Agricultural Sciences, 2014, 5, 1351-1360
Published Online November 2014 in SciRes. http://www.scirp.org/journal/as
http://dx.doi.org/10.4236/as.2014.513145

Response of Underseeded Red Clover (*Trifolium pratense* L.) to Winter Wheat (*Triticum aestivum* L.) Herbicides as Affected by Application Timing

Melody A. Robinson[1], Jocelyne Letarte[1], Michael J. Cowbrough[2], Peter H. Sikkema[3], François J. Tardif[1*]

[1]Department of Plant Agriculture, University of Guelph, Guelph, Canada
[2]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Canada
[3]University of Guelph, Ridgetown Campus, Ridgetown, Canada
Email: [*]ftardif@uoguelph.ca

Received 24 September 2014; revised 23 October 2014; accepted 10 November 2014

Abstract

Underseeding red clover in winter wheat is a beneficial agronomic practice. Still, many growers tend to forgo this approach. One reason is that herbicides used on winter wheat may injure underseeded red clover, reducing its biomass and the subsequent benefits it provides. Therefore, the effect of winter wheat herbicides on underseeded red clover needs to be evaluated. The objectives of this research were to assess the crop tolerance of underseeded red clover to ten winter wheat herbicides used in Ontario, Canada and determine if red clover tolerance differed when the herbicides were applied at various winter wheat growth stages. Experiments were conducted in 2009 and 2010 at four different Ontario locations. Each herbicide treatment was either applied at an early, normal or late timing. Overall, red clover was not affected by herbicides applied at the early timing. The likelihood of herbicides causing injury and reducing biomass of underseeded red clover increased when they were applied at the more advanced winter wheat growth stages. If timing is a constraint, the three herbicides bromoxynil/MCPA, tralkoxydim, and fenoxaprop-p-ethyl are the safest to use on red clover underseeded to winter wheat. The remaining herbicides 2,4-D, dicamba/MCPA/mecoprop, dichlorprop/2,4-D, thifensulfuron/ tribenuron + MCPA, fluroxypyr + MCPA, pyrasulfotole/bromoxynil, and prosulfuron + bromoxynil are more injurious, with the last two being the most harmful. By having identified the least damaging herbicides on underseeded red clover in winter wheat and the optimal timing for herbicide application, growers are more likely to adopt this beneficial agronomic practice, save on fertilizer costs and improve soil quality.

[*]Corresponding author.

How to cite this paper: Robinson, M.A., Letarte, J., Cowbrough, M.J., Sikkema, P.H. and Tardif, F.J. (2014) Response of Underseeded Red Clover (*Trifolium pratense* L.) to Winter Wheat (*Triticum aestivum* L.) Herbicides as Affected by Application Timing. *Agricultural Sciences*, **5**, 1351-1360. http://dx.doi.org/10.4236/as.2014.513145

Keywords

Red Clover, Herbicide Tolerance, Cover Crop, Temperature, Winter Wheat, Frost

1. Introduction

Synthetic fertilizer costs in North America have increased steadily in the last decade. This has encouraged farmers to seek other sources of nitrogen that are more cost effective. Used as a cover crop, red clover (*Trifolium pratense* L.) is a good source of nitrogen. It is a short-lived herbaceous perennial, commonly underseeded to winter wheat (*Triticum aestivum* L.).

Generally, a wheat/red clover intercrop precedes corn (*Zea mays* L.) in rotation, offsetting the high demand of synthetic nitrogen fertilizer in corn since clover is capable of fixing atmospheric nitrogen and reducing fertilizer inputs in the following crop [1]-[3]. This additional soil nitrogen following a red clover crop only occurs when the entire cover crop biomass is returned to the soil. Moreover, the nitrogen amount is relative to the biomass produced by the cover crop [3]-[5].

Aside from the extra nitrogen it releases in the soil, red clover adds non-nitrogen benefits to the cropping system. It is ideal to intercrop with winter wheat as it tolerates shading, has similar feed value to alfalfa, and helps to suppress weeds [6] [7]. In an indirect manner, cover crops also support succeeding crops by improving water infiltration, moisture conservation and decrease soil erosion [5] [8]-[11]. In fact, rotational effects, not related to nitrogen, can increase the yield of a subsequent corn crop up to 40% [5].

As elsewhere, farmers using an intercrop such as wheat and red clover are still concerned with the weed management of their crop. Of the post-emergent herbicides registered and recommended for use on winter wheat, there are few that are also registered for underseeded red clover. This is a problem for growers as control of weeds is important for maximizing crop yield. Many of the herbicides used on winter wheat may damage the underseeded red clover crop, minimizing the potential nitrogen credit and other associated benefits.

We tested several winter wheat herbicides commonly applied in Ontario: 2,4-D, dicamba/MCPA/mecoprop[1], dichlorprop/2,4-D, bromoxynil/MCPA, thifensulfuron/tribenuron + MCPA[2], fluroxypyr + MCPA, pyrasulfotole + bromoxynil, prosulfuron + bromoxynil, tralkoxydim and fenoxaprop-p-ethyl. Of these ten herbicides, only bromoxynil/MCPA and tralkoxydim are also registered for use in red clover and would therefore be assumed safe to apply on underseeded winter wheat [12]. While fenoxaprop-p-ethyl is not registered for use in red clover in Ontario, it is an aryloxyphenoxypropanoate herbicide that is safe to most dicotyledonous plant species [13]. The four herbicides thifensulfuron/tribenuron, bromoxynil, bromoxynil/MCPA and 2,4-D severely injured underseeded red clover in barley while herbicide combinations containing dicamba resulted in red clover plant death [14]. The herbicides 2,4-D and dicamba are likely to cause injury to red clover as they are often used to eliminate red clover before planting grain corn [15]. To our knowledge, there is no information in the literature on the effects of the herbicides dichlorprop/2,4-D, fluroxypyr, pyrasulfotole and prosulfuron on underseeded red clover.

Environmental factors, including temperature, have a considerable effect on the uptake, fate and efficacy of herbicides in crops and weeds. Many studies have indicated that herbicide efficacy is greatest under warm and humid conditions as a result of increased uptake and translocation [16]-[19]. Changes in cuticle composition and thickness during stress events also influence how herbicides move into and within the plant. For example, in corn, the quantity of epicuticular waxes decreases when plants are subjected to cold stress, increasing the wettability and herbicide retention on the leaves [20]. It is unknown whether red clover is more susceptible to injury when herbicides are applied during cold temperatures. It is conceivable that freezing temperatures in combination with herbicide application could cause greater injury on underseeded red clover than frost or herbicides alone.

Herbicides applied late in the growing season may result in less underseeded red clover injury as the developed winter wheat canopy provides shelter to the underseeded crop [14] [21]. For example, the herbicide application of bromoxynil/MCPA is recommended when underseeded red clover is in the first- to third-trifoliate stage

[1]Herbicide names separated by a / indicate they are formulated together in a commercial product.

[2]Herbicide names separated by a + indicate they are distinct formulated products mixed together before application.

while winter wheat forms a protective canopy over it [12].

The goal of this study was to determine the tolerance of underseeded red clover to ten herbicides registered for use on winter wheat in Ontario. Three application timings were assessed: an early timing triggered by the forecast of a frost event (0˚C), a normal timing (Zadoks stages for wheat Z21 to Z29) and a late timing (Z37 to Z39) [22]. The different application timings were used to test if winter wheat growth stage or cold temperatures at the time of herbicide application influenced the biomass and density of red clover.

2. Materials and Methods

2.1. Field Sites

Field experiments were conducted in 2009 and 2010 at the Elora, Exeter, Ridgetown and Woodstock Research Stations located in southern Ontario, Canada. At the Elora and Woodstock Research Stations, seedbed preparation consisted of conservation tillage practices that included either a chisel plow or an offset disk followed by a cultipacker. In Exeter and Ridgetown, the primary tillage was mouldboard plow followed by cultivation to ensure a level seedbed. Soft red winter wheat (cultivar Pioneer 25R47) was planted in rows 17.5 cm apart at seeding densities ranging from 3.5 to 4.5 million seeds per hectare depending on location. Potassium and phosphorous were applied in the fall according to soil test results. Sites were fertilized in the early spring (March) with 120 kg/ha of nitrogen once field conditions allowed. Red clover seeds were broadcast applied at a rate of 10 kilograms per hectare in the early spring (March-April) as soon as conditions allowed.

2.2. Experimental Design

Experiments were organized in a randomized complete block design with four replicates at each location. Plot size was 1.5 × 5.75 m at Elora and Woodstock, 2 × 10 m at Exeter and 2 × 8 m at Ridgetown.

Treatments were applied with a wheel-mounted small plot sprayer fitted with TeeJet flat fan XR 8001 nozzles at Elora and Woodstock and with a backpack sprayer fitted with Hypro FC-ULD-120-01 nozzles at Exeter and Hypro GRD-120-01 nozzles at Ridgetown.

The treatments used were either a single herbicide like 2,4-D, tralkoxydim or fenoxaprop-p-ethyl or an application of two or three herbicides combined together such as dicamba/MCPA/mecoprop, dichlorprop/2,4-D, bromoxynil/MCPA, thifensulfuron/tribenuron + MCPA, fluroxypyr + MCPA, pyrasulfotole/bromoxynil, prosulfuron + bromoxynil, at rates described in **Table 1**. Adjuvants were applied as recommended. Agral 90 (Syngenta Crop Protection Canada Inc., Guelph, ON, Canada), a non-ionic surfactant was added at a rate of 0.2%

Table 1. Active ingredient and dose for all herbicide treatments.

Treatment	Dose
	g·ai/ha
Untreated control[1]	-
2,4-D[2]	550
Dicamba/MCPA/mecoprop[3,4]	93.75/412.5/93.75
Dichlorprop/2,4-D[2]	524.2/492.8
Bromoxynil/MCPA[2]	280/280
Thifensulfuron/tribenuron[5] + MCPA[2,6]	10/5 + 280
Fluroxypyr[7] + MCPA[2]	108 + 562.5
Pyrasulfotole/bromoxynil[8]	32.3/180.7
Prosulfuron + bromoxynil[8]	10 + 140
Tralkoxydim	200
Fenoxaprop-p-ethyl	92.4

[1]Untreated control refers to unsprayed plots. [2]Formulated as 2-ethylhexyl ester. [3]Formulated as diglycolamine salt. [4]Herbicides names separated by a / indicate they are formulated together in a commercial product. [5]Thifensulfuron and tribenuron formulated as methyl esters. [6]Herbicide names separated by a + indicate they are distinct formulated products mixed together by the user. [7]Formulated as 1-methylheptyl ester. [8]Formulated as heptanoate/octanoate ester.

(v/v) to the prosulfuron + bromoxynil and thifensulfuron/tribenuron + MCPA treatments. Turbocharge emulsifiable oil (Dow AgroSciences Canada Inc., Calgary, AL, Canada) was added at a rate of 0.5% (v/v) to the tralkoxydim treatment. Ammonium sulfate (BASF Canada Inc., Mississauga, ON, Canada) was added to the pyrasulfotole/bromoxynil treatment at a rate of 1 L/ha.

Each treatment was applied at three timings: early, normal and late. The first timing was based on temperature and occurred after April 15th in 2009 and 2010 when a temperature of 0˚C was forecasted. The normal application timing occurred before wheat stem elongation, between Zadoks stages Z21 and Z29, while the late application was applied before wheat booting, at Zadoks stages Z37 to Z39. While the early application was timed based on temperature, wheat crop stage at this timing ranged between Z14 and Z21. Dates of red clover seeding, herbicide application, and winter wheat harvest and clover harvest are listed in **Table 2**. Between 4 and 8 weeks after the winter wheat harvest, underseeded red clover density and biomass were assessed by harvesting a 0.25 m^2 quadrat of red clover cut at the soil surface and dried to a constant weight at 80˚C.

2.3. Statistical Analysis

Biomass data were expressed as gram per square meter. Variances were partitioned into the random effects of block and block by year and the fixed effects of treatment. When combining locations, the fixed effects included location and location by treatment. Least square means and standard errors were computed using the PROC MIXED procedure of SAS. Data for field locations were pooled in 2009 or 2010 if type 3 tests of fixed effects location by treatment F-value were non significant ($P > 0.05$). For the data to meet the assumption of normality, data points were removed from the biomass data based on their non-alignment shown in a plot of residuals against ranked residuals and the box plot of the PROC UNIVARIATE procedure. Biomass and density data met the assumptions of the model (random, homogeneous and normal distribution of error).

Clover density data was expressed as the number of plants per square meter. Variances were partitioned into the random effects of block, block by treatment, year, year by location, year by block by treatment, and year by location by treatment. The fixed effects were treatment, location, and location by treatment. Least square means and standard errors were computed using the PROC MIXED procedure of SAS. Field locations and years were pooled for each application timing. Clover density data was subjected to a log transformation to correct for non-constancy of error variance, and then converted back to the original scale for presentation of results. The log transformation improved the distribution of clover density residuals, and data met the assumptions of the model (random, homogeneous and normal distribution of error).

3. Results

3.1. Red Clover Biomass

With some exceptions, the herbicides tested did not reduce red clover biomass in the early application timing. This is when near freezing temperatures were forecasted and winter wheat had developed to the Zadoks Z14 to Z21. Only the herbicides pyrasulfotole/bromoxynil and prosulfuron + bromoxynil caused biomass reductions

Table 2. Dates of red clover seeding, herbicide application relative to wheat growth stage, winter wheat grain harvest, and red clover biomass harvest in each year at each location.

Location	Year	Red Clover Seeding	Time of Herbicide Application			Harvest	
			Early	Normal	Late	Winter Wheat	Red Clover
Elora	2009	31-Mar	3-May	11-May	4-June	13-Aug	21-Sept
	2010	25-Mar	22-Apr	6-May	19-May	27-July	8-Sept
Exeter	2009	2-Mar	23-Apr	5-May	22-May	27-Jul	11-Sept
	2010	20-Mar	19-Apr	3-May	12-May	7-July	26-Aug
Ridgetown	2009	13-Apr	23-Apr	11-May	23-May	29-July	9-Sept
	2010	1-Apr	19-Apr	3-May	10-May	8-July	7-Sept
Woodstock	2010	26-Mar	23-Apr	10-May	28-May	1-Aug	24-Aug

compared to the untreated control at all three sites in 2009 (**Table 3**). In 2010, none of the herbicides tested reduced red clover biomass compared to untreated control. In fact, an increase of 151% in biomass was observed with bromoxynil/MCPA at Ridgetown and fenoxaprop-p-ethyl had a similar effect at Elora and Ridgetown with increases of 179% and 140%, respectively, compared to untreated red clover (**Table 3**).

A different trend was observed when herbicides were applied at the normal timing when winter wheat had reached Z21 to Z29. At this timing, more herbicides negatively affected red clover biomass. In both years, prosulfuron + bromoxynil consistently reduced biomass compared to the untreated control. Red clover biomass reduction ranged from 70% to 98% in 6 of 7 site-years (**Table 4**). The herbicide pyrasulfotole/bromoxynil was the second most harmful to red clover, reducing its biomass 71% to 96% in Exeter and Ridgetown in 2009, and also in Exeter and Woodstock in 2010. The herbicides dicamba/MCPA/mecoprop and dichlorprop/2,4-D also reduced clover biomass by 74% to 100% relative to the untreated control at Exeter and Ridgetown in 2009 and by 56% to 98% in Exeter, Ridgetown and Woodstock in 2010. Only the herbicides 2,4-D, thifensulfuron/tribenuron + MCPA and fenoxaprop-p-ethyl did not affect red clover biomass and this, regardless of sites or years. In contrast to all the other herbicides tested, bromoxynil/MCPA had a positive effect on red clover at the normal application timing. It increased red clover biomass by 74% compared to the untreated control at the Ridgetown location in 2009.

At the late application timing, when wheat is about to boot (Z37 to Z39), the majority of the herbicides tested reduced the underseeded red clover biomass. The herbicides dicamba/MCPA/mecoprop, pyrasulfotole/bromoxynil and prosulfuron + bromoxynil caused the highest reduction, with 65% to 91% less biomass in five sites out of six sites in both years (**Table 5**). Similarly, the herbicide fluroxypyr + MCPA reduced clover biomass by about 55% at all three sites in 2010 but did not have any effect in 2009. The herbicide combination of dichlorprop/2,4-D caused clover biomass reductions (55% to 73%) in 2009 at Elora and Ridgetown but had no negative effect at the other sites that year or at any of the locations in 2010. Some of the herbicides had a negative effect on red clover biomass in only one location and year. For example, the herbicides 2,4-D and thifensulfuron/tribenuron + MCPA caused a reduction in red clover biomass only at Ridgetown in 2009. It is worth noting that red clover growing at this location and year was the most affected by herbicides spraying compared to the untreated control. The only herbicides that did not affect red clover at the late timing application were bromoxynil/

Table 3. Biomass measurements of red clover underseeded to winter wheat following an early herbicide application (wheat growth stages Z14 to Z21). Results were obtained in 2009 and 2010 in four southern Ontario locations.

	Biomass							
	2009		2010[b]					
Treatment	All Locations[a]		Elora		Ridgetown		Woodstock	
	(g/m^2)							
Untreated Control	98.4	A	135.6	BC	56.0	B	122.8	AC
2,4-D	104.0	A	26.4	C	6.4	B	117.6	AC
Dicamba/MCPA/Mecoprop	55.6	AC	16.0	C	3.6	B	68.0	C
Dichlorprop/2,4-D	77.6	AC	56.8	C	12.0	B	115.2	AC
Bromoxynil/MCPA	98.8	A	99.6	BC	140.8	A	222.8	A
Thifensulfuron/Tribenuron + MCPA	79.6	AB	134.8	BC	34.8	B	210.8	AB
Fluroxypyr + MCPA	96.0	A	101.2	C	34.4	B	88.0	BC
Pyrasulfotole/Bromoxynil	40.4	BC	65.2	C	5.6	B	46.0	C
Prosulfuron + Bromoxynil	28.8	C	39.2	C	14.8	B	50.4	C
Tralkoxydim	104.4	A	240.8	AB	28.8	B	135.6	AC
Fenoxaprop-p-ethyl	103.6	A	378.4	A	134.4	A	158.4	AC

Means, within a column, followed by the same letter are not significantly different at P = 0.05 according to the Tukey's multiple means comparison test. [a]Results averaged over 3 locations: Elora, Exeter and Ridgetown. [b]Results for the Exeter location not included in analysis.

Table 4. Biomass measurements of red clover underseeded to winter wheat treated with herbicides applied at a normal timing (wheat growth stages Z21 to Z29). Results were obtained in 2009 and 2010 in different southern Ontario locations.

	2009						2010							
Treatment	Elora		Exeter		Ridgetown		Elora		Exeter		Ridgetown		Woodstock	
	g/m^2													
Untreated Control	154.8	AB	44.4	AB	51.6	B	188.0	AB	92.0	AC	268.8	AB	193.2	A
2,4-D	197.6	A	10.4	BC	14.0	BD	110.0	B	10.4	CD	105.2	BD	107.2	AB
Dicamba/MCPA/mecoprop	54.8	BC	0.0	C	6.8	D	62.8	B	0.4	D	9.6	D	76.8	B
Dichlorprop/2,4-D	146.4	AC	6.8	C	13.6	CD	109.6	B	3.2	D	30.4	CD	84.0	B
Bromoxynil/MCPA	201.2	A	27.2	AC	90.0	A	211.6	AB	120.4	AB	241.6	AC	186.0	A
Thifensulfuron/tribenuron + MCPA	141.2	AC	20.4	AC	22.4	BD	208.0	AB	16.8	CD	134.4	AD	135.6	AB
Fluroxypyr + MCPA	132.0	AC	11.6	AC	30.4	BD	210.0	AB	46.8	BD	162.4	AD	124.8	AB
Pyrasulfotole/bromoxynil	70.4	BC	3.6	C	4.8	D	64.4	B	3.2	D	70.0	BD	55.2	B
Prosulfuron + bromoxynil	28.0	C	0.8	C	4.4	D	92.4	B	2.8	D	14.8	D	58.4	B
Tralkoxydim	158.0	AB	30.4	AC	50.8	AC	304.8	A	147.6	A	206.4	AD	115.6	AB
Fenoxaprop-p-ethyl	152.0	AC	44.0	A	35.2	BD	165.6	AB	132.8	A	339.6	A	129.2	AB

Means, within a column, followed by the same letter are not significantly different at P = 0.05 according to the Tukey's multiple means comparison test.

Table 5. Biomass measurements of red clover underseeded to winter wheat following a late herbicide application (wheat growth stages Z37 to Z39). Results were obtained in 2009 and 2010 in different southern Ontario locations.

	2009						2010	
Treatment	Elora		Exeter		Ridgetown		All Locations[a]	
	No. plants/m^2							
Untreated Control	187.2	AB	25.2	AB	124.0	AB	167.2	A
2,4-D	151.2	AC	10.4	AB	34.0	CD	95.2	AC
Dicamba/MCPA/Mecoprop	46.4	EF	6.0	B	19.6	D	31.6	C
Dichlorprop/2,4-D	84.4	CF	17.2	AB	33.6	D	26.8	AC
Bromoxynil/MCPA	126.8	AE	32.8	AB	121.6	AC	142.0	AB
Thifensulfuron/Tribenuron + MCPA	105.6	BE	23.2	AB	32.8	D	89.2	AC
Fluroxypyr + MCPA	174.8	AC	25.2	AB	37.2	BD	73.6	BC
Pyrasulfotole/Bromoxynil	64.8	DF	6.4	B	23.2	D	41.6	C
Prosulfuron + Bromoxynil	21.6	F	11.6	AB	11.2	D	25.2	C
Tralkoxydim	209.6	A	36.8	A	71.6	AD	137.2	AB
Fenoxaprop-p-ethyl	140.4	AD	37.2	A	142.4	A	147.2	AB

Means, within a column, followed by the same letter are not significantly different at P = 0.05 according to the Tukey's multiple means comparison test. [a]Results averaged for 3 locations, Elora, Exeter, Woodstock.

MCPA, fenoxaprop-p-ethyl and tralkoxydim. These three herbicides did not reduce the biomass of red clover in both years and regardless of locations.

3.2. Red Clover Density

In addition to the biomass measurements taken, red clover density was also evaluated after winter wheat harvest. With the exception of dicamba/MCPA/mecoprop, none of the herbicides tested reduced red clover density at the early application timing regardless of sites and years. In the case of dicamba/MCPA/mecoprop, red clover density was reduced at all application timings. In fact, dicamba/MCPA/mecoprop was the most injurious herbicide to red clover density, with reductions of 84% for the early and normal application timings and 81% for the late timing.

The normal application timing was also the treatment time when more herbicides affected red clover density. In addition to dicamba/MCPA/mecoprop, the herbicides pyrasulfotole/bromoxynil, prosulfuron + bromoxynil and dichlorprop/2,4-D caused red clover density reductions of 75%, 64% and 63%, respectively (**Table 6**). It was the only treatment time when these herbicides had an effect on red clover density.

4. Discussion

According to our results, the best time to apply winter wheat herbicides to an underseeded red clover crop is early in the growing season when temperatures are still near freezing. This was done 4 to 6 weeks after red clover seeding when winter wheat had reached stages Z14 to Z21. None of the herbicides tested at this time reduced red clover biomass with the exception of pyrasulfotole/bromoxynil and prosulfuron + bromoxynil. In 2009, these two herbicide treatments negatively affected red clover biomass and yet, in 2010, no reduction was noted. Differences in conditions from year to year may have affected the phytotoxicity of these two herbicides. For a grower, an early application time to control weeds would be less risky in a red clover/winter wheat crop when the choice of herbicides is limited. Eight of the ten herbicides tested were safe in 2009 and all of them were in 2010. It is possible that cold conditions protected red clover against herbicide damage. Lower temperatures tend to reduce herbicide uptake because of increased viscosity and decreased permeability of the leaf cuticle [23] [24]. Moreover, cold conditions can decrease herbicide translocation [19] [25]. A combination of reduced absorption and translocation would therefore protect red clover from the phytotoxic action of the herbicides.

At the normal and late application timings, proper herbicide selection is more critical. At these times, the herbicides bromoxynil/MCPA, tralkoxydim, and fenoxaprop-p-ethyl were the most dependable. In fact, in some locations and years, these herbicides contributed to an increase of red clover biomass. As expected, tralkoxydim showed no adverse effect on red clover biomass and density at all application timings, locations and years. Our results support the registration of tralkoxydim usage on red clover underseeded to winter wheat [12]. While

Table 6. Density measurements of red clover underseeded to winter wheat. Data were obtained at the time of harvest in 2009 and 2010 following three herbicide applications timings in different southern Ontario locations[a].

	Time of Herbicide Application					
Treatment	Early		Normal		Late	
	No. plants/m^2					
Untreated Control	137.6	A	128.0	A	154.0	A
2,4-D	58.4	AB	55.2	AE	111.2	AB
Dicamba/MCPA/Mecoprop	22.0	B	20.4	E	30.0	B
Dichlorprop/2,4-D	48.0	AB	47.6	CE	97.2	AB
Bromoxynil/MCPA	155.6	AB	137.6	AB	120.8	A
Thifensulfuron/Tribenuron + MCPA	102.4	AB	103.2	AD	94.4	AB
Fluroxypyr + MCPA	68.0	AB	98.4	AD	74.4	AB
Pyrasulfotole/Bromoxynil	43.6	AB	31.6	BE	45.6	AB
Prosulfuron + Bromoxynil	28.0	AB	45.6	DE	41.2	AB
Tralkoxydim	132.4	A	123.2	AC	130.8	A
Fenoxaprop-p-ethyl	212.0	A	135.2	A	155.6	A

Means, within a column, followed by the same letter are not significantly different at P = 0.05 according to the Tukey's multiple means comparison test. [a]Locations were Elora, Exeter and Ridgetown in 2009 and all four locations in 2010.

bromoxynil/MCPA is also registered for use in winter wheat underseeded with red clover, it is recommended that applications occur when clover is in the first- to third-trifoliate stage and winter wheat provides a protective canopy [12]. In our study, bromoxynil/MCPA did not reduce red clover biomass at the early, normal or late timings. This is in contrast to Ivany *et al.* [14] who reported that bromoxynil/MCPA caused a 24% reduction in red clover density and biomass. The red clover response to fenoxaprop-p-ethyl was consistent with herbicides that control grass weeds and have no phytotoxic activity on dicotyledonous plants [12] [13].

The herbicides prosulfuron + bromoxynil and pyrasulfotole/bromoxynil consistently caused injury to red clover at the normal and late applications. Ivany *et al.* [14] and Lins *et al.* [26] both noted that bromoxynil and herbicide mixtures containing bromoxynil caused significant red clover injury and reduced biomass. The likelihood of prosulfuron + bromoxynil or pyrasulfotole/bromoxynil to reduce red clover biomass increased dramatically if they were applied to the crop at the normal or late wheat stages and these timings should be avoided in comparison to the early application. In general, enhanced metabolic degradation is the cause of crop tolerance [27] and it is possible that the rate of metabolism of these herbicides is not rapid enough in red clover to prevent phytotoxicity.

The herbicides dicamba/MCPA/mecoprop and dichlorprop/2,4-D reduced red clover biomass at the normal and late application timings. Moreover, dicamba/MCPA/mecoprop also reduced red clover density at all timings, while dichlorprop/2,4-D reduced it only at the normal timing. This is similar to Ivany *et al.* [14] who noted up to 97% injury to red clover when dicamba/MCPA/mecoprop was applied, including reduced biomass and density.

Compared to untreated red clover, the herbicides 2,4-D, fluroxypyr + MCPA and thifensulfuron/tribenuron + MCPA did not reduce red clover biomass or density at the early and normal application timings. However they affected red clover at the late application timing in some years and locations with no change in red clover density. This supports previous studies that found consistent injury and reduced biomass of red clover after applications of thifensulfuron/tribenuron and 2,4-D late in the growing season [14] [28]. In some circumstances, the use of these herbicides may be an acceptable risk for growers since they may offer the best option for controlling the species of weeds present.

Winter wheat herbicides are least injurious to use on underseeded red clover when applied at an early timing (wheat stages Z14 to Z21) when weather conditions are cold. The likelihood of herbicides negatively affecting underseeded red clover increases when they are applied at the more advanced winter wheat growth stages. Application at the late stages (Z21 to Z29 or Z37 to Z39) may result in biomass and density reduction. By identifying effective herbicides that are safe on underseeded red clover in winter wheat and the optimal timing for herbicide application, growers are more likely to adopt this beneficial agronomic practice.

If timing is an issue, the three herbicides bromoxynil/MCPA, tralkoxydim, and fenoxaprop-p-ethyl are the safest to use on red clover underseeded to winter wheat. No effect was measured in terms of biomass or density in all locations and years. The remaining herbicides might pose more risks with prosulfuron + bromoxynil and pyrasulfotole/bromoxynil being the most harmful regardless of the application time.

Since this research was conducted in the presence of winter wheat as an intercrop, we could not study the effect of herbicides on red clover in the absence of wheat. We were able to determine however if an early application timing for winter wheat would affect its yield. Cold temperature at the time of herbicide application did not affect winter wheat crop yield or plant height [29]. Some herbicides like dicamba/MCPA/mecoprop can however cause prolonged injury and affect wheat yield if applied later in the growing season. These findings support the results found for red clover, namely that applying herbicides early is safest and the use of some herbicides needs to be avoided as the season progresses.

5. Conclusion

This research is valuable to growers as it provides a framework for future management studies for underseeded red clover in winter wheat. We have identified the optimal timing for herbicide application on underseeded red clover to avoid any negative effects the herbicides might have while providing optimum weed control and support crop yield. We also found which herbicides should be avoided in this intercrop since they reduce red clover biomass and defeat the purpose of increasing nitrogen in soil.

Acknowledgements

The authors thank Lynette Brown, Todd Cowan and Peter Smith for their assistance in conducting these experiments. This research was funded in part by the Grain Farmers of Ontario, the Agricultural Adaptation Council (CanAdvance Program) and the Ontario Ministry of Agriculture, Food and Rural Affairs.

References

[1] Hesterman, O.B., Griffin, T.S., Williams, P.T., Harris, G.H. and Christenson, D.R. (1992) Forage-Legume Small-Grain Intercrops-Nitrogen Production and Response of Subsequent Corn. *Journal of Production Agriculture*, **5**, 340-348. http://dx.doi.org/10.2134/jpa1992.0340

[2] Miguez, F.E. and Bollero, G.A. (2005) Review of Corn Yield Response under Winter Cover Cropping Systems Using Meta-Analytic Methods. *Crop Science*, **45**, 2318-2329. http://dx.doi.org/10.2135/cropsci2005.0014

[3] Vyn, T.J., Faber, J.G., Janovicek, K.J. and Beauchamp, E.G. (2000) Cover Crop Effects on Nitrogen Availability to Corn Following Wheat. *Agronomy Journal*, **92**, 915-924. http://dx.doi.org/10.2134/agronj2000.925915x

[4] Bruulsema, T.W. and Christie, B.R. (1987) Nitrogen Contribution to Succeeding Corn from Alfalfa and Red Clover. *Agronomy Journal*, **79**, 96-100. http://dx.doi.org/10.2134/agronj1987.00021962007900010020x

[5] Torbert, H.A., Reeves, D.W. and Mulvaney, R.L. (1996) Winter Legume Cover Crop Benefits to Corn: Rotation vs. Fixed-Nitrogen Effects. *Agronomy Journal*, **88**, 527-535. http://dx.doi.org/10.2134/agronj1996.00021962008800040005x

[6] Fisk, J.W., Hesterman, O.B., Shrestha, A., Kells, J.J., Harwood, R.R., Squire, J.M. and Sheaffer, C.C. (2001) Weed Suppression by Annual Legume Cover Crops in No-Tillage Corn. *Agronomy Journal*, **93**, 319-325. http://dx.doi.org/10.2134/agronj2001.932319x

[7] Mutch, D.L., Martin, T.E. and Kosola, K.R. (2003) Red Clover (*Trifolium pratense*) Suppression of Common Ragweed (*Ambrosia artemisiifolia*) in Winter Wheat (*Triticum aestivum*). *Weed Technology*, **17**, 181-185. http://dx.doi.org/10.1614/0890-037X(2003)017[0181:RCTPSO]2.0.CO;2

[8] Blackshaw, R.E., Moyer, J.R. and Huang, H.C. (2005) Beneficial Effects of Cover Crops on Soil Health and Crop Management. *Recent Research Developments in Soil Science*, **1**, 15-35.

[9] Corak, S.J., Frye, W.W. and Smith, M.S. (1991) Legume Mulch and Nitrogen Fertilizer Effects on Soil Water and Corn Production. *Soil Science Society of America Journal*, **55**, 1395-1400. http://dx.doi.org/10.2136/sssaj1991.03615995005500050032x

[10] Henry, D.C., Mullen, R.W., Dygert, C.E., Diedrick, K.A. and Sundermeier, A. (2010) Nitrogen Contribution from Red Clover for Corn Following Wheat in Western Ohio. *Agronomy Journal*, **102**, 210-215. http://dx.doi.org/10.2134/agronj2009.0187

[11] Sarrantonio, M. and Scott, T.W. (1988) Tillage Effects on Availability of Nitrogen to Corn Following a Winter Green Manure Crop. *Soil Science Society of America Journal*, **52**, 1661-1668. http://dx.doi.org/10.2136/sssaj1988.03615995005200060029x

[12] Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) (2013) Guide to Weed Control 2014-2015: Publication 75. Queen's Printer for Ontario, Toronto.

[13] Burton, J.D. (1997) Acetyl-Coenzyme A Carboxylase Inhibitor. In: Roe, M.R., Burton, J.D. and Kuhr, R.J., Eds., *Herbicide Activity*: *Toxicology*, *Biochemistry and Molecular Biology*, IOS Press, Amsterdam, 187-205.

[14] Ivany, J.A., Holmstrom, D. and Kunelius, H.T. (2000) Effect of Herbicides on Two Species of Clover Underseeded into Barley. *Canadian Journal of Plant Science*, **80**, 973-976. http://dx.doi.org/10.4141/P00-015

[15] Swanton, C.J., Chandler, K. and Shrestha, A. (1998) Control of Established Alfalfa (*Medicago sativa* L.) and Red Clover (*Trifolium pratense* L.) in a No-Till Corn (*Zea mays* L.) Cropping Sequence. *Canadian Journal of Plant Science*, **78**, 175-177. http://dx.doi.org/10.4141/P97-058

[16] Coupland, D. (1983) Influence of Light, Temperature and Humidity on the Translocation and Activity of Glyphosate in *Elymus repens* (=*Agropyron repens*). *Weed Research*, **23**, 347-355. http://dx.doi.org/10.1111/j.1365-3180.1983.tb00558.x

[17] Olson, B.L.S., Al-Khatib, K., Stahlman, P., Parrish, S. and Moran, S. (1999) Absorption and Translocation of MON 37500 in Wheat and Other Grass Species. *Weed Science*, **47**, 37-40.

[18] Kumaratilake, A.R. and Preston, C. (2005) Low Temperature Reduces Glufosinate Activity and Translocation in Wild Radish (*Raphanus raphanistrum*). *Weed Science*, **53**, 10-16. http://dx.doi.org/10.1614/WS-03-140R

[19] Radosevich, S.R. and Bayer, D.E. (1979) Effect of Temperature and Photoperiod on Triclopyr, Picloram and 2,4,5-T Translocation. *Weed Science*, **27**, 22-27.

[20] Gauvrit, C. and Gaillardon, P. (1991) Effect of Low Temperatures on 2,4-D Behaviour in Maize Plants. *Weed Research*, **31**, 135-142. http://dx.doi.org/10.1111/j.1365-3180.1991.tb01752.x

[21] Cowbrough, M.J. (2007) Red Clover Tolerance to Different Herbicide Application Timings. *Field Crop Reports*, **7**, 120-122.

[22] Zadoks, J.C., Chang, T.T. and Konzak, C.F. (1974) A Decimal Code for the Growth Stages of Cereals. *Weed Research*, **14**, 415-421. http://dx.doi.org/10.1111/j.1365-3180.1974.tb01084.x

[23] Muzik, T.J. (1976) Influence of Environmental Factors on Toxicity to Plants. In: Audus, L.J., Ed., *Herbicides*: *Physiology*, *Biochemistry*, *Ecology*, Academic Press, London, 203-247.

[24] Price, C.E. (1983) The Effect of Environment on Foliage Uptake and Translocation of Herbicides. *Aspects of Applied Biology*, **4**, 157-169.

[25] McWhorter, C.G. (1981) Effect of Temperature and Relative Humidity on Translocation of 14C-Metriflufen in Johnsongrass (*Sorghum halepense*) and Soybean (*Glycine max*). *Weed Science*, **29**, 87-93.

[26] Lins, R.D., Colquhoun, J.B., Cole, C.M. and Mallory-Smith, C.A. (2005) Postemergence Small Broomrape (*Orobanche minor*) Control in Red Clover. *Weed Technology*, **19**, 411-415. http://dx.doi.org/10.1614/WT-04-175R1

[27] Devine, M., Duke, S.O. and Fedtke, C. (1993) Physiology of Herbicide Action. Prentice Hall, Englewood Cliffs.

[28] Griffin, J.L., Watson, V.H., Knight, W.E. and Cole, A.W. (1984) Forage Legume Response to Dicamba and 2,4-D Applications. *Agronomy Journal*, **76**, 487-490. http://dx.doi.org/10.2134/agronj1984.00021962007600030029x

[29] Robinson, M.A., Letarte, J., Cowbrough, M.J., Sikkema, P.H. and Tardif, F.J. (2014) Response of Winter Wheat (*Triticum aestivum* L.) to Herbicides as Affected by Application Timing and Temperature. *Canadian Journal of Plant Science*. http://dx.doi.org/10.4141/CJPS-2014-109