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Determination of Thiencarbazone in Soil by Oriental Mustard Root Length Bioassay

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Using an oriental mustard root length bioassay, thiencarbazone bioavailability and dissipation in five Saskatchewan soils was investigated under laboratory conditions. Thiencarbazone bioavailability was assessed at 0 to 3.9 μg ai kg^{-1}. Thiencarbazone concentrations corresponding to 50% inhibition (I_{50} values) obtained from dose-response curves varied from 0.56 to 1.71 μg kg^{-1}. Multiple regression analysis indicated that organic carbon content (P = 0.018) and soil pH (P = 0.017) predicted thiencarbazone bioavailability. Thiencarbazone dissipation was examined in soils incubated at 23 C and moisture content of 85% field capacity. Thiencarbazone half-lives estimated from dissipation curves were 9 to 50 d, and organic carbon content (P = 0.002) and soil pH (P = 0.008) were significant factors affecting thiencarbazone dissipation. Thiencarbazone bioavailability decreases and dissipation rate is slower in Canadian prairie soils of high organic matter content and low soil pH. Because root length of oriental mustard plants also was reduced by ammonium, therefore ammonium-containing or -producing fertilizers can cause false positive results for thiencarbazone soil residues. Canaryseed roots had the same sensitivity to ammonium as oriental mustard roots but were not inhibited by thiencarbazone. Therefore canaryseed root length bioassay was effective in identifying inhibition caused by ammonium toxicity. Use of oriental mustard root and canaryseed root bioassays together can aid in interpreting bioassay results for detection of thiencarbazone residues.

Nomenclature: Thiencarbazone, oriental mustard, *Brassica juncea* (L.) Czern. 'Cutlass'; canaryseed, *Phalaris canariensis* L. 'CDC Togo'.

Key words: Thiencarbazone, oriental mustard root length bioassay, bioavailability, dissipation, ammonium toxicity, canaryseed root length bioassay.

Thiencarbazone is an acetolactate synthase (ALS)-inhibiting herbicide belonging to the sulfonyl-amino-carbonyl-triazolinone class of herbicides and available in Canada from Bayer CropScience. It is applied POST at 5 g ai ha^{-1} providing control of certain annual grass and broadleaf weeds in spring wheat (*Triticum aestivum* L.) and durum wheat (*T. durum* Desf.) (Health Canada 2010). Thiencarbazone is the only ALS-inhibiting herbicide that controls wild oat (*Avena fatua* L.), green foxtail [*Setaria viridis* (L.) P. Beauv.], and barnyard grass [*Echinochloa crus-galli* (L.) P. Beauv.] and it can be combined in tank mixtures with other herbicides to control several broadleaf weeds (Health Canada 2010). Thiencarbazone is a weak acid with a pKa of 3.0, and therefore is ionized in soils at typical soil pH levels. Its water solubility at pH 4 and 7 is 0.172 and 0.436 g L^{-1}, respectively, thus increasing with increasing pH (Bayer CropScience 2011; Environmental Protection Agency 2008). Therefore, thiencarbazone soil solution concentration is greater in alkaline soils than in acidic soils (Goetz et al 1986; Mersie and Foy 1985; Renner et al. 1988). Thiencarbazone dissipation has reported half-lives of 3.2 to 55 d (Bayer CropScience 2011) and 17.5 to 44.5 d (Health Canada 2010). However, as is the case with other ALS-inhibiting herbicides (Anderson and Barrett 1985; Anderson and Humburg 1987; Loux and Reese 1992; Walker and Brown 1983), the low residual quantities remaining in soil might be bioactive due to the high potency of these herbicides at low concentrations. Herbicide soil residual activity is desirable for providing weed control; however, it also can cause injury to rotational crops as reported for other ALS herbicides (Moyer and Esau 1996; Moyer et al. 1990; Moyer and Hamman 2001). Rotational crop tolerance to thiencarbazone permits many recropping options; however, chickpea (*Cicer arietinum* L.), lentil *(Lens culinaris* Medik.), and timothy (*Phleum pratense* L.) have shown sensitivity to residual thiencarbazone and might require extended recropping intervals (Environmental Protection Agency 2008; Health Canada 2010).

Bioassays are effective tools for measuring the bioavailable herbicide in soil and are generally sensitive, inexpensive, and simple, as compared to analytical methods. Various plant species have been used in bioassays for determination of ALS-inhibiting herbicides in soil, including oriental mustard (Eliason et al. 2004; Szmigielski et al. 2008, 2011), corn (*Zea mays* L.) (Anderson and Humburg, 1987; Groves and Foster, 1985; Hsiao and Smith, 1983; Morishita et al. 1985), sugar beet (*Beta vulgaris* L.) (Jourdan et al. 1998), and sunflower (*Helianthus annuus* L.) (Hernández-Sevillano et al. 2001). Because ALS herbicides primarily affect root development of susceptible plants, root measurements are used most frequently in bioassays for ALS herbicides. Generally, the results of the bioassays vary with soil type and plant species.

Typically, plant response that is measured in a bioassay is not specific to one source. The lack of specificity is beneficial in that the residues of all herbicides that detrimentally affect the same plant parameter are detected. However, other soil-applied chemicals apart from herbicides also can affect the parameter measured in the bioassay and can alter the bioassay results. J. J. Schoenau (unpublished data) reported reduction of oriental mustard root length in soil that did not receive herbicide application but received a 50 kg nitrogen (N) ha^{-1} urea fertilizer application 2 d before soil sampling. Furthermore, E. N. Johnson (unpublished data) observed irregular bioassay results for control and treated plots in field dissipation studies of several ALS herbicides, and partially attributed root reduction of oriental mustard to ammonium

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Table 1. Selected characteristics of Saskatchewan soils used in the study of thiencarbazone bioavailability and dissipation.

Soil series	Canadian soil classification system	pH	Organic carbon[a]	Particle size analysis[b]			Field capacity[c]
				Sand	Silt	Clay	
			%				% w/w
Haverhill loam	Rego Brown Chernozem	7.9	0.9	51	18	31	14
Haverhill clay loam	Humic Luvic Gleysol	7.2	1.6	24	25	51	16
Scott laom	Orthic Dark Brown Chernozem	5.3	2.3	33	28	39	18
Bradwell sandy loam	Orthic Dark Brown Chenozem	7.1	1.5	60	14	26	16
Sutherland clay	Orthic Dark Brown Vertisol	7.8	2.6	14	19	67	30

[a] Organic carbon was determined via a LECO CR-12 automated combustion carbon determinator at 840 C.
[b] Particle size analysis was performed using a Horiba Laser Scattering Particle Size Distribution Analyzer.
[c] Field capacity was estimated by determining the volume of water required to completely wet the air-dried soil to the bottom of a plastic vial.

toxicity after N fertilizer application. Ammonium toxicity to plants is common and a decrease in root/shoot ratio has been reported (Britto and Kronzucker 2002). Root length reduction of oriental mustard due to the presence of ammonium can be misinterpreted as root length reduction due to residual herbicide, yielding false positive results. Confounding effects of ammonium from N fertilization on mustard root bioassay can be resolved if the toxicity can be detected by other plant species that are sensitive to ammonium but not to thiencarbazone.

The objectives of this study were (1) to investigate bioavailability and dissipation of thiencarbazone in soil using an oriental mustard root bioassay, (2) to examine the effect of ammonium from N fertilization on root inhibition of oriental mustard plants and consequently on the determination of thiencarbazone by the bioassay, and (3) to use canaryseed plants for detection of root inhibition associated with ammonium toxicity.

Materials and Methods

Soils. Five soils typical of the Canadian prairies were collected from the 0 to 10 cm depth, air-dried at room temperature and passed through a 2-mm sieve (Table 1). Soil organic carbon was determined via a LECO CR-12 automated combustion carbon determinator (LECO Corporation, 3000 Lakeview Ave., St. Joseph, MI 49085-2398) at 840 C. Carbonate carbon was excluded from analysis and only organic carbon was determined (Wang and Anderson 1998). Soil textures were assessed using a Horiba Laser Scattering Particle Size Distribution Analyzer (Model La-950, Horiba Instruments, Inc., 176871 Armstrong Ave., Irvine CA 92614). Soil pH was measured in 1 : 2 soil : water suspensions, and field-capacity water content was estimated by determining the volume of water required to completely wet the air-dried soil to the bottom of a plastic vial (Eliason et al. 2004).

Herbicide Soil Concentration. Assuming that applied thiencarbazone remains in the top 10 cm of soil and a soil bulk density of 1.3 g cm^{-3} (Eliason et al. 2004), a rate of 5 g ha^{-1} was determined to be equivalent to 3.9 μg kg^{-1} dry soil. Thiencarbazone stock solution was prepared by dissolving 0.39 g of commercial formulation (Velocity®, commercial formulation containing 10 g ai L^{-1} of thiencarbazone, Bayer CropScience, Inc., Canada, 407 Downey Rd. Saskatoon, Saskatchewan S7N 4L8, Canada) in 1 L of methanol (VWR International, 2360 Argentia Road, Mississauga, Ontario L5N 5Z7, Canada), acetone (VWR International), and water (3 : 1 : 1 by vol), yielding a thiencarbazone concentration of 3.9 mg L^{-1}. Through further dilutions, a series of solutions containing thiencarbazone at concentrations of 0, 0.029, 0.039, 0.058, 0.12, 0.19, 0.29, and 0.39 mg L^{-1} was prepared. A volume of 0.5 ml of each solution was combined with the volume of distilled water equivalent to 100% moisture content at field capacity and was added to 50 g of air-dried soil, yielding a thiencarbazone concentration in the soil from 0 to 3.9 μg kg^{-1}.

Oriental Mustard Root Length Bioassay. Oriental mustard root length bioassay was performed as described in Szmigielski et al. (2008). After adding thiencarbazone and water to the soil, the soil was hand-mixed, transferred to a 28.4-g Whirl-Pak® bag (Nasco®, VWR International), and soil in the Whirl-Pak bag was packed to form a layer 8 cm deep and 1 cm thick. Seven oriental mustard seeds (Certified seed was obtained from farm field in Saskatchewan, Canada) were planted at a 2-mm depth and the soil surface was covered with a 5-mm layer of plastic beads (Ashland, General Polymers Division, suite 404-4190 Lougheed Hwy., Burnaby, British Columbia V5C 6A8, Canada) to reduce water evaporation. Bags were watered daily to a predetermined weight to bring the soil moisture content back to 100% field capacity. Plants were grown in the laboratory with average temperature of 21 C and relative humidity of 40% under fluorescent lights that were turned on 24 h d^{-1} and had photosynthetic photon flux density of 16 μmol m^{-2} s^{-1}. Three days after seeding, plants were harvested by opening the bag and washing the soil away from the roots (or radicles that had no lateral roots) with water. Root length was measured from the soil line to the tip of the root and percent of control was calculated as:

$$y = L_\tau / L_0 \times 100\% \quad [1]$$

where y = root length (% of control), L_τ = root length for thiencarbazone-treated soil, and L_0 = root length for the nontreated (control) soil.

Thiencarbazone Bioavailability. Bioavailability was assessed by measuring oriental mustard root response to increasing concentration of thiencarbazone from 0 to 3.9 μg kg^{-1} in each soil. Thiencarbazone concentration required for 50% root length inhibition (I_{50} value) was determined from the dose-response curves that were constructed using a log-logistic regression model (Seefeldt et al. 1995):

$$y = C + (D - C)/(1 + [x/I_{50}]^b) \quad [2]$$

where y = root length (% of control), x = herbicide concentration, C = lower limit of the log-logistic curve, D =

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upper limit of the log-logistic curve, I_{50} = concentration corresponding to 50% inhibition, and b = slope of the curve around the I_{50} value.

Thiencarbazone Dissipation under Laboratory Conditions. For dissipation, soils were prepared by adding thiencarbazone at 3.9 μg kg^{-1} and water equivalent to 85% field capacity to 50 g of soil. Soils were hand-mixed and transferred to 29.6-mL Dixie foam cups (Pinnacle Distributors, 3255B Miners Ave., Saskatoon, Saskatchewan S7K 7Z1, Canada). Cups were capped with lids (Pinnacle Distributors) and placed in an incubator (Isothemp® oven, Model 630G, Fisher Scientific, 10720 – 17th St., Edmonton, Alberta T5S 1J3 Canada) with no light, set at 23 C. Soils were watered every other day to predetermined weight to bring soil moisture content back to 85% field capacity. Soil dissipation was evaluated by sampling every 5 d up to 50 d after treatment. At each sampling, soils were dried, sieved, and residual thiencarbazone determined by the oriental mustard root bioassay. The dissipation data were fitted to a first order dissipation model (Walker and Brown 1983):

$$C = C_0\, e^{-kt} \quad [3]$$

where C = herbicide concentration remaining in soil after time t, C_0 = initial herbicide concentration, and k = dissipation rate constant. Thiencarbazone half-life was then determined using the equation:

$$t_{1/2} = 1/k \ln 2 \quad [4]$$

Ammonium Effect on Oriental Mustard Root Length. The effect of ammonium on root length of oriental mustard was evaluated using ammonium nitrate (VWR International) added to Haverhill loam soil (Table 1) at 0 to 400 μg N g^{-1} soil. To assess the combined effect of ammonium and thiencarbazone on root length of oriental mustard, the soil was supplemented with combinations of ammonium nitrate at 100 μg N g^{-1} soil and thiencarbazone at 0 to 3.9 μg kg^{-1} soil. The observed inhibition due to ammonium nitrate and thiencarbazone was compared with the expected inhibition that was calculated using Colby's equation (Colby 1967):

$$E = X + Y - XY/100 \quad [5]$$

where X = root length inhibition (%) due to compound A, and Y = root length inhibition (%) due to compound B. If observed inhibition is similar to expected inhibition, the combined effect is additive; if observed is larger than expected, the combined effect is synergistic, and if observed is smaller than expected, the combined effect is antagonistic

Canaryseed Root Length Bioassay. Because roots of oriental mustard are inhibited by both ammonium and thiencarbazone, detection of ammonium toxicity by another indicator plant could aid in determining the cause of mustard root reduction. To select a plant species sensitive to ammonium but not susceptible to thiencarbazone, the response of barley (*Hordeum vulgare* L.), wheat, and canaryseed (Canaryseed, Crop Development Centre, Plant Sciences Department, University of Saskatchewan, 51 Campus Dr., Saskatoon, Saskatchewan S7N 5A8, Canada) to ammonium nitrate added at 100 μg N g^{-1} soil and separately to thiencarbazone added at 0.58 μg kg^{-1} soil was assessed in the Haverhill loam soil (Table 1). These species exhibited similar root length inhibition of approximately 30% to ammonium nitrate at 100 μg N g^{-1} soil and no response to thiencarbazone at 0.58 μg kg^{-1} soil. Because canaryseed root (a radicle with no lateral roots) was easier to measure than roots of barley and wheat, canaryseed was selected as an indicator species for detection of ammonium toxicity. To examine the response of canaryseed roots to ammonium nitrate in the range from 0 to 400 μg N g^{-1} soil and to thiencarbazone in the range from 0 to 3.9 μg kg^{-1} soil, canaryseed plants were grown for 5 d in a bioassay conducted in the same way as the oriental mustard bioassay in Whirl-Pak bags.

Experiments were conducted as randomized complete block designs and a Whirl-Pak bag seeded with seven plants was the experimental unit. Experiments were replicated three times and were repeated as two independent runs. Data from two runs in each experiment were combined because the run-by-treatment interactions were not significant, as indicated by ANOVA that was performed using Excel (Microsoft Corporation, One Microsoft Way, Redmond, WA 98052-6399). Dose-response curves (Equation 2) from which I_{50} values were estimated, and dissipation curves (Equation 3) from which dissipation half-lives were calculated (Equation 4), were obtained after fitting the data to nonlinear regressions using Sigma Plot (Sigma Plot, Jandel Scientific, P.O. Box 3457, San Rafael, CA 94912-3457). To examine the relationship of I_{50} values and dissipation half-lives with organic carbon content, soil pH, and clay content of the investigated soils, multiple regression analysis was performed using SPSS (SPSS, 233 S Wacker Dr. #1100, Chicago, IL 60606-6412).

Results and Discussion

Thiencarbazone Bioavailability. The effect of thiencarbazone on root length inhibition of oriental mustard plants varied among the investigated soils (Figure 1) and the concentrations corresponding to 50% inhibition (I_{50} values) obtained from the dose-response curves ranged from 0.56 to 1.71 μg kg^{-1}. Multiple regression analysis showed that organic carbon content and soil pH were significant predictors of thiencarbazone bioavailability in Canadian prairie soils (Table 2). Typically, high organic matter content decreases bioavailability of herbicides because organic matter is highly adsorptive due to its reactive surface (Schoenau et al. 2005; Senesi et al. 1997). Soil pH affects the ionization of weak acidic herbicides as well as the ionic charges of the soil surfaces; at alkaline soil pH these herbicides are more soluble in soil solution and are less adsorbed to negatively charged soil surfaces (Renner et al. 1988; Schoenau et al. 2005). High organic matter content and low soil pH have been reported to increase adsorption and consequently decrease bioavailability of ALS-inhibiting herbicides including chlorsulfuron (Mersie and Foy 1985), imazaquin and imazethapyr (Che et al. 1992; Goetz et al. 1986; Loux and Reese 1992; Renner et al. 1988), imazapyr (Wang and Liu 1999; Wehtje et al. 1987), chlorsulfuron (Thirunarayanan et al. 1985), and chlorimuron (Goetz et al. 1989). Because enhanced adsorption to soil particles reduces thiencarbazone efficacy, higher application rates might be required to achieve adequate weed control. The oriental mustard root bioassay was very sensitive to

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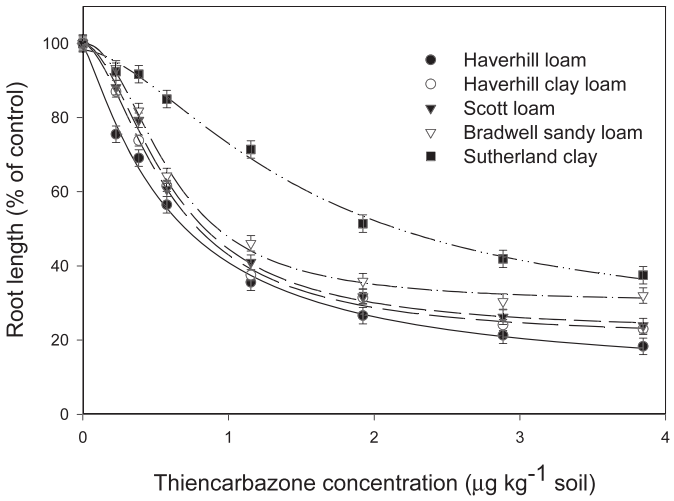

Figure 1. Dose-response curves for thiencarbazone in five soils determined by the oriental mustard root length bioassay: Haverhill loam, $y = 9.1 + 90.5/(1 + [x/0.56]^{1.21})$; Haverhill clay loam, $y = 20.2 + 79.8/(1 + [x/0.59]^{1.73})$; Scott loam, $y = 22.0 + 78.0/(1 + [x/0.60]^{1.83})$; Bradwell sandy loam, $y = 30.0 + 70.0/(1 + [x/0.62]^{2.14})$; Sutherland loam, $y = 23.2 + 75.1/(1 + [x/1.71]^{1.65})$. Each data point represents mean ± standard error.

thiencarbazone, and a concentration as low as 0.29 µg kg^{-1} was detectable in some soils.

Thiencarbazone Dissipation under Laboratory Conditions. Thiencarbazone dissipation differed among the investigated soils (Figure 2); the estimated half-lives ranged from 9 to 50 d and are comparable to reported half-lives from the literature (Bayer CropScience 2011; Health Canada 2010). Multiple regression analysis indicated that organic carbon content and soil pH were significant in affecting thiencarbazone half-life in Canadian prairie soil (Table 3). Enhanced thiencarbazone adsorption in soils of high organic matter and low soil pH decreases the dissipation rate because less thiencarbazone is available for microbial and chemical degradation. Microbial degradation is the primary mechanism for many ALS-inhibiting herbicides because dissipation has been shown to be slower in autoclaved soils than in nonsterile soils with the increase of dissipation half-lives varying with soil type and herbicide (Brown 1990; Joshi et al. 1985). Although thiencarbazone dissipation is less rapid in soils of high organic carbon and low soil pH, its bioavailability in these soils is reduced, and therefore residual thiencarbazone might not pose a risk of injury to sensitive crops. Similar relationships of dissipation rates and soil properties have been reported for many ALS-inhibiting herbicides with organic matter, clay content, and soil pH playing an important role in persistence of these herbicides (Beckie and McKercher 1989; Eliason et al. 2004; Goetz et al. 1990; Hultgren et al. 2002; Smith and Aubin 1992; Thirunarayanan et al. 1985). Topography of the field might have an effect on thiencarbazone dissipation because shorter half-life was observed in the Haverhill loam soil that was collected from the upper landscape position compared to the Haverhill clay loam soil that was collected from the lower landscape position of the same farm field. Similar trends have been reported for other ALS-inhibiting herbicides in the undulating landscape of western Canada (Moyer et al. 2010; Schoenau et al. 2005). Therefore, when testing soil for residual thiencarbazone from a farm field with varying landscape, sampling different parts of the field separately may be advisable to better understand thiencarbazone activity and persistence in the field.

Table 2. Multiple regression analysis for thiencarbazone I_{50} values (concentrations corresponding to 50% inhibition of oriental mustard root length) and selected soil characteristics.

Model	Coefficient	Standard error	P value[a]
Constant	−3.42	0.97	0.013
Organic carbon	0.88	0.27	0.018
Soil pH	0.42	0.13	0.017
Clay	−0.01	0.01	0.563

[a] Significance.

Table 3. Multiple regression analysis for thiencarbazone dissipation half-lives and selected soil characteristics.

Model	Coefficient	Standard error	P value[a]
Constant	−109.20	24.96	0.005
Organic carbon	35.37	6.99	0.002
Soil pH	12.90	3.28	0.008
Clay	−0.47	0.27	0.131

[a] Significance.

Ammonium Effect on Oriental Mustard Root Length. To examine the effect of ammonium toxicity on root length of oriental mustard, ammonium nitrate was used rather than urea to avoid the confounding effect of ammonia (NH_3 gas) that is first produced when urea is hydrolyzed in the soil by the urease enzyme. Free ammonia gas is highly toxic to seedlings. The ammonia gas that is produced from urea hydrolysis then reacts with soil water to form ammonium ions in the water, which also are toxic. We focussed on ammonium toxicity so that the scope of N fertilizer sources covered would be wider; for example, ammonium nitrate, ammonium sulfate, and ammonium phosphate.

Bioassaying the soil with added ammonium nitrate revealed that oriental mustard root length was inhibited by ammonium nitrate and that the root length inhibition increased with the increase of ammonium nitrate concentration (Figure 3).

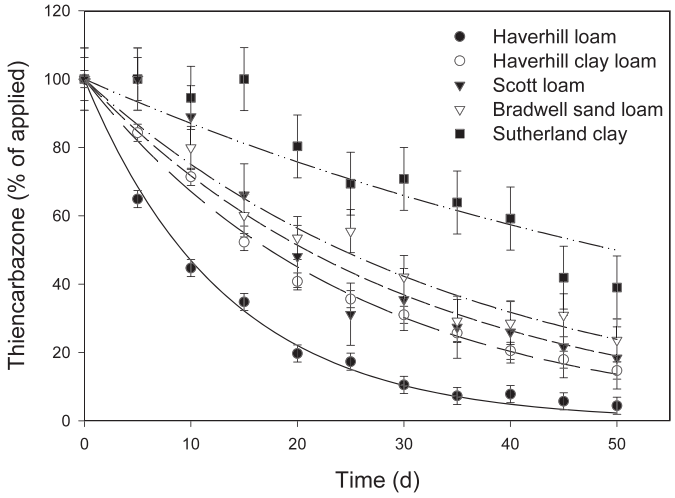

Figure 2. Thiencarbazone dissipation in five soils under laboratory conditions of 23 C and moisture content of 85% field capacity: Haverhill loam, $y = 3.9\ e^{-0.077t}$ ($t_{½} = 9$ d); Haverhill clay loam, $y = 3.9\ e^{-0.040t}$ ($t_{½} = 17$ d); Scott loam, $y = 3.9\ e^{-0.033t}$ ($t_{½} = 21$ d); Bradwell sandy loam, $y = 3.9\ e^{-0.025t}$ ($t_{½} = 24$ d); Sutherland loam, $y = 3.9\ e^{-0.014t}$ ($t_{½} = 50$ d). Each data point represents mean ± standard error.

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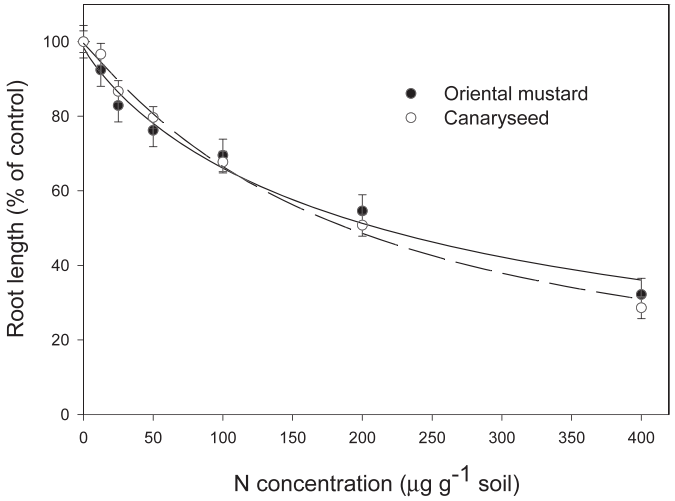

Figure 3. Effect of increasing ammonium nitrate concentration on root length inhibition of oriental mustard and canaryseed plants in the Haverhill loam soil: oriental mustard, $y = 0 + 98.4/(1 + [x/219.2]^{0.91})$; canaryseed, $y = 0 + 99.6/(1 + [x/191.1]^{1.08})$. Each data point represents mean ± standard error.

Biosassaying the soil with added combinations of ammonium nitrate and thiencarbazone demonstrated that the combined effect of ammonium nitrate and thiencarbazone on oriental mustard roots was additive because the observed and expected root length inhibition were similar (Figure 4), and the observed I_{50} value was not different from the expected I_{50} value at 0.05 level based on the asymptotic z-test. Because oriental mustard root length reduction due to recent addition of ammonium containing or ammonium producing N fertilizer can be misinterpreted as reduction due to thiencarbazone, mustard root bioassay can yield false positive results. Ideally, soil sampling for the detection of residual thiencarbazone by the oriental mustard root bioassay should be completed before N fertilizer field application. Also, in the field dissipation studies during the growing season, the impact

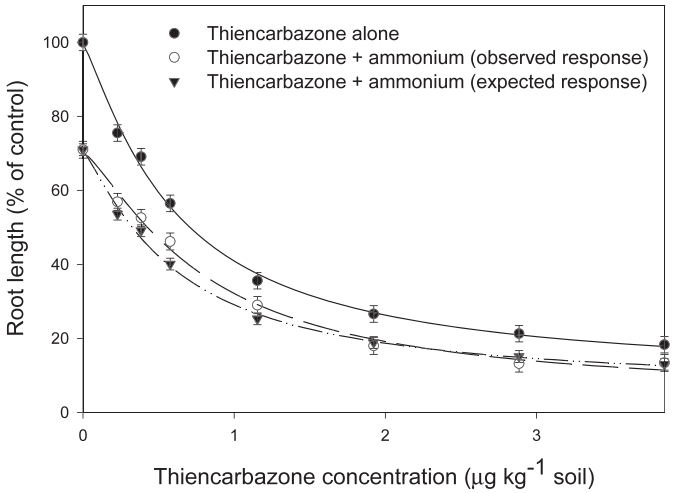

Figure 4. Root length inhibition of oriental mustard plants in response to increasing thiencarbazone concentration with ammonium nitrate (observed and expected) and without ammonium nitrate in the Haverhill loam soil: thiencarbazone + ammonium nitrate (observed response), $y = 4.2 + 66.0/(1 + [x/0.79]^{1.33})$; thiencarbazone + ammonium nitrate (expected response), $y = 6.5 + 64.2/(1 + [x/0.61]^{1.21})$; thiencarbazone alone, $y = 9.1 + 90.5/(1 + [x/0.56]^{1.21})$. Each data point represents mean ± standard error.

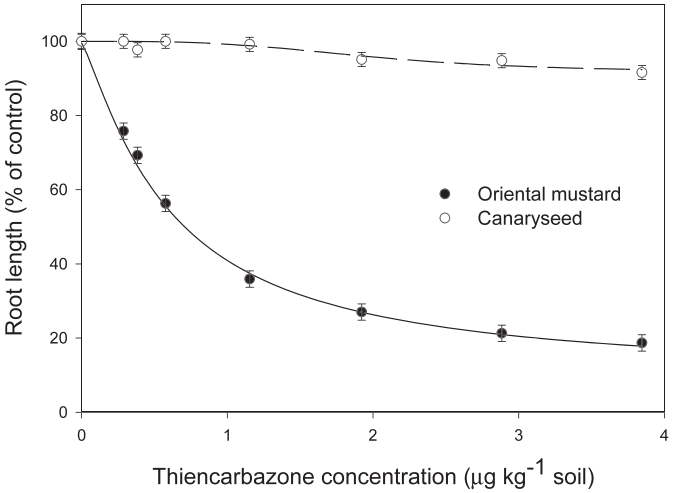

Figure 5. Effect of increasing thiencarbazone concentration on root length inhibition of oriental mustard and canaryseed plants in the Haverhill loam soil: oriental mustard, $y = 9.1 + 90.5/(1 + [x/0.56]^{1.21})$; canaryseed, $y = 91.4 + 8.6/(1 + [x/2.02]^{3.27})$. Each data point represents mean ± standard error.

of N fertilization on the results of the oriental mustard root bioassay should be taken into consideration.

Detection of Ammonium Toxicity by the Canaryseed Root Length Bioassay. Ability to detect ammonium toxicity by another plant species could prevent false positive results from the oriental mustard root bioassay for thiencarbazone residues. Canaryseed plants proved to be a useful plant species for this purpose because roots of canaryseed plants were sensitive to ammonium nitrate to the similar extent as roots of oriental mustard plants (Figure 3), whereas the canaryseed root length inhibition due to thiencarbazone was very small and was less than 10% at the highest thiencarbazone concentrations tested (Figure 5). Thus, canaryseed root bioassay can be effective in identifying ammonium toxicity, and use of both oriental mustard root and canaryseed root bioassay can potentially minimize the likelihood of false positive results while testing soils for residual thiencarbazone.

Practical Considerations for Use of the Oriental Mustard and Canaryseed Root Length Bioassay. Root length of plants grown in Whirl–Pak bags is reproducible because root development is not restricted because the bioassay is completed before roots grow to the bottom of the bag (Szmigielski et al. 2008). In soils free of thiencarbazone and of ammonium, root length of oriental mustard is 7 ± 1 cm in a 3-d bioassay, and 8 ± 1 cm for canaryseed plants in a 5-d bioassay. The results of the two bioassays with respect to thiencarbazone and ammonium detection are summarized in Table 4. No

Table 4. Summary of the results for the oriental mustard root length and canaryseed root length bioassay in soil.

Oriental mustard root length	Canaryseed root length	Thiencarbazone in soil	Ammonium in soil
cm			
7 ± 1	8 ± 1	No	No
< 6	8 ± 1	Yes	No
< 6	< 7	?[a]	Yes

[a] Not conclusive because oriental mustard root reduction might be due to ammonium alone or a combination of ammonium and thiencarbazone.

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reduction of oriental mustard and canaryseed root length indicates that there is no detectable thiencarbazone residue and no ammonium in soil. Reduction of oriental mustard but no reduction of canaryseed root length indicates that there is a detectable thiencarbazone residue but no ammonium in soil. Reduction of both oriental mustard and canaryseed root length indicates that thiencarbazone residue could be present; however, ammonium thiencarbazone detection is not conclusive because oriental mustard root inhibition could result from ammonium alone or from a combination of ammonium and thiencarbazone. In this case, field history of soil treatments, particularly previous herbicide uses and ammonium N fertilization is helpful in interpreting bioassay results.

In conclusion, oriental mustard root length inhibition bioassay proved to be useful for assessment of thiencarbazone bioavailability and dissipation in Canadian prairie soils. However, because oriental mustard roots are also inhibited by ammonium, the presence of freshly applied ammonium containing or ammonium producing N fertilizer to soil can interfere with the outcome of the bioassay. Because canaryseed roots are sensitive to ammonium but are not sensitive to thiencarbazone, use of a canaryseed root bioassay is effective in detecting root inhibition associated with ammonium toxicity and improves the reliability of the oriental mustard root bioassay for thiencarbazone soil residues.

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