotrione carryover from 2006 to 2007 than from 2005 to 2006.

3.5 Conclusions

Results from this study show that mesotrione residues can cause injury and yield loss in sensitive crops grown in rotation. Response to mesotrione residues differed among crops. Sugar beet was the most sensitive crop with injury, plant dry weight reductions and yield loss as high as 100% at the higher mesotrione application doses. Green bean was the next most sensitive crop, based on injury as high as 73% and a yield loss of 95% in 2006 at the 560 g ai ha^{-1} application dose of mesotrione, applied the previous year. Despite injury on cucumber and pea in the conventional residue carryover study, there was no yield loss on either of these crops when compared to the untreated control, even at higher doses of mesotrione. Mesotrione injury of 26% in soybean did not result in yield loss at a mesotrione application dose of 560 g ai ha^{-1}.

Carryover of mesotrione residues to rotation crops has been observed, particularly in soils of low pH, low soil organic matter and low cation exchange capacity (Felix et al. 2007). Mesotrione is broken down in the soil by microbial activity. Field observations indicate that injury in soybean from mesotrione residues is usually limited to sandy soils with low soil organic matter and low cation exchange capacity as well as soils with a pH lower than 6.0 (M. Cowbrough, personal communication). Any soil characteristic that inhibits microbial activity, such as dry conditions or changes in soil pH, could potentially lead to a decrease in the breakdown of mesotrione residues. Because mesotrione is dependent on microbial degradation, it persists longer under low soil moisture and cooler temperatures which reduce plant metabolism.

Generally, injury increased as mesotrione application dose increased. Soil moisture and soil pH had a significant effect on mesotrione carryover. In all studies and for all crops, injury in 2007 was less severe than in 2006. This is most likely related to difference in weather conditions (rainfall) and soil pH. Temperatures during the two

months following mesotrione application in 2005 and 2006 were similar, however, in July 2006 rainfall was considerably higher than in July 2005 (Table 3.1). Maeghe et al. (2002) found that there was more mesotrione degradation in years with heavy rainfall, especially within two months of application, and thus crop yield loss was greater in years with low rainfall. Soil pH also differed between the two experiment locations; in 2005 soil pH was 6.0 compared to the soil pH of 6.7 in 2006. Mesotrione is more likely to adsorb to soil at low soil pH and thus less likely to degrade than at high soil pH (Dyson et al. 2002). Less degradation of mesotrione in the year of application could result in more being available the following year to injure sensitive crops grown in the rotation (Felix et al. 2007).

Low soil pH and drier conditions immediately following application in 2005 caused more mesotrione to be adsorbed to the soil, making it less available for degradation and potentially more available for the following year. In 2006, when the sensitive crops were planted, rainfall was higher, making it more favourable for mesotrione to become available for plant uptake and causing greater injury and yield reduction. The conditions were reversed for the study initiated in 2006. A higher soil pH and higher soil moisture conditions immediately following application in 2006 caused less mesotrione adsorption to the soil, making it more available for degradation and less available to persist into the following year. In 2007, the conditions were once again drier than 2006, thus causing more mesotrione adsorption to the soil, making it less available for plant uptake, leading to decreased injury and yield reductions. The soil organic matter at the two experiment sites was similar; 1.7% in 2005 and 1.6% in 2006 and likely does not account for the difference between the two locations. The levels of mesotrione residues, measured by chemical analysis, in the soil at planting in 2006 and 2007 support observations on the impact of soil pH and rainfall, in explaining differences between rotational crop response to mesotrione residues in 2006 versus 2007. Differences in rotational crop response to

simulated residues of mesotrione between years can also be explained by differences in soil pH and soil moisture between the two locations in 2006 and 2007.

These studies support the current mesotrione label prohibiting re-cropping with sensitive crops for two years after application of mesotrione, without a field bioassay. Under similar environmental conditions and soil characteristics to those observed in these studies, sugar beet, green bean and to a lesser extent cucumber injury, plant dry weight and yield reductions are likely when these crops are grown in soils containing mesotrione residues.

Simulated residue carryover studies provided a more rigorous test of rotational crop sensitivity to mesotrione residues than conventional residue studies but only at the higher application doses and for the most sensitive crops like sugar beet and green bean. Otherwise results for simulated residue studies in terms of plant injury, plant dry weight reductions and yields were similar to results from the conventional residue carryover studies. This agrees with the findings of Barnes and Lavy (1991) who reported that at low concentrations of imazaquin and norflurazon there were no corn yield differences between the simulated and actual carryover studies. These studies verified the applicability of using a one- year field bioassay (simulated residue carryover) for all crops with the possible exception of sugar beet, the most sensitive crop. With sugar beet, the injury risk may be over estimated by using a one-year in-field bioassay. However, this is preferable to underestimating the risk in crop rotation planning. Sugar beet data suggested that higher concentrations of the newly-applied herbicides have a greater effect on sensitive crops than similar concentrations of a herbicide that has been present in the soil for at least a one year period. Other researchers have shown decreased herbicide desorption and bioactivity in soils over time (Weber et al. 1986). Differences in the magnitude of the bioavailable fraction or differences in the strength of

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binding mechanisms following prolonged contact with soil may be responsible for the reduced activity of these herbicides in conventional residue carryover studies compared with simulated residue carryover studies (Barnes and Lavy 1991).

When these studies are compared with the reported literature it can be concluded that the simulated residue carryover study was successful in measuring persistence of mesotrione and in understanding rotational crop sensitivity. Simulated residue carryover studies are appropriate for determining the potential for injury to sensitive crops grown in the rotation. When compared to a two-year conventional residue carryover study, a one-year simulated residue carryover study provides a more rigorous test of crop sensitivity to herbicide residues, provides more timely results and provides a significant cost savings to researchers, the agricultural chemical and vegetable crop industries.

Table 3.1. Monthly precipitation (mm) and maximum, minimum and mean temperatures (°C) during the 2005, 2006 and 2007 growing seasons at the Simcoe Research Station, Simcoe, Ontario[a].

	2005				2006				2007			
	Precipitation	Temperature			Precipitation	Temperature			Precipitation	Temperature		
	total	max	min	mean	total	max	min	mean	total	max	min	mean
	mm	°C			mm	°C			mm	°C		
April	95.6	12.7	2.4	7.6	72.1	14.5	1.3	7.9	70.6	10.4	1.6	8.2
May	44.2	18.2	7.0	12.7	75.7	19.9	6.9	13.5	19.8	21.2	8.8	15.6
June	69.4	27.1	16.1	21.6	52.3	25.1	12.0	18.5	49.0	26.4	13.6	20.4
July	49.6	28.8	16.9	22.9	132.1	28.4	15.9	22.2	49.4	26.1	14.4	20.4
August	114.2	27.1	16.3	21.7	93.4	26.0	13.7	19.9	59.6	26.6	15.5	21.0
September	87.6	24.0	13.0	18.5	167.2	19.5	9.9	14.7	73.0	23.1	11.5	17.5

[a] Automated weather station based on a Campbell Scientific CR-21X data logger, located at the Simcoe Research Station, Simcoe, ON. GPS coordinates: Latitude 42 ° 51'N; Longitude 80 ° 16'W

Table 3.2. Effect of mesotrione soil residues on injury, plant dry weight and yield of sugar beet in 2006 and 2007, one year after mesotrione application to corn.

Mesotrione	2006				2007			
	Injury		Dry weight	Yield	Injury		Dry weight	Yield
g ai ha^{-1}	21 DAP[a]	39 DAP	40 DAP		21 DAP	39 DAP	40 DAP	
	—— % ——		g	t ha^{-1}	—— % ——		g	t ha^{-1}
0	0 b[b]	0 a	23.8 a	72.3 a	0 a	0 a	51.3 a	65.2 a
70	38 bc	42 b	13.6 b	62.7 a	0 a	6 a	57.9 a	61.5 a
140	33 bc	29 b	13.9 b	51.6 b	5 ac	8 a	79.8 a	54.8 a
280	63 ac	76 c	14.4 b	15.7 c	68 b	80 b	0.0 b	0.0 b
420	100 a	100 c	0.0 c	0.0 c	50 bc	86 b	0.0 b	0.0 b
560	88 a	88 c	8.9 c	0.0 c	94 b	89 b	6.7 b	2.0 b

[a] Abbreviations: DAP, days after planting

[b] Means followed by the same letter in each column are not signficantly different according to Fisher's protected LSD test (P<0.05).

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Table 3.3. Effect of mesotrione, applied before planting, on injury, plant dy weight and yield of sugar beet in 2006 and 2007.

Mesotrione	2006				2007			
	Injury		Dry weight	Yield	Injury		Dry weight	Yield
g ai ha^{-1}	21 DAP[a]	39 DAP	40 DAP		21 DAP	39 DAP	40 DAP	
	—— % ——		g	t ha^{-1}	—— % ——		g	t ha^{-1}
0	0 a[b]	0 d	16.8 a	59.4 a	0 a	0 a	62.9 a	58.6 a
7	51 b	58 c	0.0 b	1.4 b	0 a	5 a	46.3 a	66.1 a
14	99 c	61 bc	0.7 b	0.0 b	80 b	86 b	2.8 b	17.0 b
28	98 c	98 a	0.0 b	2.7 b	100 c	100 c	0.0 b	0.0 c
42	98 c	88 abc	0.0 b	0.0 b	100 c	100 c	0.0 b	0.0 c
56	99 c	94 ab	0.0 b	0.0 b	100 c	100 c	0.0 b	0.0 c

[a] Abbrevations: DAP, days after planting

[b] Means followed by the same letter in each column are not significantly different according to Fisher's protected LSD test (P<0.05)

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Table 3.4. Effect of mesotrione soil residues on injury, plant dry weight and yield of cucumber in 2006 and 2007, one year after mesotrione application to corn. Data for 2006 and 2007 is combined.

Mesotrione	Injury		Dry weight	Yield
g ai ha^{-1}	21 DAP[a]	39 DAP	40 DAP	
	——— % ———		g	t ha^{-1}
0	0 a[b]	0 c	60.0 a	22.4 a
70	21 abc	14 bc	54.8 a	21.8 a
140	13 ac	10 bc	58.5 a	21.7 a
280	24 cb	19 ab	54.7 a	21.8 a
420	33 cb	25 ab	49.2 a	15.1 a
560	39 b	33 a	51.8 a	16.3 a

[a] Abbrevations: DAP, days after planting

[b] Means followed by the same letter in each column are not signficantly different according to Fisher's protected LSD test (P<0.05).

Table 3.5. Effect of mesotrione, applied before planting, on injury, plant dry weight and yield of cucumber in 2006 and 2007.

Mesotrione	2006				2007			
	Injury		Dry weight	Yield	Injury		Dry weight	Yield
g ai ha⁻¹	21 DAP[a]	39 DAP	40 DAP		21 DAP	39 DAP	40 DAP	
	—— % ——		g	t ha⁻¹	—— % ——		g	t ha⁻¹
0	0 a[b]	0 a	32.3 a	18.3 a	0 b	0 b	85.8 bcd	18.6 a
7	28 a	28 ab	23.2 a	11.3 a	0 b	0 b	92.5 bcd	23.5 a
14	25 a	14 ab	32.4 a	22.9 a	18 ab	6 ab	113.3 b	27.1 a
28	60 b	40 b	23.0 a	10.8 a	43 a	16 ab	74.3 ad	20.1 a
42	65 b	34 b	20.4 a	17.1 a	20 ab	29 a	82.6 bd	19.3 a
56	63 b	36 b	40.2 a	16.6 a	53 a	26 a	45.1 a	23.9 a

[a] Abbreviations: DAP, days after planting

[b] Means followed by the same letter in each column are not significantly different according to Fisher's protected LSD test (P<0.05).

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Table 3.6. Effect of mesotrione soil residues on injury, plant dry weight and yield of green bean in 2006 and 2007, one year after mesotrione application to corn.

Mesotrione	2006				2007			
	Injury		Dry weight	Yield	Injury		Dry weight	Yield
g ai ha^{-1}	21 DAP[a]	39 DAP	40 DAP		21 DAP	39 DAP	40 DAP	
	—— % ——		g	t ha^{-1}	—— % ——		g	t ha^{-1}
0	0 a[b]	0 c	69.0 a	10.6 ab	0 a	0 b	69.6 ab	9.3 a
70	14 ac	30 bc	42.6 ab	13.2 a	0 a	0 b	86.2 a	10.4 a
140	19 ac	44 ab	46.5 ab	6.7 bc	11 a	18 b	83.1 a	8.2 a
280	33 bc	64 ab	29.1 bc	3.8 dc	38 b	40 a	69.2 ab	10.8 a
420	64 d	81 a	14.1 bc	1.4 d	49 b	59 a	36.9 b	7.8 a
560	64 d	73 a	6.0 c	0.5 d	49 b	59 a	43.6 b	8.2 a

[a] Abbrevations: DAP, days after planting
[b] Means followed by the same letter in each column are not significantly different according to Fisher's protected LSD test (P<0.05).

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Table 3.7. Effect of mesotrione, applied before planting, on injury, plant dry weight and yield of green bean in 2006 and 2007.

Mesotrione g ai ha^{-1}	2006				2007			
	Injury		Dry weight	Yield	Injury		Dry weight	Yield
	21 DAP[a]	39 DAP	40 DAP		21 DAP	39 DAP	40 DAP	
	%		g	t ha^{-1}	%		g	t ha^{-1}
0	0 d[b]	0 c	54.2 a	12.3 a	0 d	0 c	80.4 a	7.8 ab
7	19 c	26 b	42.0 ab	8.0 b	0 d	5 c	77.9 a	10.1 a
14	58 b	43 b	25.9 bc	5.2 bc	59 c	41 b	36.1 b	8.0 ab
28	58 b	74 a	13.6 cd	2.3 cd	74 b	61 ab	29.0 b	6.0 bc
42	80 a	86 a	6.3 d	0.7 d	69 bc	46 ab	21.5 b	7.3 ac
56	88 a	88 a	6.6 d	0.4 d	85 a	78 ab	24.6 b	3.9 c

[a] Abbreviations: DAP, days after planting
[b] Means followed by the same letter in each column are not significantly different according to Fisher's protected LSD test (P<0.05).

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Table 3.8. Effect of mesotrione soil residues on injury, plant dry weight and yield of pea, one year after mesotrione application to corn (conventional residue carryover) and mesotrione applied before planting (simulated residue carryover). Data for 2006 and 2007 is combined.

Conventional Residue Carryover

Mesotrione g ai ha⁻¹	Injury 21 DAP[a]	Injury 39 DAP	Dry weight 40 DAP	Yield
	—— % ——		g	t ha⁻¹
0	0 c[b]	0 c	76.5 ab	5.4 ab
70	1 c	4 bc	79.2 ab	6.5 ab
140	0 c	1 bc	89.7 a	7.0 a
280	24 a	18 ab	72.0 ab	5.8 ab
420	49 b	29 a	57.1 b	5.3 ab
560	43 b	28 a	63.6 ab	5.1 b

Simulated Residue Carryover

Mesotrione g ai ha⁻¹	Injury 21 DAP	Injury 39 DAP	Dry weight 40 DAP	Yield
	—— % ——		g	t ha⁻¹
0	0 a	0 a	72.5 ac	7.0 a
7	1 a	1 ab	78.8 c	6.1 ab
14	11 a	3 b	80.6 c	7.5 a
28	46 b	11 c	60.6 acd	5.7 ab
42	66 c	21 cd	48.8 ab	4.3 b
56	73 c	40 d	35.4 bd	3.7 b

[a] Abbreviations: DAP, days after planting

[b] Means followed by the same letter in each column are not significantly different according to Fisher's protected LSD test (P<0.05).

Table 3.9. Effect of mesotrione soil residues on injury, plant dry weight and yield of soybean, one year after mesotrione application to corn (conventional residue carryover) and mesotrione applied before planting (simulated residue carryover). Data for 2006 and 2007 is combined.

	Conventional Residue Carryover					Simulated Residue Carryover			
	Injury		Dry weight	Yield		Injury		Dry weight	Yield
Mesotrione g ai ha^{-1}	21 DAP[a]	39 DAP	40 DAP		Mesotrione g ai ha^{-1}	21 DAP	39 DAP	40 DAP	
	—— % ——		g	t ha^{-1}		—— % ——		g	t ha^{-1}
0	0 a[b]	0 d	45.7 ab	5.3 a	0	0 a	0 a	51.4 a	4.5 a
70	2 ac	6 bcd	42.5 b	4.2 a	7	3 a	2 a	48.4 a	4.7 a
140	2 a	4 cd	58.0 a	4.2 a	14	6 a	2 a	48.9 a	5.1 a
280	6 bc	13 bc	47.4 ab	4.7 a	28	28 b	10 b	42.0 a	5.3 a
420	10 b	19 ab	54.3 ab	4.9 a	42	38 b	12 b	38.5 a	5.3 a
560	18 b	26 a	45.3 ab	5.4 a	56	35 b	10 b	41.9 a	5.1 a

[a] Abbreviations: DAP, days after planting

[b] Means followed by the same letter in each column are not significantly different according to Fisher's protected LSD test (P<0.05).

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Table 3.10. Mesotrione residues (μg kg^{-1}) from chemical analaysis of soil samples taken one year after mesotrione application to corn.

Mesotrione	Mesotrione Residues[a]	
g ai ha^{-1}	2006	2007
	μg kg^{-1}	
0	0.00 c[b]	0.00 d
70	1.18 c	0.43 cd
140	1.58 c	0.93 bcd
280	3.55 bc	2.80 ab
420	6.75 ab	2.40 abc
560	8.83 a	3.65 a

[a]Chemical analysis method was by liquid chromatography–tandem mass spectrometry/ (LC-MS/MS).

[b]Means followed by the same letter in each column are not signficantly different according to Fisher's protected LSD test (P<0.05).

4.0 GREENHOUSE BIOASSAY TO DETERMINE CROP RESPONSE TO MESOTRIONE RESIDUES

4.1 Abstract

A whole- plant bioassay was developed, using sugar beet, lettuce, cucumber, green bean, pea and soybean as test crops to determine injurious soil residual concentrations of mesotrione. Two greenhouse bioassay studies were conducted in 2006 and 2007 at the Simcoe Research Station, Simcoe, Ontario. The test crops were planted into soil treated with mesotrione in the field the previous year at doses of 0 to 560 g ai ha^{-1} and untreated soil from the same field, spiked with mesotrione concentrations of 0 to 320 µg kg^{-1}. Experiments were conducted in the greenhouse for a 21-day period. Whole plant dry weight (shoot and root) response was determined and expressed as percent of mean controls. I$_{50}$ values were predicted using a log- logistic non-linear regression model. Injury and plant dry weight reduction in the greenhouse was also compared to the injury and yield reduction in the field. I$_{50}$ values of 9.5 (se ± 2.0), 16.4 (se ± 2.2), 32.8 (se ± 12.1), 45.9 (se ± 8.1), 58.4 (se ± 7.0) and 74.9 (se ± 33.4) g ai ha^{-1} for sugar beet, lettuce, green bean, cucumber, pea and soybean respectively, indicate that these crops were effective bioassay test species for quantifying mesotrione residues.

Nomenclature: Mesotrione, 2-(4-methylsulfonyl-2-nitrobenzoyl)-1,3-cyclohexanedione; soybean, *Glycine max* Merr. L.; green bean, *Phaseolus vulgaris* L; pea , *Pisum sativum* L.; cucumber, *Cucumis sativus* L.; sugar beet, *Beta vulgaris* L., leaf lettuce, *Lactuca sativa* L.

Key words: Mesotrione, greenhouse bioassay, residue carryover, I$_{50,}$ dose-response curves.

4.2 Introduction

Greenhouse bioassays are an alternative to field bioassays where low concentrations of residual herbicides can be detected and results available in a shorter time period than in the field. Soil bioassays have been used to detect different herbicide residues, in particular sulfonylureas (Eliason et al. 2004; Geisel et al. 2008; Hernandez-Sevillano et al. 2001; Stork and Hannah 1996; Sunderland et al. 1991). O'Sullivan (2005) developed a grower-friendly greenhouse bioassay to determine the effect of imazethapyr soil residues on vegetable crops. There are fewer published details on using a greenhouse bioassay to detect mesotrione residues. Maeghe et al. (2002) used a greenhouse bioassay to detect mesotrione, sulcotrione and isoxaflutole residues in different soil types. Sugar beet and red clover were regarded as extremely sensitive to these herbicides and were selected as the test species for their bioassay.

Mesotrione is applied at relatively low doses compared to traditional herbicides (g ai ha^{-1} compared to kg ai ha^{-1}), and undergoes soil dissipation in a manner similar to other herbicides by following first order kinetics. However, soil residues of this herbicide can remain to cause significant biological and economic damage to highly sensitive crop species when grown in rotation. Dose-response curves are often used to quantify plant sensitivity to a herbicide (Seefeldt et al. 1995) and can be developed from herbicide bioassays. Usually the biological response is explained by nonlinear regression models, which has an upper limit, defined by a very low herbicide dose, a lower limit, defined by a very high or lethal herbicide dose, and the midpoint between these two points, I_{50} (dose giving 50% response) (Streibig and Kudsk 1993). Doses near the I_{50}, the range in which the greatest plant response occur, should allow for shifts in the curve from one experiment to another (Seefeldt et al. 1995). Assuming that the dose-response is sufficiently described by the logistic curve and that the I_{50} value is a proper measurement

of the potency of the herbicide, dose-response curves can be compared by using the I_{50} values (Nielsen et al. 2004).

The objective of this study was to develop a rapid, whole-plant bioassay to accurately detect plant-available concentrations of mesotrione residues in soil that are injurious to sensitive crops grown in rotation. This bioassay can then be used to quantify the concentration of mesotrione residues in the soil relative to their impact on the growth of a range of crop species, such as sugar beet, lettuce, cucumber, green bean, pea and soybean. Two greenhouse bioassays were conducted using 1) soil treated with mesotrione in the field the previous year and 2) untreated soil from the same field, "spiked" with known concentrations of mesotrione.

4.3 Materials and Methods

Two greenhouse bioassay studies were conducted over a two year period (2006 and 2007) at the University of Guelph, Simcoe Research Station, Simcoe, Ontario, Canada. One study consisted of spiking untreated soil from the field with known concentrations of mesotrione. The second study sampled soil, at two different depths, treated with mesotrione in the field the previous-year, as previously described in Chapter 3. The experiment area was cultivated, fertilized, planted with field corn and mesotrione was applied as previously described for the conventional residue carryover study in Chapter 3 (3.2.1). For both years of the study, the soil was a fine sandy loam (Wattford) consisting of 70, 25 and 5 percent sandy, silt and clay respectively. In 2006 the pH of the soil was 6.0, organic matter 1.7%, and CEC 6.1. In 2007 the pH of the soil was 6.7, organic matter 1.6% and CEC 5.4.

For the spiked-soil greenhouse bioassay, eight cored soil samples were taken from untreated areas of the field. For the greenhouse bioassay, based on the previous-year

mesotrione application, eight cored soil samples were collected from the untreated and

the mesotrione treated field plots at the 0-15 cm depth and at the 0-7.5 cm depth. Soil

samples were taken on May 16, 2006 and May 17, 2007. For both bioassays, large

pieces of organic matter were removed from the soil by hand. The soil was then

screened through a 5-mm sieve. The soil was left to dry at room temperature for 48

hours. Soil field capacity was determined by using methods similar to Eliason et al.

(2004). Dried soil (100 g) was placed in filter paper and suspended above a small glass

cup. Water was continuously added to completely wet the soil without leaving standing

water in the bottom of the cup. The saturated soil was then weighed. Soil field capacity

was calculated as the ratio of the weighed:

$$\frac{\text{saturated soil} - \text{dry soil}}{\text{dry soil}} \text{ x } 100\%$$

The soil field capacity was calculated to ensure that each experimental unit for the

greenhouse bioassays was maintained at 80% field capacity.

Stock solutions for the spiked-soil bioassay were prepared using the commercial

formulation of mesotrione (480 g ai L^{-1} of solution) by adding 52.1 µL of the formulation

in 0.05 L of water, yielding a mesotrione concentration of 0.5 g L^{-1}. Stock solutions were

left at room temperature for no more than 24 hours before being applied to the soil. In

order to spike the untreated soil collected from the field at mesotrione concentrations of

0, 1, 5, 10, 20, 40, 80, 120, 160, 320 µg kg^{-1}, the following amounts were transferred

from the stock solution and added to 100 mL- volumetric flasks; 0, 2, 10, 20, 40, 80, 160,

240, 320 and 640 µL. Water was added to the mesotrione solution to reach a total

volume of 100 mL. One kilogram of soil from the untreated field plots was laid out evenly

on a tray lined with a plastic bag for each mesotrione dose. The soil was spiked by

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slowly adding each solution using a Gustafson® Batch Lab Treater. The bag, with soil

lying on top, was then turned inside out to ensure the soil was sealed inside the bag. It

was mixed thoroughly by hand for five minutes. The soil was left sealed in the bags for

14-16 hours at room temperature. Soil was again mixed thoroughly by hand.

The experiment design for both greenhouse bioassays was a randomized complete

block design with four replicates per species for each dose. The test species chosen for

evaluation were: sugar beet (*Beta vulgaris* L. 'Crystal 963'); lettuce (*Lactuca sativa* L.

'Lasting Green M.I.'); cucumber (*Cucumis sativus* L. 'Vlaspik'); green bean (*Phaseolus

vulgaris* L. 'Matador'); pea (*Pisum sativum* L. 'Durango') and soybean (*Glycine max* (L.)

Merr. 'S15-B1'). Mesotrione concentrations varied with test crop. Based on 2006 results,

concentrations were adjusted in 2007 (Table 4.3). To compare the greenhouse bioassay

mesotrione concentrations to field concentrations, $\mu g \ kg^{-1}$ was converted to g ai ha^{-1}

using the following equation:

$$\frac{x \text{ g ai mesotrione}}{ha} \times \frac{ha}{1 \times 15^{9} cm^{3}} \times \frac{1 \times 10^{6} \mu g}{g} \times \frac{cm^{3}}{0.0015 \text{ kg}} = x \ \mu g \ kg^{-1} \text{ soil}$$

[Eq. 1]

This equation was derived from Geisel (2007). 1×15^{9} cm^{3} is based upon mesotrione

remaining in the top 15 cm of the soil and application rates applied to soil based upon a

soil bulk density of 1.5 g cm^{-3}.

For both bioassays, 150 g of treated soil (either spiked with mesotrione or with

mesotrione applied the previous-year) was added to labeled 250 mL Styrofoam® cups.

Seeds of the test crops were then planted into the soil (15 seeds for sugar beet and

lettuce, 5 seeds for cucumber, pea, green bean and soybean). Twenty grams of the

treated soil and 10 g of turface (to prevent rapid evaporation) were added to cover the seeds. A double Styrofoam® cup technique was used in which the inside cup (holding the soil and seeds) had five small holes to allow drainage, and the outside cup captured leachate from the inside cup. Water was then added to a total weight (cups, soil, turface and seeds) of 240 g. This total weight was determined by measuring the total of the cups, turface, seeds and soil at 80% field capacity in the cups. The cups were placed randomly into Fiber Grow Garden Paks® (to prevent cup tipping) and placed into the greenhouse. Each cup was individually weighed on a daily basis and watered up to the 240 g total weight to maintain 80% field capacity. Once per week the plants were watered with nutrient solution (20% N, 8% P_2O_5, 20% K_2O and micronutrients, 3 g L^{-1}) to ensure that fertility was not a limiting factor in plant growth. The cups were re-randomized after each watering. Upon plant emergence, sugar beet and lettuce seedlings were thinned to five per cup and No-Damp® (3.2% oxine benzoate) was applied to all crops at 10 mL per 1 L of water to prevent damping off diseases.

Visible crop injury ratings were made on each crop for both experiments using a 0 to 100% scale, where 0 = no effect and 100 = crop death. Injury ratings were taken at 17 days after planting (DAP). Plant shoot and roots were harvested at 21 DAP by gently removing the plant from the soil and then gently washing the roots under a stream of water. The shoots and roots were patted dry and weighed. The plants were then placed into a paper bag and dried at 70°C and then weighed.

All data were subjected to analysis of variance (ANOVA) using the MIXED procedure of SAS statistical software (Ver. 9.2, SAS Inst., Cary, NC). The variances were partitioned into the fixed effects of mesotrione dose and into the random effects of year and replicated block-by-year interaction. For the greenhouse bioassay in which two soil sample depths were used, data was also analyzed using the MIXED procedure of SAS

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where variances were partitioned into the fixed effects of mesotrione dose and into the random effects of soil sample depth and the interaction between soil sample depth and mesotrione dose, and block-by-soil sample depth interaction. The assumptions of the variance analysis were tested ensuring that the residuals were random, homogeneous, with a normal distribution about a mean of zero using residual plots and the Shapiro-Wilk normality test. To meet assumptions of the variance analysis, green bean dry matter for the spiked soil greenhouse bioassay was subjected to a log transformation. Means were back-transformed to the original scale for presentation of results. When the effects of year and the interaction with mesotrione dose were not significant after analysis the data were pooled by year. The effect of soil sample depth and the interaction with mesotrione dose was not significant, however, because there was a year by mesotrione dose interaction for most crops at the 0-7.5 cm soil depth study, data for both depths was not pooled. Results presented are from data taken from the 0-15 cm depth based on the study by Rouchaud et al. (2000) who found mesotrione present in the 10-15 cm soil layer.

In the spiked-soil greenhouse bioassay, several doses were decreased from 2006 to 2007 for all crops except for sugar beet and lettuce because crop sensitivity was greater than expected. Thus this data could not be pooled and is reported by year. Mesotrione dose means were separated using Fisher's protected LSD. The Type I error was set at 0.05 for all statistical comparisons.

An experimental unit was a single Styrofoam® cup consisting of five plants of each crop. The average weight of the four untreated experimental units for each crop species was calculated. The percent growth response relative to the mean weight of untreated plants was determined for each experimental unit, as suggested by Streibig and Kudsk (1993). To assess crop sensitivity to mesotrione and estimate the dose of mesotrione

required for 50% (I_{50}) crop response (50% dry plant weight reduction), regression of crop dry matter over mesotrione dose was performed using the log-logistic model described by Seefeldt et al. (1995).

$$y = C + \frac{D - C}{1 + \exp[b(\log(X) - \log(I_{50}))]} \quad \text{[Eq.2]}$$

Where y is crop dry matter as a percent of the mean untreated control, C is the lower limit, D is the upper limit, b is the slope of the, I_{50} is the dose giving 50% response and X is the herbicide dose. The curve described by Eq. 2 is sigmoidal in shape, using a logarithmic scale on the x-axis for herbicide dose and using a linear scale on the y-axis for the dry plant weight as a percent of the mean of the untreated control. The regression parameters were generated using PROC NLIN in SAS and dose-response curves were produced using these parameters in Sigma Plot (Ver. 10.0 for Windows, Systat Software Inc., Point Richmond, CA). SAS statements are summarized in the Appendix.

4.4 Results and Discussion

4.4.1 *Greenhouse Bioassay (using field samples of mesotrione treated soil)*

Since there was a year by treatment interaction for some rating parameters for some crops, the 2006 and 2007 data are presented separately. Figure 4.1 shows the visual response of all test crops to mesotrione concentrations ranging from 0 to 560 g ai ha^{-1} at 21 DAP, just prior to shoot/root harvest.

In 2006, sugar beet showed the highest level of injury, due to mesotrione residues, among all crops evaluated (Table 4.1). There was injury of 8 to 100%, compared to the untreated check for mesotrione applied at 70 to 560 g ai ha^{-1} in the previous year. Injury

in lettuce ranged from 8 to 89%, green bean from 0 to 79%, cucumber from 0 to 43%,

pea from 3 to 38% and soybean from 0 to 25%, over the same range of mesotrione

application doses. In 2007, sugar beet also showed the highest level of injury, due to

mesotrione residues (Table 4.1). There was injury of 23 to 100% compared to the

untreated check, for mesotrione applied at 70 to 560 g ai ha^{-1} in the previous year. Injury

in lettuce ranged from 5 to 99%, green bean from 0 to 60%, cucumber from 0 to 55%,

pea from 0 to 33% and soybean had 0%, over the same range of mesotrione application

doses. Injury levels followed the same pattern with respect to application dose in both

years. In 2007, injury levels were higher in the most sensitive crops (sugar beet and

lettuce) but lower in the other crops, compared to 2006.

In 2006, sugar beet showed the highest level of plant dry weight reduction, due to

mesotrione residues, among all crops evaluated (Table 4.2). There was plant dry weight

reduction of 25 to 100%, compared to the untreated check, for mesotrione applied at 70

to 560 g ai ha^{-1} in the previous year. Plant dry weight reduction in lettuce ranged from 15

to 94%, green bean from 6 to 75%, cucumber from 0 to 55%, pea from 9 to 51% and

soybean from 6 to 7%, over the same range of mesotrione application doses. In 2007,

sugar beet also showed the highest level of plant dry weight reduction, due to

mesotrione residues, among all crops evaluated (Table 4.2). There was plant dry weight

reduction of 0 to 100%, compared to the untreated check, for mesotrione applied at 70 to

560 g ai ha^{-1} in the previous year. Plant dry weight reduction in lettuce ranged from 0 to

100%, green bean from 14 to 77%, cucumber from 0 to 36%, pea from 2 to 50% and

soybean from 1 to 6%, over the same range of mesotrione application doses. Plant dry

weight reduction levels were very similar and followed the same pattern with respect to

crop sensitivity and application dose in both years.

Log-logistic models were tested for each crop and experiment using the plant dry weight as a percent of the untreated check. However only sugar beet and green bean for the 2006 experiment could be described by the models in which convergence was met and parameters estimated. These two crops were the most sensitive to mesotrione residues and had a greater response to the mesotrione doses used in this study, whereas the other crops did respond to the increasing doses but to a lesser degree. Despite this, the dose-response curves for sugar beet and green bean for the 2006 experiment are useful for indicating plant sensitivity to mesotrione and to compare plant response observed in the greenhouse to plant response observed in the field which was discussed in Chapter 3. This greenhouse/field relationship will be discussed in 4.4.3.

4.4.2   *Greenhouse Bioassay (soil spiked with mesotrione of known concentrations)*

Results in Table 4.3 indicate differences in crop sensitivity to mesotrione. The spiked bioassay was designed specifically to develop dose-response curves for each of the crops, thus different doses of mesotrione were applied to each crop. Log-logistic models were tested for each crop and experiment using the dry plant weight as a percent of the untreated check. Doses from the 2006 experiment were adjusted for the 2007 experiment, and thus data could not be pooled for analysis. Dose-response curves were developed for each crop based on the experimental data that fit the models the best. For example, the parameters produced from the nonlinear regression for pea in 2006 showed less standard error than the parameters produced from the 2007 data. The parameters estimated by nonlinear regression in the fitted model are presented in Table 4.4. I_{50} values, the dose of mesotrione in g ai ha^{-1} resulting in a plant dry weight response of 50%, indicated that sugar beet were the most sensitive (9.5), followed by lettuce (16.4), green bean (32.8), cucumber (45.9), pea (58.4) and soybean (74.9).

4.4.3 Greenhouse bioassay related to field residue carryover study

The response of several crops grown in the field, previously treated with mesotrione (conventional residue field carryover study described in Chapter 3), was compared to the response of these crops grown in the greenhouse, in soil sampled from the field previously treated with mesotrione (greenhouse bioassay described in 4.4.1). The sugar beet dose-response curves for the conventional residue carryover study and the greenhouse bioassay study completed the sigmodial pattern over the range of concentrations used and both curves are similar in shape. The greenhouse bioassay relates dry matter as a percent of the untreated mean whereas the conventional field residue carryover study relates marketable yield as a percent of the untreated mean. The greenhouse bioassay dose-response curves indicate slightly more sensitivity in the sugar beet at the higher and lower end of the concentration ranges compared to the conventional residue carryover dose-response curve, but the two curves converge approximately at the I_{50} value (Figure 4.2). The green bean dose-response curves for the conventional field residue carryover study and the greenhouse bioassay study also completed the sigmodial pattern over the range of concentrations used (Figure 4.3). Similar to the sugar beet dose-response curves, the greenhouse bioassay dose-response curves indicated more sensitivity in green bean at the lower end of the concentration range compared to the conventional field residue carryover dose-response curve. However, at the higher end of the concentration range, the greenhouse bioassay and the conventional residue carryover curves indicate similar sensitivity.

4.5 Conclusions

Sugar beet and lettuce were effective test species for mesotrione residue detection. No important difference between these test species was observed, suggesting that either would be appropriate in whole-plant bioassay studies for the detection of residues of this herbicide. The bioassay system described here, conducted over a 21-day period, exposes the test species to only plant-available herbicide concentrations. Dose-response curves developed for sugar beet and green bean indicate similarities between results from the greenhouse bioassays and results in the conventional field mesotrione carryover study. As the curves show, the bioassay overestimates the concentration of herbicide present probably because the impact of soil and environmental factors that influence adsorption and herbicidal degradation were unlikely to be identical to those experienced under field conditions. Maintaining soils at field capacity with optimal temperature is likely to result in greater bioavailability of the test herbicides in the greenhouse compared to field conditions because of desorption from soil binding sites. Although sugar beet and lettuce proved to be highly sensitive test species, their value in real world situations may be limited. Cucumber, green bean, pea and especially soybean crops are widely grown in Ontario and are typically grown in rotation with corn. These crops show the correlation between the responses of highly sensitive species and commonly grown crops that exhibit sensitivity to mesotrione residues.

The whole-plant bioassay system, using untreated field soil, spiked with known concentrations of mesotrione, was able to detect low concentrations of mesotrione in soil. The test species used exhibited a range of responses to mesotrione residues. The responses were reflective of results from other bioassay studies and were well represented by the log-logistic regression model (Seefeldt et al. 1995) based on the fit to the observed data. This bioassay methodology was therefore, appropriate for

examination of soil residues of mesotrione with the test species used. Based on the

predicted I_{50} values determined in these experiments, this bioassay system can detect

mesotrione at concentrations of less than 1.0 µg kg^{-1}.
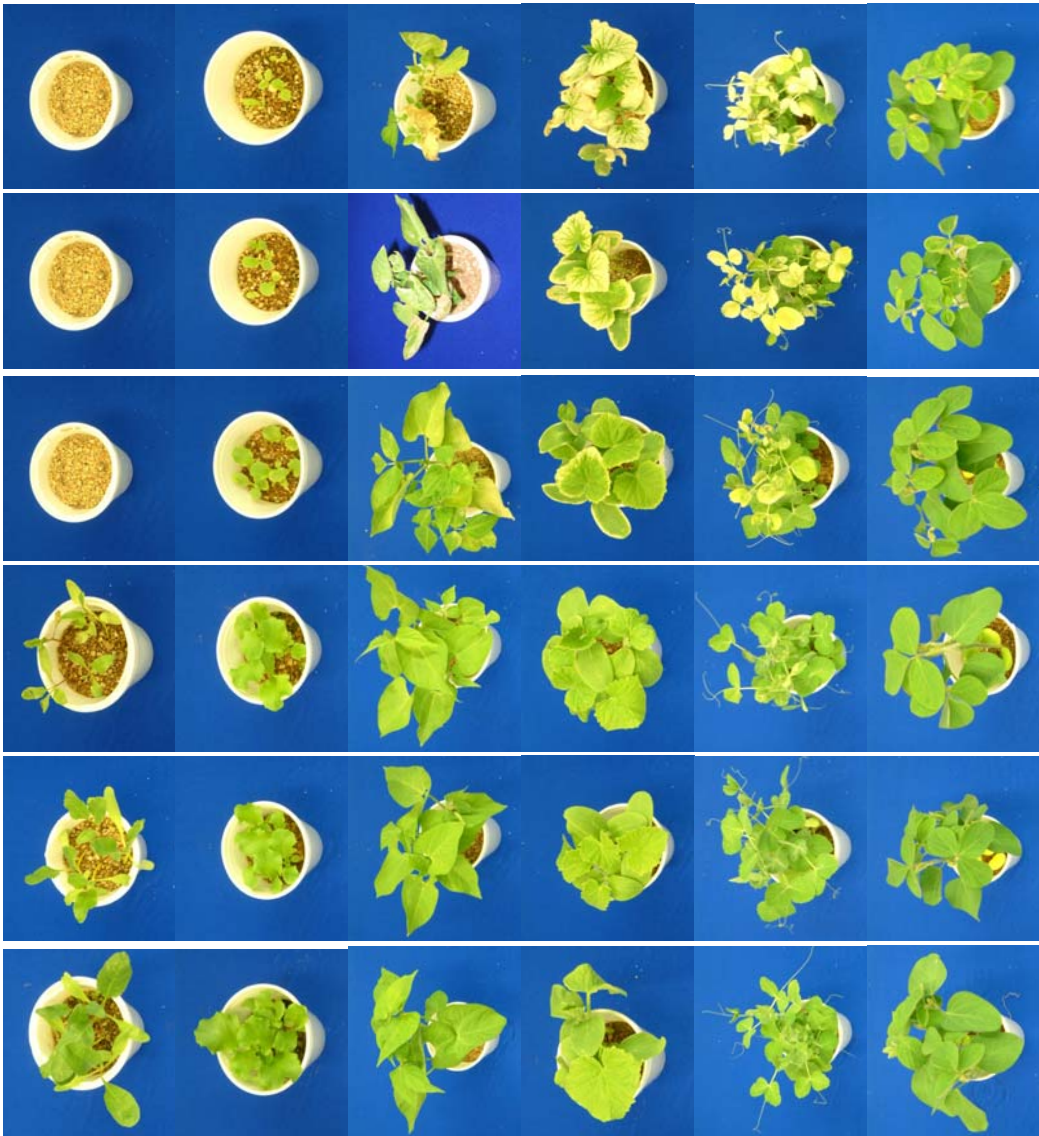
Figure 4.1. Photographs of, from top to bottom, sugar beet, lettuce, green bean, cucumber, pea and soybean injury response to mesotrione residues 21 days after planting. Crops were greenhouse-grown in field soil treated with mesotrione the previous year at concentrations of, from left to right, 0, 70, 140, 280, 420, 560 g ai ha^{-1}.

Table 4.1. Effect of mesotrione soil residues on visual injury (%) to greenhouse-grown crops in 2006 and 2007, one year after mesotrione application to corn.

Mesotrione g ai ha^{-1}	Injury - 2006					
	sugar beet	lettuce	green bean	cucumber	pea	soybean
				%		
0	0 a[a]	0 c	0 d	0 b	0 c	0 a
70	8 a	8 bc	0 d	0 b	3 c	0 a
140	15 a	8 bc	8 cd	0 b	4 bc	1 a
280	78 b	31 bc	18 c	3 b	13 b	13 b
420	100 b	89 a	63 a	41 a	38 a	25 c
560	94 b	83 a	79 a	43 a	35 a	25 c

	Injury - 2007					
	sugar beet	lettuce	green bean	cucumber	pea	soybean
				%		
0	0 a	0 c	0 b	0 b	0 b	0 a
70	23 b	5 c	0 b	0 b	0 b	0 a
140	83 c	6 c	0 b	0 b	0 b	0 a
280	99 d	48 b	4 b	3 b	2 b	0 a
420	100 d	89 a	26 b	6 b	3 b	0 a
560	100 d	99 a	60 a	55 a	33 a	0 a

[a] Means followed by the same letter in each column are not significantly different according to Fisher's protected LSD test (P<0.05).

Table 4.2. Effect of mesotrione soil residues on plant dry weight (g) to greenhouse-grown crops in 2006 and 2007, one year after mesotrione application to corn.

Mesotrione g ai ha^{-1}	Plant Dry Weight - 2006					
	sugar beet	lettuce	green bean	cucumber	pea	soybean
	g					
0	0.51 a[a]	0.32 a	2.36 a	1.60 a	1.84 a	2.02 a
70	0.36 a	0.27 ab	2.21 a	1.64 a	1.68 ab	2.48 b
140	0.38 a	0.27 ab	1.59 b	1.94 a	1.37 bc	1.90 a
280	0.07 b	0.17 b	1.45 b	1.37 ab	1.31 cd	2.04 ab
420	0.00 b	0.02 c	0.82 c	0.73 b	1.00 de	1.88 a
560	0.02 b	0.05 c	0.58 c	0.77 b	0.90 e	1.91 a

	Plant Dry Weight - 2007					
	sugar beet	lettuce	green bean	cucumber	pea	soybean
	g					
0	0.48 a	0.70 ab	2.97 a	1.43 cd	1.82 ab	2.30 a
70	0.70 a	1.04 a	2.43 abc	2.33 ab	1.84 ab	2.41 a
140	0.05 b	0.56 bc	2.54 ab	2.88 a	1.94 a	2.38 a
280	0.03 b	0.23 cd	1.47 cd	1.74 bc	1.78 ab	2.15 a
420	0.00 b	0.12 d	1.52 bcd	1.76 bc	1.42 b	2.22 a
560	0.00 b	0.00 d	0.69 d	0.91 d	0.91 c	2.26 a

[a] Means followed by the same letter in each column are not significantly different according to Fisher's protected LSD test ($P<0.05$).

Table 4.3. Visual injury (%) and plant dry weight (g) in six crop species grown in a greenhouse bioassay using field soils spiked with known concentrations of mesotrione in 2006 and 2007.

Mesotrione	2006		Mesotrione	2007	
µg kg^{-1}	Injury	Dry Wt	µg kg^{-1}	Injury	Dry Wt
	— % —	— g —		— % —	— g —
Sugar beet					
0	0 a[a]	0.78 a	0	0 a	0.66 a
1	9 b	0.64 ab	1	99 b	0.00 b
5	9 b	0.48 b	5	100 b	0.00 b
10	100 c	0.00 c	10	86 b	0.03 b
20	100 c	0.00 c	20	100 b	0.00 b
40	100 c	0.00 c	40	100 b	0.00 b
Lettuce					
0	0 a	0.43 a	0	0 a	0.62 a
1	0 a	0.63 b	1	74 b	0.12 b
5	0 a	0.46 a	5	70 b	0.12 b
10	73 b	0.11 c	10	23 a	0.31 a
20	93 c	0.02 c	20	100 b	0.00 b
40	100 c	0.00 c	40	100 b	0.00 b
Cucumber					
0	0 a	1.86 a	0	0 a	1.20 a
40	93 b	0.21 b	10	6 a	1.09 a
80	100 c	0.00 b	20	70 b	0.43 b
120	100 c	0.00 b	40	75 b	0.43 b
160	100 c	0.00 b	80	100 c	0.00 c
200	100 c	0.00 b	120	100 c	0.00 c
Pea					
0	0 a	3.13 a	0	0 a	1.06 a
40	9 b	0.92 b	10	0 a	1.11 a
80	9 b	0.52 c	20	0 a	1.14 a
120	100 c	0.00 d	40	19 b	1.00 a
160	100 c	0.00 d	80	89 c	0.24 b
200	100 c	0.00 d	120	98 c	0.00 b
Green bean					
0	0 a	4.32 a	0	0 a	2.19 a
40	39 b	1.76 b	20	59 b	0.90 b
80	66 c	1.66 b	40	83 c	0.34 c
160	81 d	1.43 c	80	93 cd	0.35 c
320	95 e	0.00 d	160	99 d	0.05 c
			320	99 d	0.00 c
Soybean					
0	0 a	2.31 a	0	0 a	3.37 a
40	15 b	1.46 b	20	0 a	2.40 b
80	40 c	1.11 c	40	0 a	2.39 b
160	60 d	1.12 c	80	25 b	1.63 c
320	75 e	0.98 c	160	40 c	1.30 d
			320	60 d	1.41 d

[a] Means followed by the same letter in each column are not signficantly different according to Fisher's protected LSD test (P<0.05).

Table 4.4. Estimated regression parameters for plant response curves for greenhouse bioassay studies using field soils spiked with known mesotrione concentrations conducted in 2006 and 2007 determined by the dry plant weight for each test species.

Crop	year[a]	Regression Parameter Estimates (±SE)				
		c^b	d^c	b^d	I_{50}^e	R^2
Sugar beet	2006	0.0 (8.5)	100.7 (8.5)	0.2 (0.04)	9.5 (2.0)	0.85
Lettuce	2006	1.8 (8.6)	119.1 (8.1)	4.6 (1.6)	16.4 (2.2)	0.93
Green bean	2007	0.0 (10.9)	100.1 (8.5)	1.3 (0.9)	32.8 (12.1)	0.97
Cucumber	2007	0.0 (9.0)	103.0 (6.8)	1.8 (0.5)	45.9 (8.1)	0.99
Pea	2006	0.0 (11.0)	100.0 (2.1)	1.1 (0.4)	58.4 (7.0)	0.91
Soybean	2007	33.3 (12.6)	99.5 (5.7)	1.1 (0.6)	74.9 (33.4)	0.84

[a] Different rates were used for each of the two years of this study, thus data not pooled.

[b] c, lower limit; [c] d, upper limit; [d] b, slope

[e] I_{50}, dose of mesotrione (g ai ha^{-1}) resulting in plant dry weight reduction of 50%.
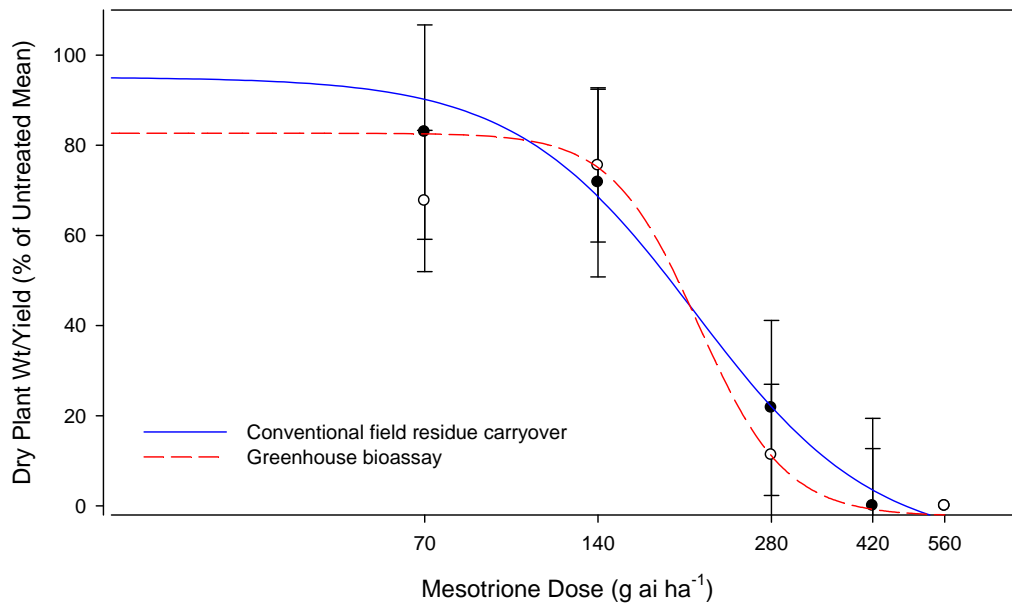
Figure 4.2. Response curves for plant dry weight (wt) and yield, of sugar beet, as a percent of the untreated control. Sugar beet was grown in 2006 in the field and in the greenhouse, in soil treated with mesotrione the previous year. Each data point represents mean ± SE. Dose-response curves are based on the following regression parameter estimates (±SE); c, lower limit = 0 (21.4); 0.6 (9.4), d, upper limit = 96.8 (12.0); 85.6(8.3), b, slope = 2.9 (1.8); 5.7 (3.6) and I_{50}, dose of mesotrione (g ai ha^{-1}) resulting in a dry plant weight or yield reduction of 50% = 201.4 (55.1); 208.9 (43.8) for the conventional field residue carryover and greenhouse bioassay respectively.
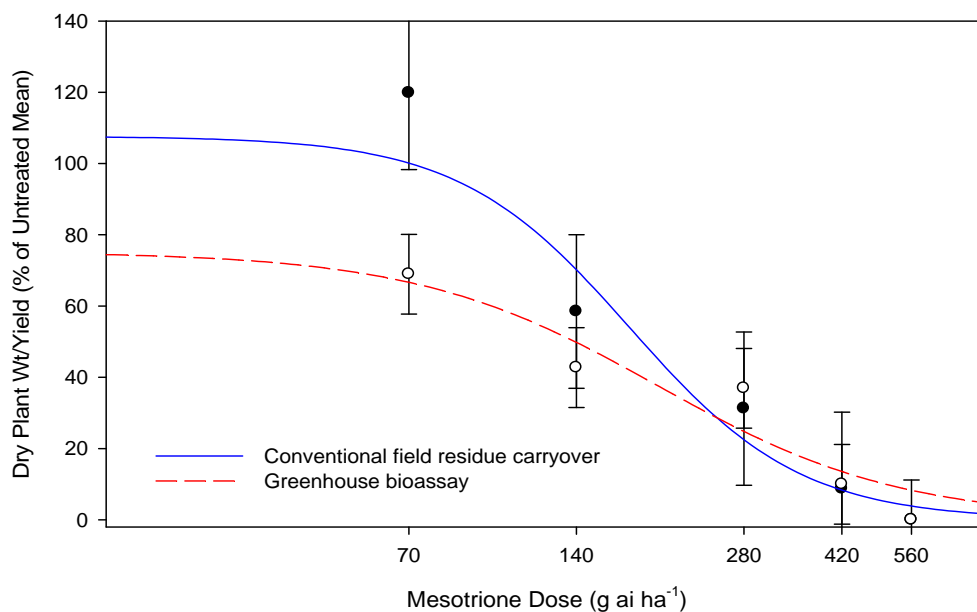
Figure 4.3. Response curves for plant dry weight (wt) and yield, of green bean, as a percent of the untreated control. Green bean was grown in 2006 in the field and in the greenhouse, in soil treated with mesotrione the previous year. Each data point represents mean ± SE. Dose-response curves are based on the following regression parameter estimates (±SE); c, lower limit = 0 (20.9); 0 (8.5), d, upper limit = 107.7 (17.2); 75.2 (6.4), b, slope = 2.8 (1.6); 2.0 (0.9) and I_{50}, dose of mesotrione (g ai ha^{-1}) resulting in a dry plant weight or yield reduction of 50% = 174.8 (47.7); 196.1 (52.0) for the conventional field residue carryover and greenhouse bioassay respectively.

5.0 GENERAL DISCUSSION AND CONCLUSION

Two field residue studies were conducted from 2005 to 2007 to evaluate the effects of mesotrione soil residues on injury, plant dry weight and yield of sugar beet, cucumber, pea, green bean and soybean and to verify the potential of reducing a two-year field residue study (conventional residue carryover study) to a one-year field study (simulated residue carryover study) by growing these crops in soil treated with reduced doses of mesotrione applied in the same year. Two whole-plant, greenhouse bioassays, using sugar beet, lettuce, cucumber, green bean, pea and soybean as test crops, were used to determine soil residual concentrations of mesotrione. Bioassays were conducted using 1) soil treated with mesotrione in the field the previous year and 2) untreated soil from the same field "spiked" with known concentrations of mesotrione. Untreated soil, collected from the field in 2006 and 2007, was spiked with mesotrione at concentrations of 0 to 320 μg kg^{-1}. Whole plant dry weight (shoot and root) response was determined and expressed as percent of control means and I_{50} values were predicted using a log- logistic non-linear regression model.

5.1 Research contributions

This research shows that mesotrione residues can cause injury and yield loss at several mesotrione doses in sensitive crops grown in rotation. Soil factors had a significant effect on mesotrione carryover with differences between years explained by differences in soil moisture and soil pH. Response to mesotrione residues differed between crops. In the field, sugar beet was the most sensitive crop with injury, plant dry weight reductions and yield loss as high as 100%. Green bean was the next most

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sensitive crop. Despite injury on cucumber, pea and soybean there was no yield loss on any of these crops.

This research verified the applicability of using a simulated residue carryover study for determining the carryover potential of mesotrione to sensitive rotational crops. The one-year field bioassay was suitable for all crops except sugar beet, the most sensitive crop. Crop yields were similar in both the conventional and the simulated carryover studies, especially at the lowest doses of mesotrione. Crop yield response to herbicide residues differed in conventional and simulated residue carryover studies only for sugar beet. Sugar beet data suggests that the higher concentration of the freshly applied herbicide has a greater effect on sensitive crops than similar concentration of a herbicide that has been present in the soil for at least a one-year period. This confirms decreased herbicide desorption and bioactivity in soils over time. Differences in the magnitude of the bioavailable fraction or differences in the strength of binding mechanisms following prolonged contact with soil may be responsible for the reduced activity of these herbicides in conventional residue carryover studies compared with simulated residue carryover studies.

In-field bioassays successfully measured mesotrione persistence and rotational crop sensitivity. When these studies are compared with the reported literature it can be concluded that in-field bioassays were successful in measuring persistence of mesotrione and in understanding of rotational crop sensitivity. Under similar environmental conditions and soil characteristics to those observed in these studies, sugar beet, green bean and to a lesser extent cucumber injury, plant dry weight and yield reductions are likely when these crops are grown in soils containing mesotrione residues. In addition, these studies support the current mesotrione label prohibiting re-

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cropping of sensitive crops like sugar beet, green bean and cucumber for two years after mesotrione application.

Generally, injury increased as application dose of mesotrione increased. For all studies conducted, injury on most crops was more severe in 2006 than in 2007. The adsorption of mesotrione was affected by soil pH and soil moisture. Increased crop injury in 2006, compared to 2007 could be attributed to higher rainfall in the first weeks following mesotrione application in 2006. The amount of mesotrione carryover from the previous year was measured by chemical analysis and verifies that there was less mesotrione carryover from 2006 to 2007 than from 2005 to 2006. The levels of mesotrione residues in the soil at planting in 2006 and 2007 support observations on the impact of soil moisture and soil pH, in explaining differences between rotational crop response to mesotrione residues between years. This research indicated that soil properties such a soil moisture and soil pH greatly influenced the phytotoxicity of the applied herbicides. Variations in mesotrione adsorption accounted for much of the variation and the degradation on mesotrione and carryover injury to sensitive crops in the rotation. Based on high mesotrione bioavailability in soils of high soil moisture and high pH, there is less risk of carryover injury associated with mesotrione.

Soil bioassays are methods commonly applied in ecotoxicology in the determination of the levels of bioavailable phytotoxic residues of herbicide active ingredients in soil. Results from this research indicate that sugar beet and lettuce are effective greenhouse bioassay test species for mesotrione residue detection and for quantifying mesotrione residues. No important difference between these test species was observed, suggesting that either would be appropriate in whole-plant bioassay studies for the detection of residues of this herbicide. The greenhouse bioassay was found to be a simple and sensitive tool in detecting small amounts of herbicides present in the soil. This bioassay

can be used to quantify the concentration of mesotrione residues in the soil, relative to their impact on the growth of a range of crop species, such as sugar beet, lettuce, cucumber, green bean, pea and soybean. Bioassays have several very important advantages, compared to analytical methods (liquid chromatography, gas chromatography, mass spectrometry, capillary electrophoresis and immunoassays), as they are inexpensive and easy to perform, do not require expensive laboratory equipment and they yield reproducible results.

Dose-response curves developed for sugar beet and green bean indicate similarities between results from the greenhouse bioassay and results from the conventional field mesotrione residue carryover study. The curves indicate that the bioassay overestimates the concentration of herbicide present probably because the impact of soil and environmental factors that influence adsorption and herbicidal degradation were unlikely to be identical to those experienced under field conditions.

The whole-plant bioassay, in which untreated field soil was spiked with known concentration of mesotrione, was successful in detecting low concentrations of mesotrione in soil. The test species used exhibited a range of responses to mesotrione residues. The responses were reflective of results from other bioassay studies and were well represented by the log-logistic regression model based on the fit to the observed data. This bioassay methodology was therefore, appropriate for examination of soil residues of mesotrione with the test species used. Based on the predicted I_{50} values determined in these experiments, this bioassay system can detect mesotrione at concentrations below 1.0 µg kg^{-1}.

This research will help identify the potential for injury to rotation crops following mesotrione application to corn or other crops. For carryover injury to rotational crops to occur, the herbicide must be available to plants at phytotoxic levels, must show sufficient persistence in the soil and the rotational crop must be susceptible to the residual

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concentration of the herbicide remaining in the soil one year after herbicide application. Simulated residue carryover studies are appropriate for determining the potential for injury to sensitive crops grown in the rotation. When compared to a two-year conventional residue carryover study, a one-year simulated residue carryover study provides a more rigorous test of crop sensitivity to herbicide residues, provides more timely results and provides a significant cost savings to researchers, the agricultural chemical and vegetable crop industries. The greenhouse bioassay helps to predict potential herbicide residue problems so the grower can make better decisions about crop rotation, herbicide selection, planting date and other cultural practices. Sugar beet and lettuce were sensitive indicators of injury risk to field grown vegetable crops following mesotrione application to corn. Growers can use this information to minimize risk of crop injury by choosing appropriate rotation crops that suffer little or no yield reduction. Knowing the level of mesotrione residues in the soil, growers have flexibility in crop rotations following mesotrione use on corn. If the bioassay crops were correlated with field results and soil spatial variability is accounted for in sampling, bioassays can be effectively used to predict follow-crop injury. In this way mesotrione herbicide can be used in agriculture by learning to manage the residue problems that it presents.

5.2 Research limitations

The greenhouse bioassay described here, conducted over a 21-day period, exposed the test species to plant-available herbicide concentrations only. This bioassay may overestimate the concentration of herbicide present because the impact of soil and environmental factors that influence adsorption and herbicidal degradation are unlikely to be identical to those experienced under field conditions. The distribution of herbicide between sorbed and solution phases can be affected by changes in soil moisture levels.

Maintaining soils at field capacity with optimal temperature is likely to result in greater bioavailability of the test herbicides in the greenhouse compared to field conditions because of desorption from soil binding sites.

Mesotrione doses in this study were chosen to represent the anticipated dissipation of mesotrione over time with respect to half life of the compound. This simulated residue carryover study is based on 10% of mesotrione remaining in the soil one year after application. This amount may not be appropriate for all soil types and environments or other residual herbicides as it is clearly affected by environmental conditions and varies with year and location.

Herbicide molecules adsorbed are chemically bound within the solid phase and are not absorbed by plants. Under field conditions this balance is constantly disturbed as a result of various actions and through changes in temperature and moisture content of the soil which affects the availability of herbicides to crops. Only a portion of herbicide active ingredient residue found in the soil is available to plants and, depending on the soil and climatic conditions, may be phytotoxic. The phytotoxic affect of mesotrione residues was quantified on the basis of plant injury and reduction of plant dry weight. Limitations of bioassays include the fact that it is impossible to identify the tested active ingredient. Bioassays may be a good supplementation or even an alternative to classical instrumental measurements used in the detection of phytotoxic residue of herbicide active ingredients in the soil.

Several researchers have reported increased persistence and reduced bioactivity of herbicides in a dry season. Under dry soil conditions, enhanced herbicide adsorption makes the herbicides less available for plant uptake. In trying to predict rotational crop response to mesotrione residues, based on this research, it is important to consider the impact of rainfall during the two-month period after application. Due to mesotrione

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dependence on microbial degradation, it persists longer under low soil moisture and low temperature conditions. Lower temperatures reduce plant metabolism and delay detoxification, so greater crop injury has been observed in cooler temperatures than in optimum or above optimum growing conditions.

In this research organic matter was not a variable. However, organic matter appears to be an important soil property that should be considered when attempting to predict the phytotoxicity and persistence of mesotrione residues. The organic matter content is one of the main factors controlling the ability of soils to adsorb mesotrione residues and render them less plant available. Soil pH also plays a role with mesotrione adsorbed at a lower soil pH. Therefore, the effective recropping intervals for mesotrione may be different for different soil types.

5.3 Future research

Future research on simulated, in-field bioassays should focus on contrasting the chemical dissipation and biological response of sensitive crops to herbicide residues. This would allow for a greater understanding of how accurately simulated carryover experiments model the injury potential of herbicides used in a production setting. Future research towards a better understanding of mesotrione persistence is recommended to include bioassays of a wide range of soils to fully understand the implications of mesotrione residue phytotoxicity.

The simulated residue carryover study reported here was based on 10% of mesotrione remaining in the soil one year after application. In this study approximately 3.5% of mesotrione applied in 2005 was carried over to 2006 while approximately 2% of mesotrione applied in 2006 was carried over to 2007. Future simulated residue studies

need a range of rates, based on the best estimate on the amount of the herbicide carried over from one year to the next.

The nature and degree to which newly applied compounds versus aged compounds and their metabolites affect phytotoxicity persistence and interaction in soil needs further research. Research should examine the persistence of mesotrione over time as affected by soil and environmental conditions and the relationship between plant growth observed in the bioassay and carryover injury observed in the field.

Further research should also be conducted on the greenhouse spiked-soil bioassay. Mesotrione concentrations need more refining so that dose-response curves, for various test species, could be created that would accurately estimate bioavailability of mesotrione residues in the field by correlating results from the greenhouse bioassay to results from the field residue carryover studies.

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7.0 Appendix 1: Cucumber grade distribution[1]

Grade Distribution	Size in diameter
Nubs	Any sized misshapen fruit
No. 1	Up to 25 mm
No. 2	25 to 32 mm
No. 3	32 to 41 mm
No. 4	41 to 51 mm
Oversize	greater than 51 mm

[1] Source: Ontario Processing Vegetable Growers and the Farm Products Marketing Act.

8.0 Appendix 2: SAS code to analyze experiments

Summary of SAS Version 9.2 statements used in the analysis reported in Chapters 3
and 4.

Proc MIXED - Example conventional residue carryover study:

Year – 2006 or 2007
Block – 1 to 4
Dose – 0 to 560 gai ha^{-1}

```
proc mixed covtest;
class year block dose;
model yield=block dose/outp=second;
random year block(year) year*dose;
lsmeans dose/pdiff;
run;
```

Residual Analysis – Example conventional residue carryover study:

```
proc glm;
class year block dose;
model yield=year block dose;
output out=second p=pyield r=ryield;
proc plot;
plot ryield*pyield ryield*dose ryield*block/vref=0;
run;

proc univariate normal; var ryield;
proc sort; by dose; run;
proc univariate normal; var ryield; by dose;
run;

proc sort;
by ryield;
run;
proc print;
run;
```

Non-linear regression analayis using proc NLIN – Example greenhouse bioassay using

soil treated with mesotrione the previous year in the field (Seefeldt et al. 1995):

```
proc nlin;
parameters d=100, c=0, i50=70, b=2;
if dose=0 then predict=d;
else predict=c+(d-c)/(1+exp(b*logdose-log(i50))));
model yield=predict;
output out=second p=ppct r=rpct l95m=lower95 u95m=upper95;
proc plot;
plot ppct*logdose='*'yield*logdose='o'/overlay;
plot rpct*ppct/vref=0;
run;
```