An integrated weed management strategy for the control of horseweed (*Conyza canadensis*)

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Abstract

Multiple herbicide-resistant populations of horseweed [*Conyza canadensis* (L.) Cronquist] continue to spread rapidly throughout Ontario, notably in areas where no-till soybean [*Glycine max* (L.) Merr.] is grown. The occurrence of multiple herbicide resistance within these populations suggests that the future role of herbicide tank mixtures as a means of control will be limited. An integrated weed management strategy utilizing complementary selection pressures is needed to reduce the selection intensity of relying solely on herbicides for control. Field studies were conducted in 2018 and 2019 to test the hypothesis: if fall-seeded cereal rye (*Secale cereale* L.) can reduce *C. canadensis* seedling density and suppress seedling growth, then the interaction(s) of complementary selection pressures of tillage, cereal rye, and herbicides would improve the level of *C. canadensis* control. Laboratory studies were conducted to determine whether the allelopathic compound 2-benzoxazolinone (BOA) affected the root development of *C. canadensis* seedlings. The interactions observed among multiple selection pressures of tillage, cereal rye, and herbicides were inconsistent between the 2 yr of study. A monoculture of cereal rye seeded in the fall, however, did reduce seedling height and biomass of *C. canadensis* consistently, but not density. This reduction in seedling height and biomass was likely caused by the allelopathic compound BOA, which reduced seedling root development. Control of *C. canadensis* seedlings in the spring required the higher registered rates of dicamba or saflufenacil. The addition of shallow fall tillage and the presence of cereal rye did not improve the variability in control observed notably with 2,4-D or the lower rates of saflufenacil or dicamba. With the implementation of complementary weed management strategies, environmental variables in any given year will likely have a direct influence on whether these interactions are additive or synergistic.

Introduction

The number of reported herbicide-resistant weeds continues to rise globally at an increasing rate, despite current efforts to tank mix and rotate herbicides to mitigate this issue (Heap 2019). Weed management is reliant upon herbicides; however, the problem of herbicide resistance cannot be solved solely through use of more herbicides (Gressel 1992; Norsworthy et al. 2012). In the United States, the repeated use of glyphosate resulted in the first reports of glyphosate-resistant (GR) horseweed [*Conyza canadensis* (L.) Cronquist] in Delaware in 2001 (VanGessel 2001). In 2010, GR *C. canadensis* was documented in Essex County, Ontario (Byker et al. 2013a). To date, GR *C. canadensis* has been observed in 30 counties across Ontario. Furthermore, 23 counties have GR *C. canadensis* populations that also exhibit resistance to acetolactate synthase (ALS)-inhibiting herbicides, specifically cloransulam-methyl (Budd et al. 2018).

The occurrence of GR and ALS inhibitor–resistant *C. canadensis* in Ontario has created unique challenges for soybean [*Glycine max* (L.) Merr.] producers (Byker et al. 2013c). First, *Conyza canadensis* seedlings that emerge with soybean are very competitive, reducing yields by 60% to 90% (Bruce and Kells 1990; Byker et al. 2013a). Second, although several PRE and POST herbicide treatments involving dicamba or 2,4-D in combination with crop-specific herbicides can be applied to dicamba or 2,4-D–resistant cultivars (Budd et al. 2016; Byker et al. 2013a, 2013b; Kruger et al. 2010), later-emerging seedlings continue to escape the residual control provided by these herbicide treatments. These escaped seedlings can complete their life cycles and produce thousands of wind-disseminated seeds per plant (Davis and Johnson 2008). Despite the confirmed utility of selected herbicides for control of *C. canadensis*, further research is required to explore alternative integrated strategies that utilize a combination of

complementary selection pressures to reduce the selection intensity of relying solely on herbicides for control (Swanton et al. 2008; Swanton and Weise 1991).

Alternative strategies including crop rotation and the potential for cover crops have been reported to reduce *C. canadensis* density and biomass. In a 4-yr crop rotation study, Davis et al. (2009) reported a reduction in field and seedbank densities of *C. canadensis* in a soybean–corn (*Zea mays* L.) rotation compared with continuous soybean. Cholette et al. (2018) reported that the residue of a crimson clover (*Trifolium incarnatum* L.)/cereal rye (*Secale cereale* L.) cover crop seeded after winter wheat (*Triticum aestivum* L.) harvest was effective in reducing density and biomass of *C. canadensis* the following year in a succeeding corn crop. A recent paper by Wallace et al. (2019) observed that a monoculture of cereal rye had the highest and most consistent reduction in seedling density relative to the control before spring burndown herbicide application. Similar results have been reported by Pittman et al. (2019) and Sherman et al. (2019). Although these studies have reported on cereal rye's ability to reduce seedling density, height, and biomass, no study has determined a possible mechanism that would account for this response.

Cereal rye is well known to produce allelopathic compounds (Barnes and Putman 1987). Przepiorkowski and Gorski (1994) observed aqueous extracts of cereal rye tissues inhibited germination of *C. canadensis* by 50%. Cereal rye produces a group of compounds called benzoxazinones (Barnes and Putman 1987; Schulz et al. 2013). Two specific benzoxazinones produced by cereal rye are 2,4-dihydroxy-1,4(2H)-benzoxazin-3-one and 2-benzoxazolinone (BOA). BOA was reported to inhibit the germination and root-and-shoot length of lettuce (*Lactuca sativa* L. var. iceberg) (Barnes and Putman 1987). It is unknown whether BOA has a similar effect on *C. canadensis*.

To the best of our knowledge, no research has explored the possible complementary interactions that may occur when shallow tillage, cereal rye cover crops, and herbicides are used as a multiple selection strategy to control *C. canadensis*. Tillage is an effective method of reducing seedling emergence and controlling established *C. canadensis* seedlings. Tillage has been reported to disrupt the recruitment, dormancy, and viability of seed through burial (see Cici and Van Acker 2009). Furthermore, fall tillage may be useful to control fall-emerged *C. canadensis* by uprooting the rosettes. Managing fall-emerged *C. canadensis* rosettes is essential, as it controls the individuals that have a competitive advantage and are more likely to escape herbicide treatments in the following spring. To reduce the adverse effects deep tillage has on soil health, shallow tillage was used in this study. Shallow tillage, defined in this experiment as tillage less than 5-cm depth in the soil profile, was considered appropriate, as *C. canadensis* is a surface-germinating species, with 80% of the germinated seeds located in the top 2 cm of the soil profile (Bhowmik and Bekech 1993). In addition, numerous studies have reported on the effectiveness of spring-applied herbicides to control emerged seedlings. Research performed by Chahal and Jhala (2019), Kruger et al. (2010), and Soltani et al. (2017) highlighted saflufenacil at 25 g ai ha^{-1}, dicamba at 280 g ae ha^{-1}, and 2,4-D ester at 560 g ae ha^{-1} as chemical options with good to excellent activity on *C. canadensis* populations. With the exception of dicamba, this collective research, however, displays considerable variability in the reported control of *C. canadensis* by these herbicides.

To address this variability in control, we tested the hypothesis: if fall-seeded cereal rye can reduce *C. canadensis* seedling density and suppress seedling growth, then the interaction(s) of complementary selection pressures of tillage, cereal rye, and herbicides would improve the level of *C. canadensis* control. The specific objectives were, first, to confirm that fall-seeded cereal rye alone could reduce *C. canadensis* seedling biomass, density, and height. Second, to explore whether shallow fall tillage followed by fall-seeded cereal rye reduced spring seedling biomass, density, and height of *C. canadensis*, thereby reducing the variability in control with spring-applied herbicides. Finally, in an effort to explain the reported reduction in seedling density, height, and biomass of *C. canadensis* seedlings when grown in the presence of a cereal rye cover crop, studies were conducted to determine whether the allelopathic compound BOA affected seedling root development of *C. canadensis* seedlings. This research was conducted to explore these complementary selection pressures in an effort to develop a more preemptive herbicide-resistant weed management strategy.

Table 1. *Conyza canadensis* seedbank distribution within the soil profile.

Depth	Number of *C. canadensis* seeds in the seedbank	Proportion of total seedbank
cm	seeds m^{-2}	%
0–5	728	80
5–10	182	20
Total	910	—

Materials and Methods

Field Experiments

Field experiments were initiated in the fall of 2017 and 2018 and continued throughout the growing seasons of 2018 and 2019. The soil type was a sandy soil with 1.8% organic matter and a pH of 5.4. At this experimental site, the previous cropping system consisted of a no-till corn–soybean rotation with repeated applications of glyphosate, which led to the occurrence of GR *C. canadensis*. This was first observed on this site in 2015 (S Rupert, personal communication).

Soil cores from across the experimental area were collected in both years to discern the existing amount of *C. canadensis* seed present in the soil profile. Seventy cores from each experimental area were collected randomly from two soil depths (0 to 5 cm and 5 to 10 cm). A 2-cm-diameter soil corer was used to collect 10-cm cores of the soil profile. The cores were then halved, and the 0 to 5 and 5 to 10 cm halves separated. The samples were placed in a freezer set at −4 C for 3 mo to simulate winter. Afterward, the seedling emergence method (Ter Heerdt et al. 1996) was utilized to estimate the *C. canadensis* seedbank. The two soil segments were thinly layered in separate 26 cm by 52.5 cm by 6 cm potting trays. The trays were placed in a growth room with 16-h daylength, 25 C daytime temperature, 20 C nighttime temperature, 75% relative humidity, 275 μmol photons m^{-2} s^{-1}, and water as needed. Daily counts of emerged *C. canadensis* seedlings were made, and the counted individuals were immediately removed from the sample. The trays were left in the growth room until the emergence of *C. canadensis* ceased. The samples were then placed again in the freezer. After 3 mo, the samples were placed in the growth room again. This cycle was repeated three times in total. The seed count data results were then scaled to a square meter basis for each soil depth. The seedbank was found to contain approximately 910 *C. canadensis* seeds m^{-2} in the top 10 cm of the soil profile (Table 1). It is recognized that this method may underestimate the actual number of seeds in the seedbank.

Table 2. List of herbicides and rates applied for control of *Conyza canadensis.*

Common name	Trade name	Active ingredient concentration	Application rates	Manufacturer
			g ae ha^{-1}	
2,4-D ester	2,4-D ester 700 liquid herbicide	660 g ae L^{-1} (2-ethylhexyl ester)	350, 600, 850	Nufarm Canada 333 96 Avenue NE, Calgary, AB T3K OS3, Canada
Dicamba	Banvel® II	480 g ae L^{-1} (diglycolamine salt)	300, 600	BASF Canada Inc. 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada
			g ai ha^{-1}	
Saflufenacil	Eragon® LQ	342 g L^{-1}	25.2, 74.8, 101.1	BASF Canada Inc. 100 Milverton Drive, Mississauga, ON L5R 4H1, Canada

In the fall of 2017 and 2018, trials were established as a randomized complete block design with three replicates and strip plots. The plots were 1 m by 4 m, with each plot halved, containing a 2-m strip of fallow and a 2-m strip of cereal rye cover. This experimental design was chosen to ensure that all possible treatment combinations of fall tillage, cereal rye cover crops, and spring-applied herbicide treatments were contained within the experimental area. Shallow fall tillage treatments consisted of two levels of disturbance, passive tillage and aggressive tillage. A no-tillage control was included in this study. The tillage implement used was sold commercially as a CurseBuster (Soil Regeneration Unlimited, 4560 S 390E, Wabash, IN, USA). This implement allowed for a shallow tillage of approximately 2.5-cm depth and easy adjustment of the harrow settings. The shallow depth was important, because the majority, 80% of the seed, was located within the top 5 cm of the soil profile (Table 1). Fall tillage was implemented to reduce the occurrence of fall-established *C. canadensis* rosettes. To facilitate control of fall-established *C. canadensis* rosettes, the angle of the harrows was adjusted relative to the soil surface. There were two angles that the harrows could be set to, either perpendicular to the ground or at approximately 45°. These two settings were referred to as passive and aggressive tillage and specify the intensity of soil disturbance caused by each setting, respectively. Immediately following fall tillage, Common No. 1 winter cereal rye 'AC® Hazlet' was planted at 67 kg ha^{-1}, approximately 19-cm row spacing, using an eight-row no-till Tye seed drill on November 8 in 2017 and 2018. No fertilizer was applied in the fall or spring to any of the treatments.

On May 31, 2018, and June 5, 2019, populations of *C. canadensis* were sufficiently established to perform the initial assessment before the herbicide burndown treatments. Across the entire experimental plot area, seedling densities ranged from 0 to 2,241 and 0 to 474 plants m^{-2}, heights of individual seedlings ranged from 0.5 to 16.4 and 0.5 to 16.2 cm for 2018 and 2019, respectively. In both years, the established populations were considered to have emerged in the spring, as no fall rosettes were present at the time of assessment; only spring-emerged seedlings, which do not develop initially as rosettes, were present. The cereal rye cover crop at this time was in Feekes growth stages 10.1 to 10.5 and 9 to 10.1 in 2018 and 2019, respectively. Herbicide treatments were applied the day after this assessment.

All herbicide treatments were applied POST on June 1, 2018 and June 6, 2019. Herbicide treatments included 2,4-D ester at rates of 350, 600, and 850 g ae ha^{-1}; saflufenacil at rates of 25.2, 74.8, and 101.1 g ai ha^{-1}; and dicamba at rates of 300 and 600 g ae ha^{-1} (Table 2). The rates selected were based on the labeled rates recommended for different crops and fallow ground. These herbicides were applied using a 50-cm-spaced two-nozzle boom with flat-fan AIXR11002 TeeJet® nozzles (TeeJet®Technologies, 1801 Business Park Dr, Springfield, IL, USA) and pressure set to 207 kPa. A no-herbicide treatment control was included in both years. The herbicides used in this study had no impact on the growth of the cereal rye cover. On June 19 in 2018 and 2019, 2 wk after the herbicide treatment, visual control ratings were recorded for all treatments. Density counts and height measurements of *C. canadensis* were recorded only in the no-herbicide treatment controls. The population counts were performed by randomly placing a 232-cm^2 quadrat in the plot and counting the number of plants in the quadrat. Height measurements of the tallest and smallest plants within the quadrat were recorded. At 4 wk after treatment, on July 4 in 2018 and 2019, visual control ratings, population per square meter counts, and plant height measurements were collected from all treatments. Immediately following this assessment, *C. canadensis* plants growing within the quadrat were hand harvested by clipping the shoots at the soil surface. Plants were then placed into a paper bag and dried to a constant weight at 80 C.

Throughout the experiment, the growth of the cereal rye was left uncontrolled, although it is recognized that a grower would terminate the cereal rye cover crop before planting. This was done to observe the effect of standing cereal rye on *C. canadensis* throughout the growing season. The height of individual cereal rye plants was measured weekly in both years. Light measurements of the photosynthetic photon flux density (PPFD) above and below the cereal rye canopy were recorded in 2018 only, weather permitting. Light measurements were collected using a Li-1400 DataLogger (Li-Cor® Biosciences, 4647 Superior Street, Lincoln, NE, USA). A light bar was placed on the ground beneath the cereal rye canopy, and a light point sensor was placed above the canopy. No light measurements were recorded before the herbicide treatment, because the cereal rye at this time was too short, and no shade was present. Furthermore, no biomass measurements of the cereal rye cover crop were collected in either year of study.

Allelopathy Bioassays

Laboratory experiments were conducted to determine the dose response of *C. canadensis* and lettuce to varying doses of BOA. BOA has been identified as a compound strongly associated with cereal rye's allelopathic characteristics (Barnes and Putnam 1987; Chiapusio et al. 2004; Schulz et al. 2013). As well, BOA was commercially readily available and was purchased from Sigma-Aldrich (2149 Winston Park Drive, Oakville, ON, Canada).

Doses of BOA were selected based on previous allelopathic studies involving lettuce (Hussain et al. 2011). Lettuce was included

Table 3. Results of fixed effects from ANOVA (linear mixed model) of *Conyza canadensis* biomass, population, and height metrics for 2018 and 2019.

	Fixed effects						
Metric	Tillage	Herbicide	Rye	Tillage × herbicide	Tillage × rye	Herbicide × rye	Tillage × herbicide × rye
2018							
Biomass[a]	*	*	*	*	*	ns	ns
Population[a]	*	*	*	*	*	ns	ns
Height[b]	ns	n/a	*	n/a	ns	n/a	n/a
2019							
Biomass[a]	ns	*	*	ns	ns	ns	ns
Population[a]	ns	*	*	ns	ns	*	ns
Height[b]	ns	n/a	*	n/a	ns	n/a	n/a

[a]*, Significant at $P \le 0.05$; ns, nonsignificant
[b]Height measurement analysis did not include data on herbicide treatments; n/a, not available.

in this experiment to act as a positive control. The doses included 0.0 (untreated control), 0.01, 0.1, 0.5, 1.0, 2.5, 5.0, and 10.0 mM of BOA. For each BOA treatment concentration, 0.8 g of water agar was mixed with 90 ml of water and brought to a boil in a microwave. As the solution cooled, 10 ml of an appropriate stock concentrated BOA solution was added to the agar to create the desired treatment concentrations. Four Fisherbrand square disposable petri dishes with grids (Fisher Scientific, 112 Colonnade Road, Ottawa, ON, Canada) received 25 ml of the treated agar solution for each BOA dose, which was left to solidify. Following solidification of the agar, 10 seeds of *C. canadensis* or lettuce were placed onto the upper grid line of the dish and sealed with self-sealing thermoplastic (Parafilm® M, Bemis Company, Neenah, WI, USA). The petri dishes were then placed randomly into a tray; the tray contained every treatment once. These trays held the dishes upright along the narrow edge, thus ensuring that the seedling roots would grow down the surface of the petri dish. Two trays were then placed in a growth chamber set to 25 C daytime and 20 C nighttime temperature, with 16 h of light at 350 µmol m^{-2} s^{-1} PPFD. These conditions were selected based on previous *C. canadensis* germination studies (Buhler and Owen 1997). Within this temperature range, lettuce germination was >90%. This was repeated six times for a total of 12 replications. After 3 d, root length measurements of the lettuce seedlings were recorded. The lettuce was measured at this time because of its rapid seed germination and root growth compared with *C. canadensis*. At 7 d after planting, the same measurements were collected from the *C. canadensis* seedlings only.

Statistical Analysis

Field Experiments. The trial was set as a randomized complete block design with strip plots, with each possible treatment combination replicated three times. Weed seedbank measurements were scaled to a square meter area per 5 cm of soil depth. A PROC TTEST was computed using SAS® v. 9.4 (SAS, 100 SAS Campus Drive, Cary, NC, USA) to perform a test of heterogeneity. This test determined that no data sets from the 2018 and 2019 trials could be pooled. Data variance was divided between random and fixed effects. Random effects included replication, replication by tillage, replication by herbicides, and replication by tillage by herbicides. Fixed effects included tillage, herbicide, cereal rye, cereal rye by herbicides, cereal rye by tillage, tillage by herbicides, and cereal rye by tillage by herbicides. An ANOVA was calculated using PROC GLIMMIX. An alpha value was set ≤0.05 to determine significant interactions among the fixed effects and differences among treatment groups. Tukey's multiple comparison test was used to determine differences among the treatment means. Finally, to test

the fit of the regression model, the normality of the residuals was tested by graphing the studentized residuals and performing a univariate test. If the analyzed data set's residuals had a poor gaussian distribution, when appropriate, the data set was transformed into the log scale to improve the distribution of the residuals. All transformed data were back-transformed for presentation in the tables. As a result of the significant interactions that occurred between years, the interactions will be addressed first, followed by the individual selection pressures.

Allelopathy Bioassays. The experiment was designed as a randomized complete block design with 12 replications. The average percent root length reduction for each petri dish was calculated. Random effects included the tray and growth chamber. Dose of BOA was the only fixed effect. PROC GLM (SAS v. 9.4) was used to generate an ANOVA and graph the dose–response curve. Due to the linear response within the biologically effective doses of BOA, PROC REG was used to determine the regression parameters in lieu of other procedures. The relationship between BOA and root length was modeled using the following equation:

$$Y = mx + b \qquad [1]$$

where Y is the percent reduction in root length compared with the untreated control, m is the slope of the line, x is the dose of BOA, and b is the y-axis intercept. The procedure was instructed to calculate the IC$_{50}$, the concentration of BOA required to decrease the average root length by 50% compared with the untreated control. The IC$_{50}$ of *C. canadensis* was compared with that of lettuce to gauge its relative sensitivity to BOA. Finally, an adjusted R^2 value was calculated to test the fit of the model.

Results and Discussion

Interactions of Alternative Selection Pressures under Field Conditions

The interactions of tillage by cereal rye and tillage by herbicides on aboveground *C. canadensis* biomass were inconsistent between the 2 yr of study (Table 3). In 2018, these two interactions were found to be significant at $P < 0.0001$ and $P = 0.0133$, respectively. In 2019, however, there were no significant interactions between tillage by cereal rye or tillage by herbicides. In 2018, the addition of cereal rye to the no-tillage treatment reduced biomass of *C. canadensis* by 96% (Table 4). Passive or aggressive tillage with no cereal rye reduced total aboveground biomass per square meter by 100% compared with the no cereal rye, no-till control. When passive or aggressive tillage was paired with cereal rye, there were no further biomass reductions than tillage alone. Pairing passive or aggressive tillage

Table 4. Response of *Conyza canadensis* biomass to fall-planted cereal rye cover crops and shallow fall tillage treatments by July 4, 2018.

Treatment[a]	Biomass[b]	Reduction in biomass relative to the no-till, no cereal rye control
	g m^{-2}	%
No-till, no rye control	46.961 a	—
No-till, rye	1.668 b	96
Passive tillage, no rye control	0.017 c	100
Passive tillage, rye	0.004 c	100
Aggressive tillage, no rye control	0.004 c	100
Aggressive tillage, rye	0.004 c	100

[a]The 2018 biomass measurements tillage by cereal rye interaction significant at $P < 0.0001$.
[b]Log-scale was applied to the data set; means presented are back-transformed log values. Means with the same lowercase letter in the same column are not significantly different at $P \le 0.05$ using Tukey's multiple comparison test.

Table 5. Response of *Conyza canadensis* biomass to shallow fall tillage and spring-applied herbicide treatments at 4 wk after herbicide treatment in 2018.

Treatment[a]	Herbicide rate	Biomass[b]	Reduction of biomass relative to the no-tillage, no-herbicide control
	g ae ha^{-1}	g m^{-2}	%
No-tillage, no-herbicide control		146.991 a	—
Passive tillage, no-herbicide control		0.190 bcd	100
Aggressive tillage, no-herbicide control		0.004 cd	100
No-tillage, 2,4-D	350	20.073 ab	86
No-tillage, 2,4-D	600	29.815 ab	80
No-tillage, 2,4-D	850	42.659 ab	71
Passive tillage, 2,4-D	350	0.026 cd	100
Passive tillage, 2,4-D	600	0.009 cd	100
Passive tillage, 2,4-D	850	0.012 cd	100
Aggressive tillage, 2,4-D	350	0.004 d	100
Aggressive tillage, 2,4-D	600	0.009 cd	100
Aggressive tillage, 2,4-D	850	0.004 d	100
No-tillage, dicamba	300	6.789 ab	95
No-tillage, dicamba	600	1.422 bc	99
Passive tillage, dicamba	300	0.004 d	100
Passive tillage, dicamba	600	0.004 d	100
Aggressive tillage, dicamba	300	0.004 d	100
Aggressive tillage, dicamba	600	0.004 d	100
	g ai ha^{-1}		
No-tillage, saflufenacil	25.2	47.897 ab	67
No-tillage, saflufenacil	74.8	0.466 bcd	100
No-tillage, saflufenacil	101.1	0.409 bcd	100
Passive tillage, saflufenacil	25.2	0.009 cd	100
Passive tillage, saflufenacil	74.8	0.004 d	100
Passive tillage, saflufenacil	101.1	0.004 d	100
Aggressive tillage, saflufenacil	25.2	0.004 cd	100
Aggressive tillage, saflufenacil	74.8	0.004 d	100
Aggressive tillage, saflufenacil	101.1	0.004 d	100

[a]The 2018 biomass measurements tillage by herbicide interaction significant at $P = 0.0133$.
[b]Log-scale was applied to the data set; means presented are back-transformed log values. Means with the same lowercase letter in the same column are not significantly different at $P \le 0.05$ using Tukey's multiple comparison test.

Table 6. Response of *Conyza canadensis* biomass to fall-planted cereal rye cover crops by July 4, 2019.

Treatment[a]	Biomass[b]	Reduction relative to the no cereal rye control
	g m^{-2}	%
No rye control	0.090 a	—
Rye	0.005 b	94

[a]The 2019 biomass measurements cereal rye did not interact with tillage or herbicide treatments.
[b]Log-scale was applied to the data set; means presented are back-transformed log values. Means with the same lowercase letter in the same column are not significantly different at $P \le 0.05$ using Tukey's multiple comparison test.

Table 7. Response of *Conyza canadensis* biomass at 4 wk after spring-applied herbicide treatment in 2019.

Treatments[a]	Herbicide rate	Biomass[b]	Reduction in biomass relative to the no-herbicide control
	g ae ha^{-1}	g m^{-2}	%
No-herbicide control		3.339 ab	—
2,4-D	350	6.033 a	0
2,4-D	600	0.006 abc	100
2,4-D	850	0.071 abc	98
Dicamba	300	0.003 bc	100
Dicamba	600	0.002 c	100
	g ai ha^{-1}		
Saflufenacil	25.2	0.002 c	100
Saflufenacil	74.8	0.003 bc	100
Saflufenacil	101.1	0.004 bc	100

[a]The 2019 biomass measurements for herbicide treatments did not interact with cereal rye or tillage treatments.
[b]Log-scale was applied to the data set; means presented are back-transformed log values. Means with the same lowercase letter in the same column are not significantly different at $P \le 0.05$ using Tukey's multiple comparison test.

in combination with herbicides reduced biomass of *C. canadensis* compared with the no-till, no-herbicide treatment (Table 5). Tillage enhanced control (i.e., reduced plant biomass) notably for all rates of 2,4-D and dicamba treatments and the 25.2 g ha^{-1} rate of saflufenacil. No benefit to control was observed when tillage was paired with the 74.8 and 101.1 g ha^{-1} rates of saflufenacil, because both rates provided a 100% reduction in plant biomass compared with the no-tillage, no-herbicide control. In 2019, cereal rye averaged across all treatments reduced the aboveground biomass of *C. canadensis* by 94% compared with the no cereal rye control (Table 6). Passive or aggressive tillage had no effect on the biomass of *C. canadensis* compared with the no-tillage control. Dicamba at 600 g ha^{-1} was the most efficacious herbicide (Table 7), on average, resulting in a 100% reduction in biomass of *C. canadensis* compared with the no-herbicide control.

The interactions of tillage by cereal rye, tillage by herbicides, and herbicides by cereal rye on *C. canadensis* population per square meter counts were also inconsistent between the 2 yr of study (see also Table 3). In 2018, significant interactions between tillage by cereal rye and tillage by herbicides were observed at $P < 0.0001$ and $P = 0.00033$, respectively. In 2019, tillage did not interact with cereal rye or herbicides to reduce the overall *C. canadensis* population. Herbicide by cereal rye was the only interaction observed in 2019, $P = 0.00033$. In 2018, adding cereal rye to the no-tillage treatments reduced the population of *C. canadensis* by 48% compared with the no cereal rye, no-tillage control (Table 8). Passive or aggressive tillage reduced the population by more than 82% compared with the no cereal rye, no-tillage

Table 8. Response of *Conyza canadensis* population per square meter counts to fall-planted cereal rye cover crops and shallow fall tillage treatments by July 4, 2018.

Treatment[a]	Population[b]	Reduction of weed population relative to the no-till, no cereal rye control
	plants m^{-2}	%
No-till, no rye control	265 a	0
No-till, rye	138 b	48
Passive tillage, no rye control	49 c	82
Passive tillage, rye	44 c	84
Aggressive tillage, no rye control	42 c	84
Aggressive tillage, rye	43 c	84

[a]The 2018 population per square meter count measurements for tillage by cereal rye interaction significant at P < 0.0001.
[b]Means with the same lowercase letter in the same column are not significantly different at P ≤ 0.05 using Tukey's multiple comparison test.

control. Adding cereal rye to either passive or aggressive tillage did not result in any further reduction in the *C. canadensis* population. All herbicide treatments, when paired with tillage, resulted in a further decrease in the population of *C. canadensis*, except for saflufenacil applied at 74.8 and 101.1 g ha^{-1} (Table 9). These higher rates of saflufenacil provided >75% reduction in population compared with the no-herbicide, no-till control. Within the 2019 herbicide by cereal rye interaction, there were no reductions in population among the cereal rye, no-herbicide treatment compared with the no cereal rye, no-herbicide control (Table 10). All rates of 2,4-D resulted in a further population reduction when paired with cereal rye. No rate of saflufenacil or dicamba resulted in any further reduction in population with the addition of cereal rye. All rates of saflufenacil or dicamba provided >80% reduction in the population of *C. canadensis* compared with the no cereal rye, no-herbicide control.

Response of Individual Selection Pressures

Over the 2 yr of study, the most consistent herbicide treatments were the 600 g ha^{-1} rate of dicamba and saflufenacil applied at rates of 74.8 and 101.1 g ha^{-1} when paired with tillage treatments or cereal rye cover crops. Crespo et al. (2013) developed a dose–response equation evaluating dicamba's effect on 10 Nebraskan *C. canadensis* populations. They calculated that, on average, to achieve 90% visual control, a rate of dicamba of 560 g ha^{-1} or more was required. Alternatively, Kruger et al. (2010) noted that 280 g ha^{-1} of dicamba, applied POST to *C. canadensis* 7 to 15 cm in height, consistently reduced the biomass of *C. canadensis* by 81% compared with the no-herbicide control. Saflufenacil at 25.2 g ha^{-1} was found to be inconsistent in reducing the population counts and plant biomass of *C. canadensis*. Previous research has suggested that rates of 25 g ha^{-1} were sufficient to provide commercially acceptable control (Budd et al. 2016; Soltani et al. 2017; Waggoner et al. 2011). For example, Budd et al. (2016) conducted a dose–response experiment and calculated that the biologically effective rate of saflufenacil to achieve a 90% reduction in biomass of *C. canadensis* was 25 g ha^{-1}. All treatments of 2,4-D did not provide a consistent reduction in plant biomass or population of *C. canadensis*. This concurs with the conclusions drawn by Mahoney et al. (2016) and Byker et al. (2013b). In both studies, for 2,4-D ester applied at 528 and 500 g ha^{-1}, respectively, at

Table 9. Response of *Conyza canadensis* population per square meter counts to shallow fall tillage and spring-applied herbicide treatments at 4 wk after herbicide treatment in 2018.

Treatment[a]	Herbicide rate	Population[b]	Reduction of weed population relative to the no-till, no-herbicide control
	g ae ha^{-1}	plants m^{-2}	%
No-tillage, no-herbicide control		369 a	––
Passive tillage, no-herbicide control		51 def	86
Aggressive tillage, no-herbicide control		48 ef	87
No-tillage, 2,4-D	350	217 abcd	41
No-tillage, 2,4-D	600	248 abc	33
No-tillage, 2,4-D	850	283 ab	23
Passive tillage, 2,4-D	350	49 ef	87
Passive tillage, 2,4-D	600	45 f	88
Passive tillage, 2,4-D	850	45 f	88
Aggressive tillage, 2,4-D	350	40 f	89
Aggressive tillage, 2,4-D	600	48 ef	87
Aggressive tillage, 2,4-D	850	40 f	89
No-tillage, dicamba	300	183 bcd	50
No-tillage, dicamba	600	141 cde	62
Passive tillage, dicamba	300	41 f	89
Passive tillage, dicamba	600	41 f	89
Aggressive tillage, dicamba	300	40 f	89
Aggressive tillage, dicamba	600	40 f	89
	g ai ha^{-1}		
No-tillage, saflufenacil	25.2	265 abc	28
No-tillage, saflufenacil	74.8	94 def	75
No-tillage, saflufenacil	101.1	94 def	75
Passive tillage, saflufenacil	25.2	45 f	88
Passive tillage, saflufenacil	74.8	41 f	89
Passive tillage, saflufenacil	101.1	41 f	89
Aggressive tillage, saflufenacil	25.2	44 f	88
Aggressive tillage, saflufenacil	74.8	40 f	89
Aggressive tillage, saflufenacil	101.1	40 f	89

[a]The 2018 population per square meter count measurements for tillage by herbicide interaction significant at P = 0.00033.
[b]Means with the same lowercase letter in the same column are not significantly different at P ≤ 0.05 using Tukey's multiple comparison test.

4 wk after treatment, visual control ratings ranged from and 78% to 82% and 74 to 92%, respectively.

Shallow fall tillage did not consistently control fall-established *C. canadensis* seedlings. This result contrasted with the research performed by Brown and Whitwell (1988) and Chahal and Jhala (2019). Both groups of researchers looked at fall tillage's ability to control *C. canadensis* in the following growing season. The former study performed tillage with a disk cultivator at a depth of 10 cm in the soil profile, while the latter study rototilled to a depth of 10 cm. Both groups observed that tillage consistently controlled *C. canadensis*. In these studies, the effectiveness of deep fall tillage may be attributed to the large number (68% to 95% of the seedbank) of the *C. canadensis* seedlings emerging and establishing rosettes in the fall (Buhler and Owen 1997). In the present study, however, seedlings and rosettes were identified at the time of fall tillage; by spring, there was no evidence of fall-emerged rosette

Table 10. Response of *Conyza canadensis* population per square meter counts to fall-planted cereal rye cover crops and spring-applied herbicide treatments at 4 wk after herbicide treatment in 2019.

Treatment[a]	Herbicide rate	Population[b]	Reduction of weed population relative to the no cereal rye, no-herbicide control
	g ae ha^{-1}	plants m^{-2}	%
No rye, no-herbicide control		103 a	—
Rye, no-herbicide control		86 a	16
No rye, 2,4-D	350	81 ab	21
No rye, 2,4-D	600	50 c	51
No rye, 2,4-D	850	53 bc	49
Rye, 2,4-D	350	46 cd	56
Rye, 2,4-D	600	5 e	95
Rye, 2,4-D	850	17 de	84
No rye, dicamba	300	17 de	84
No rye, dicamba	600	0 e	100
Rye, dicamba	300	2 e	98
Rye, dicamba	600	0 e	100
	g ai ha^{-1}		
No rye, saflufenacil	25.2	2 e	98
No rye, saflufenacil	74.8	0 e	100
No rye, saflufenacil	101.1	0 e	100
Rye, saflufenacil	25.2	0 e	100
Rye, saflufenacil	74.8	5 e	95
Rye, saflufenacil	101.1	0 e	100

[a]The 2019 population per square meter count measurements for herbicide by cereal rye interaction significant at $P < 0.0001$.
[b]Means with the same lowercase letter in the same column are not significantly different at $P \leq 0.05$ using Tukey's multiple comparison test.

Table 11. Response of *Conyza canadensis* height to fall-planted cereal rye cover crop by July 4 in 2018 and 2019.

Treatment[a]	Plant height[b]		Reduction in weed height relative to the no cereal rye control[c]	
	2018	2019	2018	2019
	——cm——		——%——	
No rye control	33.01 a	25.99 a	—	—
Rye	12.74 b	3.00 b	61	88

[a]No significant tillage by cereal rye interaction was observed among 2018 and 2019 height measurements.
[b]Log-scale was applied to the data set; means presented are back-transformed log values. Means with the same lowercase letter in the same column are not significantly different at $P \leq 0.05$ using Tukey's multiple comparison test.
[c]Calculations made within the same year of data.

Table 12. Photosynthetic photon flux density (PPFD) measurements above and below the fall-planted cereal rye canopy each week from June 5 to July 4, 2018.

Date	PPFD[a]	
	Above cereal rye canopy	Below cereal rye canopy to soil surface
	——μmol m^{-2} s^{-1}——	
June 5	1,987 ± 11.5	1,548 ± 23.4
June 12	1,969 ± 9.3	1,431 ± 20.1
June 26	1,993 ± 3.2	1,599 ± 22.0
July 4	N/A	N/A

[a]Means (± SE).

survival at the time of pre-spray assessment. According to Tozzi et al. (2013), southern Ontario populations of *C. canadensis* required 94 growing degree days (GDD, $T_{base} = 0$ C) to stimulate 50% germination of *C. canadensis* seed. In the fall of 2017, at 4 wk before the tillage treatments, a total of 255 GDD accumulated compared with 156 GDD over the same period in 2018 (Government of Canada 2019). This difference in GDD between the 2 yr would suggest that in the fall of 2017, more seedlings of the *C. canadensis* emerged before tillage than in 2018. This difference in seedling emergence patterns between years may have contributed to the inconsistency in the reduction of both biomass and population of *C. canadensis* by tillage. Therefore, the 2017 tillage treatments may have disrupted a larger proportion of emerging *C. canadensis* seedlings than in 2018.

The fall-planted cereal rye cover crop was consistent in reducing both biomass and height of *C. canadensis* over the two growing seasons (Figure 1). Biomass, on average, was reduced by 96% and 94%, as well as individual plant height by 61% and 88%, in 2018 and 2019, respectively (Tables 4, 6, and 11). These results support the conclusions drawn from research conducted by Pittman et al. (2019) and Sherman et al. (2019). Possible mechanisms accounting for these observations would be either a change in PPFD as the cereal rye canopy developed or allelopathy. In 2018, light measurements recorded at the soil surface underneath the cereal rye canopy ranged from 1,431 to 1,599 μmol photons m^{-2} s^{-1} (Table 12). This range exceeded the light required for successful germination and seedling establishment of *C. canadensis* (Main et al. 2004; Nandula et al. 2006). If not due to light interception, the observed reduction in plant biomass and height was likely caused by the allelopathic compound BOA.

Figure 1. Cereal rye suppression of *Conyza canadensis* (left) compared with the uncontrolled *C. canadensis* in the adjacent untreated (no rye; right) area in 2018.

Allelopathy Bioassay

Exposure of *C. canadensis* and lettuce seedlings to varying rates of BOA reduced root length by 50% at doses of 0.25 mM and 0.28 mM, respectively (Table 13). These results suggested that *C. canadensis* seedlings were as sensitive to BOA as lettuce. Lettuce, when exposed to BOA, is known to undergo a reduction in photosynthetic capability, water retention, and growth of the shoots and roots (Hussain et al. 2011). At the cellular level, BOA decreased the mitotic activity in root tips, increased the cytoplasmic vacuolation, decreased the number of mitochondria, and

Table 13. The half maximal inhibitory concentration (IC$_{50}$) of 2-benzoxazolinone (BOA) required to reduce the root length of lettuce and *Conyza canadensis* seedlings and their linear relationships.

Species	IC$_{50}$[a]	Lower	Upper	Relationship[b]	Adjusted R^2 value
		mM			
Lettuce	0.2802	0.2439	0.3210	$Y = 122.96x + 24.21$	0.81
C. canadensis	0.2537	0.1940	0.3225	$Y = 159.64x + 9.50$	0.60

[a]The half maximal inhibitory concentration: concentration of BOA required to decrease the average root length compared with the untreated control by 50%.
[b]*Y*, percent root length reduction; *x*, concentration of BOA.

reduced lipid degradation (Burgos et al. 2004; Singh et al. 2005). Furthermore, BOA has been detected in roots and cotyledons of radish (*Raphanus sativus* L.) seedlings and is known to decrease the overall efficiency of photosystem II (Chiapusio et al. 2004; Sánchez-Moreiras et al. 2010). This allelopathic effect on the roots of *C. canadensis* seedlings may be considered as a possible mechanism contributing to the observed reduction in *C. canadensis* seedling biomass and height. Research by La Hovary et al. (2016) demonstrated that cereal rye produced BOA throughout the plant's entire life cycle, with a cereal rye stand (Feekes growth stage 5) capable of producing 5 kg ha^{-1} of total BOA and its chemical derivatives. It is recognized, however, that other mechanisms, such as light quality and nutrient competition, may also contribute to the observations discovered within the field trials.

In summary, we had hoped that the complementary selection pressures of fall tillage, a cereal rye cover crop, and herbicides would interact consistently in a synergistic manner to facilitate the management of herbicide-resistant *C. canadensis*. This did not occur in this study. These interactions, for example, may be initially influenced by the fall and spring environmental conditions and timing and depth of the tillage relative to seedling emergence of *C. canadensis*. A monoculture of cereal rye seeded in the fall, however, did reduce plant height and biomass of *C. canadensis* consistently, but not density. This reduction in seedling height and biomass was likely caused by the allelopathic compound BOA, which reduced seedling root development. Control of *C. canadensis* seedlings in the spring required the higher rates of dicamba or saflufenacil. The addition of shallow fall tillage and the presence of cereal rye did not reduce variability in the control, although this was observed notably with the 2,4-D or the lower rates of saflufenacil or dicamba. The hypothesis "if fall-seeded cereal rye can reduce *C. canadensis* seedling density and suppress seedling growth, then the interaction(s) of complementary selection pressures of tillage, cereal rye and herbicides would improve the level of *C. canadensis* control" is therefore rejected. Although this hypothesis is rejected, it is recognized that the interactions and effectiveness of each individual selection pressure will vary among years. With the implementation of complementary weed management strategies, environmental variables in any given year will likely have a direct influence on whether these interactions are additive or synergistic.

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