

Efficacy of 2,4-D Choline/Glyphosate Dimethylamine on Glyphosate Resistant Canada Fleabane (*Conyza canadensis*) at Different Sizes

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Abstract

Glyphosate resistant (GR) Canada fleabane has spread quickly across southwestern Ontario and new strategies for the control of this competitive weed must be developed especially in no-tillage crops. A premix of 2,4-D choline and glyphosate dimethylamine (DMA) has been developed for application on tolerant corn, soybean and cotton crops that provides an option for the control of this problematic GR weed. The objective of this research was to determine the required dose needed to effectively control GR Canada fleabane at different size categories in field and greenhouse experiments. In the field experiments, nine rates of 2,4-D choline/glyphosate DMA (53.8 to 13,760 g·ae·ha⁻¹) were applied to GR Canada fleabane that were 10 cm in diameter/tall, 20 cm tall or 30 cm tall. Similarly, in the greenhouse, seven rates of 2,4-D choline/glyphosate DMA (0 to 3440 g·ae·ha⁻¹) were applied to 10, 20 and 30 cm tall GR Canada fleabane plants. The three different size classes of GR Canada fleabane responded similarly to 2,4-D choline/glyphosate DMA in the field experiment. In the greenhouse there were some differences in control for the three size classes of GR Canada fleabane with 2,4-D choline/glyphosate DMA; the 20 and 30 cm tall plants required similar rates to provide equivalent control, but the 10 cm plants required a lower rate. In all situations, greater than 1720 g·ae·ha⁻¹ of 2,4-D choline/glyphosate DMA was required to provide 95% control of 10, 20 and 30 cm tall Canada fleabane in greenhouse (35 DAA) and field experiments (8 WAA), respectively.

Keywords

Glyphosate Resistance, Multiple Herbicide-Resistant Crops, Preplant Herbicides, Postemergence Herbicides

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1. Introduction

Glyphosate resistant (GR) Canada fleabane [*Conyza canadensis* (L.) Cronq.] was first discovered in Ontario, Canada in 2010 at eight sites in Essex County [1]. By 2012, 155 sites in eight Ontario counties had been confirmed with GR Canada fleabane [1]. Glyphosate resistant Canada fleabane has spread rapidly across south-western Ontario because of its fecundity (up to 1 million seeds/plant) [2] and the long pappus attached to the small seed (1.3 mm long by 0.3 mm wide) which allows the seed to be wind dispersed up to 500 km from the parent population [3] [4]. Canada fleabane is well adapted to no-tillage crop production systems because its germination success is the greatest when the seed is on the soil surface [2]. Canada fleabane begins growth as a rosette and bolts later in the growing season, reaching up to 180 cm in height [5]. Canada fleabane seeds can germinate year round, with the majority of seeds germinating in autumn and overwintering as a rosette and a smaller proportion of seeds germinating in the spring; this prolonged germination makes herbicide application timing very difficult [6].

With the development of GR weeds, older herbicide modes of action, including 2,4-D, are being used for control. The herbicide, 2,4-D, was developed during the 1940s and provides control of broadleaf weeds [7]. Although the first herbicide resistant weed was 2,4-D resistant wild carrot (*Daucus carota* L.) in the 1950s there are only 17 species resistant to 2,4-D today [8]. Wild carrot is the only specie in Ontario that has previously been found to be resistant to 2,4-D [8]. 2,4-D is a growth hormone that causes the transcription of genes that are normally repressed leading to uncontrolled plant growth and eventually plant death [9]. This herbicide is rapidly broken down in the soil within 17 - 38 days, and therefore does not provide long residual activity [10]. One concern with the use of 2,4-D, is that it may result in injury to sensitive crops in adjacent fields because it is volatile and can result in vapour drift [11] [12]. The 2,4-D ester formulation is more volatile than the 2,4-D amine [13]. The 2,4-D choline formulation is designed and has demonstrated to be less volatile as well [9]. The 2,4-D choline formulation will be sold in a pre-mix with glyphosate (dimethylamine) DMA [9]. The combination of the choline salt with the manufacturer's formulation science of this herbicide has resulted in 88% - 96% less vapour loss compared to other forms of 2,4-D [14]. The 2,4-D choline/glyphosate DMA formulation combined with coarse droplet sized nozzle tips has resulted in 90% less drift compared to a tank mix of 2,4-D amine and glyphosate applied with medium droplet sized nozzle tips [14]. The 2,4-D choline/glyphosate DMA pre-mix herbicide will be introduced in combination with soybean, cotton and corn cultivars/hybrids that are resistant to this herbicide pre-mix [9]. Some other positive attributes of this new formulation include a reduction in odour, and an increase in stability at cold storage temperatures [9]. The 2,4-D choline/glyphosate DMA pre-mix also reduces tank mix problems that can occur with other salt formulations, for example 2,4-D amine combined with potassium salt formulation of glyphosate produces a precipitate that clogs nozzles [9].

The labelled rate of the 2,4-D choline/glyphosate DMA in Canada will be 1720 g·ae·ha⁻¹ which includes 840 g·ae·ha⁻¹ of 2,4-D and 880 g·ae·ha⁻¹ of glyphosate. There have been conflicting reports in the literature on the effect of Canada fleabane size on 2,4-D efficacy. Kruger *et al.* [15] reported that 2,4-D ester and amine (560 g·ae·ha⁻¹) provided equivalent control (90%) of Canada fleabane at 0.5 to 30 cm in height. However, Kruger *et al.* [16] found that 2,4-D amine applied to Canada fleabane over 30 cm in height provided 81% control. Keeling *et al.* [17] found that 2,4-D ester (600 g·ae·ha⁻¹) provided 97% and 50% - 60% control of rosette sized (less than 10 cm) and 10 - 15 cm tall Canada fleabane, respectively. These studies show variable control of Canada fleabane with 2,4-D ester and amine. In addition, Kruger *et al.* [16] reported that the various salt formulations of 2,4-D and dicamba influence efficacy on GR Canada fleabane biotypes differently. These studies show variable control of Canada fleabane with 2,4-D ester. Consequently, it is important to determine the efficacy of 2,4-D choline/glyphosate DMA applied to Canada fleabane at different sizes.

The objective of this research was to determine the required dose of 2,4-D choline/glyphosate DMA needed to effectively control GR Canada fleabane at 10, 20 and 30 cm height.

2. Materials and Methods

2.1. Greenhouse Studies

This experiment was repeated twice in the summer of 2012 with four replications for each run. The replications were randomly arranged in the greenhouse to ensure lighting was similar for the three size classes of Canada fleabane. Seven rates of 2,4-D choline/glyphosate DMA (0, 107.5, 215, 430, 860, 1720, 3440 g·ae·ha⁻¹) were

applied to three different size classes of Canada fleabane, 10, 20 and 30 cm tall plants. Plants of each size were transplanted from a field near Leamington that had been confirmed resistant in 2011 [1]. These plants were placed in the greenhouse and given a chance to recover from the transplant shock before the herbicide treatments were applied. This experiment was conducted in the summer and therefore only natural light was used, with the shade cloth across, the photoperiod during this time of the year was 16 h of natural light. The GR Canada fleabane was watered with tap water after transplanting and every morning for the duration of the experiment, fertilizer was not applied.

The plants were sprayed with the different rates of 2,4-D choline/glyphosate DMA in a chamber sprayer with a single 80-02 flat fan nozzle. The spray chamber shelf was adjusted for the different sized plants, so that the top of the canopy was 45 cm below the spray nozzle. The spray chamber was set at $2.15 \text{ km}\cdot\text{h}^{-1}$ with a pressure of 280 kPa. Control ratings were taken 1, 7, 14, 21 and 35 days after the application (DAA). At day 35, any living plants were harvested at the soil line, placed in the dryer at 60°C (approx. 2 weeks), and the dry weight was recorded.

2.2. Field Studies

Field trials were completed over two summers (2012 and 2013) at Ontario farms with confirmed glyphosate resistant (GR) Canada fleabane [1]. There were six sites in total; three sites near Harrow (L2, L3, and L5), two near Windsor (L1 and L6) and one near Ridgeway (L4). At each of these six sites, three experiments were established where nine rates of 2,4-D choline/glyphosate DMA (53.8, 107.5, 215, 430, 860, 1720, 3440, 6880 or $13,760 \text{ g}\cdot\text{ae}\cdot\text{ha}^{-1}$) were applied to Canada fleabane that were 10, 20 or 30 cm tall. The diameter/height was used for the 10 cm trial because if the plant bolted before it reached 10 cm in diameter the height was used. The 10 cm Canada fleabane experiment from the L3 site (Harrow) in 2012 was moved to the Windsor location (L1), due to limited space at the Harrow location. Roundup Ready corn was no-tilled at all sites in the spring. Soil samples were taken and characteristics for each site are summarized in Table 1.

All three experiments were established using a randomized complete block design with four replications. Experimental units were 2.5 m wide by 8 m long, with a 2 m walkway between each replication. There was an untreated (weedy) and weed-free control in each replication. The treatment timing was dependent on the Canada fleabane size and not the corn growth stage; application date and corn growth stage at the time of the application are summarized in Table 2. Herbicide treatments were applied using a CO_2 -pressurized backpack sprayer calibrated to deliver $200 \text{ L}\cdot\text{ha}^{-1}$ at 240 kPa. The boom was 1.5 m long with four ultra-low drift nozzles (ULD120-02, Hypro, New Brighton, MN) spaced 50 cm apart.

Canada fleabane control ratings were based on a 0% - 100% scale, 0 meaning no control and 100 meaning complete control. Canada fleabane control ratings occurred 1, 2, 4, and 8 WAA. At 8 WAA, weed counts and weed harvests were taken using two 0.25 m^2 quadrats/treatment, the Canada fleabane in each quadrat was counted and cut at the soil surface. These samples were dried at 60°C , and the dry weight was recorded.

Table 1. Location information, soil characteristics, crop description and planting date for the application of 2,4-D choline/glyphosate DMA choline on different sizes of Canada fleabane (10 cm, 20 cm and 30 cm tall) conducted in field trials in 2012 and 2013.

Location ^z	Year	Nearest town	Soil texture	Soil % OM	Soil pH	Planting date	Corn hybrid	Planting population (seeds·ha ⁻¹)	Row spacing (cm)
L1	2012	Windsor	Sandy loam	2.5	6.7	18-May-12	P0118AR	46930	76.2
L2	2012	Harrow	Loam	3.3	7.2	04-May-12	P0245	83900	76.2
L3	2012	Harrow	Loam	3.3	7.2	04-May-12	P0245	83900	76.2
L3 - 10 cm trial	2012	Windsor	Sandy loam	2.5	6.7	18-May-12	P0118AR	46930	76.2
L4	2013	Ridgeway	Loam	2.2	5.7	15-May-13	P 35F50	79781	96.5
L5	2013	Harrow	Sandy loam	1.4	7.5	22-May-13	DK 49-94 RIB	79040	76.2
L6	2013	Windsor	Clay loam	2.6	7.2	07-Jun-13	DK 49-94 RIB	79040	76.2

^zAbbreviations: RIB is refuge in the bag; OM is organic matter.

Table 2. Application date, corn growth stage at the time of the application and the density of Canada fleabane across the field sites in 2012 and 2013.

Location ^z	Year	Application date 10 cm	Application date 20 cm	Application date 30 cm	Corn stage at 10 cm	Corn stage at 20 cm	Corn stage at 30 cm	Density 10 cm (#m ⁻²)	Density 20 cm (#m ⁻²)	Density 30 cm (#m ⁻²)
L1	2012	14-May-12	30-May-12	8-Jun-12	Preplant	3 LF	4 LF	920	112	332
L2	2012	18-Apr-12	1-May-12	17-May-12	Preplant	Preplant	2 LF	12	12	12
L3	2012	-	8-May-12	23-May-12	-	Preemergence	4 LF	-	6	21
L3 - 10 cm trial	2012	17-May-12	-	-	Preplant	-	-	86	-	-
L4	2013	9-May-13	29-May-13	17-Jun-13	Preplant	2 LF	7 LF	134	47	70
L5	2013	30-Apr-13	14-May-13	30-May-13	Preplant	Preplant	Spike	35	154	363
L6	2013	19-Jun-13	26-Jun-13	3-July-13	2 - 3 LF	3 - 4 LF	6 LF	23	19	21

^zAbbreviations: L1, L6 are windsor; L2, L3 and L5 are harrow; L4 is ridgetown; LF is leaf.

2.3. Statistical Analysis

The data were subjected to an ANOVA using the PROC MIXED procedure in the SAS 9.1 program. Variance of Canada fleabane control for each size (10, 20, 30 cm) was partitioned into fixed effects (herbicide treatment) and random effects (environment/run (for greenhouse), replication within environment/run and treatment by environment/run interactions). A Type 1 error rate of 0.05 was used for all statistical tests. Z-tests were used to determine the significance of the random effects and F-tests were used to test the significance of the fixed effects. The significance of the environment/run by treatment interaction was used to determine if the sites could be combined. The assumptions of the analysis (errors are random, homogenous, independent of effects and normally distributed) were examined by plotting the residuals as well as examining the Shapiro-Wilk test of normality. If necessary, a transformation of the data (natural log, square root or arcsine square root) was applied to help meet the assumptions.

A non-linear regression analysis was conducted on all data using the PROC NLIN procedure in the SAS 9.1 (SAS Institute, Cary, NC). The I50 values were compared to determine if there was a difference in control with 2,4-D choline/glyphosate DMA when applied to different sizes of Canada fleabane. The log-logistic model was used [18]:

$$Y = C + \frac{D - C}{1 + \exp[b(\log(X) - \log(I50))]}$$

In this equation Y stands for the percent control of Canada fleabane, C is the lower limit, D is the upper limit, b is the slope of the line (negative for control, positive for dry weight and density) and the I50 is the rate that provides a 50% response between the upper and lower limits [18]. The ED50, 80 and 95 represent the rate that provides 50%, 80% and 95% control of Canada fleabane, respectively. These values were determined by entering 50, 80 and 95 as the Y value and plugging the D, C, B and I50 values from the PROC NLIN procedure to solve for X or the rate. For the dry weight and density 50, 20 and 5 were used as the Y values.

3. Results and Discussion

3.1. Greenhouse Studies

The D values (upper limit) 1 day after application (DAA) of 2,4-D choline/glyphosate DMA on 10 cm, 20 cm and 30 cm tall Canada fleabane plants were 100, 46 and 46, respectively (Tables 3-5). The I50 values 1 DAA, varied as well, 52,093, 335 and 965 for the 10, 20 and 30 cm trials, respectively (Tables 3-5). To achieve 50%, 80% and 95% control 1 DAA of 2,4-D choline/glyphosate DMA on 10 cm tall Canada fleabane a rate greater than the highest rate applied, 3440 g·ae·ha⁻¹, was needed (Table 3). There were no rates 1 DAA of 2,4-D choline/glyphosate DMA applied to 20 cm and 30 cm tall plants that provided 50%, 80% or 95% control of the Canada fleabane (Table 4, Table 5). The application of 2,4-D choline/glyphosate DMA on 10 cm tall plants at 7 DAA had a lower I50 value of 190 compared to the 20 cm trial, 5132, and 30 cm trial, 3747 (Tables 3-5). At 7 DAA, the rate of 2,4-D choline/glyphosate DMA required to provide 50% and 80% control of 10 cm tall Canada

Table 3. The rate response for the control of 10 cm tall glyphosate resistant Canada fleabane with 2,4-D choline/glyphosate DMA 1, 7, 14, 21 and 35 days after application (DAA) and dry weight data in controlled environment experiments in 2012.

Rate response ^v	Run	D ^z	C	B ^y	I50 ^x	ED50 ^w	ED80	ED95
1 DAA	R1 and R2	100 (0)	0.2 (1.4)	0.2 (0)	52,093 (24455)	>13,760	>13,760	>13,760
7 DAA	R1 and R2	100 (0)	0 (0.0)	0.4 (0.1)	190 (54)	190	9624	>13,760
14 DAA	R1 and R2	100 (0)	0.1 (0.3)	0.5 (0.1)	648 (138)	645	10334	>13,760
21 DAA	R1 and R2	100 (0)	0 (0)	0.6 (0.1)	175 (39)	175	1767	>13,760
35 DAA	R1 and R2	100 (0)	0.8 (6.0)	0.9 (0.2)	638 (137)	627	2944	>13,760
Dry weight	R1 and R2	100 (1)	24.8 (1.0)	1.3 (1.4)	62 (45)	143	-	-

^zD is the upper limit and C is the lower limit; ^yB is the slope of the line; ^xI50 is the rate where there is a 50% response; ^wED = the rate of 2,4-D choline/glyphosate DMA where there is 50%, 80% and 95% control; ^vAbbreviations: DAA, days after application; R1 and R2 are the two runs of this experiment.

Table 4. Rate response for the control of 20 cm tall glyphosate resistant Canada fleabane with 2,4-D choline/glyphosate DMA 1, 7, 14, 21 and 35 DAA and dry weight data at 35 DAA in controlled environment experiments in 2012.

Rate response ^v	Run	D ^z	C	B ^y	I50 ^x	ED50 ^w	ED80	ED95
1 DAA	R1 and R2	46 (15)	0 (0)	0.5 (0.2)	335 (469)	-	-	-
7 DAA	R1 and R2	100 (0)	0 (0.1)	0.3 (0.0)	5132 (1403)	5132	>13,760	>13,760
14 DAA	R1 and R2	100 (0)	0 (0)	0.5 (0)	220 (31)	220	3660	>13,760
21 DAA	R1 and R2	100 (0)	0 (0)	0.5 (0.1)	271 (46)	271	3444	>13,760
35 DAA	R1 and R2	100 (0)	1.1 (3.4)	0.8 (0.1)	702 (91)	683	3543	>13,760
Dry weight	R1 and R2	98 (8)	0 (0)	0.6 (0.2)	1084 (448)	3305	>13,760	>13,760

^zD is the upper limit and C is the lower limit; ^yB is the slope of the line; ^xI50 is the rate where there is a 50% response; ^wED = the rate of glyphosate/2,4-D choline where there is 50%, 80% and 95% control; ^vAbbreviations: DAA, days after application; R1 and R2 are the two runs of this experiment.

Table 5. Rate response for the control of 30 cm tall glyphosate resistant Canada fleabane with 2,4-D choline/glyphosate DMA 1, 7, 14, 21 and 35 DAA and dry weight data at 35 DAA in controlled environment in 2012.

Rate response ^v	Run	D ^z	C	B ^y	I50 ^x	ED50 ^w	ED80	ED95
1 DAA	R1 and R2	46 (14)	0 (0)	0.6 (0.2)	965 (1047)	-	-	-
7 DAA	R1 and R2	94 (38)	0 (0)	0.4 (0.1)	3747 (7396)	5238	>13,760	-
14 DAA	R1 and R2	100 (0)	0.1 (0)	0.6 (0.1)	346 (45)	345	3215	>13,760
21 DAA	R1 and R2	100 (0)	1.0 (3.4)	0.9 (0.1)	987 (127)	965	4651	>13,760
35 DAA	R1 and R2	100 (0)	0.2 (0.4)	0.8 (0.1)	708 (125)	704	4424	>13,760
Dry weight	R1 and R2	89 (5)	0 (0)	1.3 (0.4)	2499 (512)	3933	8045	>13,760

^zD is the upper limit and C is the lower limit; ^yB is the slope of the line; ^xI50 is the rate where there is a 50% response; ^wED = the rate of 2,4-D choline/glyphosate DMA where there is 50%, 80% and 95% control; ^vAbbreviations: DAA, days after application; R1 and R2 are the two runs of this experiment.

fleabane was 190 g·ae·ha⁻¹ and 9624 g·ae·ha⁻¹, respectively (**Table 3**). At 7 DAA, the rate of 2,4-D choline/glyphosate DMA required to provide 50% control of the 20 and 30 cm tall Canada fleabane was 5132 g·ae·ha⁻¹ and 5238 g·ae·ha⁻¹, respectively (**Table 4** and **Table 5**). These rates are higher than the highest rate of 2,4-D choline/glyphosate DMA applied in the greenhouse (3440 g·ae·ha⁻¹). At 14 DAA, the rate of 2,4-D choline/glyphosate DMA providing 50% control of the 10, 20 and 30 cm tall Canada fleabane was 645, 220 and 346 g·ae·ha⁻¹, respectively (**Tables 3-5**). At 21 DAA, the rate of 2,4-D choline/glyphosate DMA required to provide 50%, 80% and 95% control of Canada fleabane that was 10 cm in height was 175, 1767 and >13,760 g·ae·ha⁻¹, respectively (**Table 3**). At 21 DAA, the plants that were 20 cm tall at the time of the application required rates of 271 g·ae·ha⁻¹, 3444 g·ae·ha⁻¹ and greater than 13,760 g·ae·ha⁻¹ to provide 50%, 80% and 95% control, respectively (**Table 4**). Where 2,4-D choline/glyphosate DMA was applied to 30 cm tall Canada fleabane, 21 DAA a rate of 965 g·ae·ha⁻¹ was needed to provide 50% control, 4651 g·ae·ha⁻¹ to provide 80% control and greater than

13,760 g·ae·ha⁻¹ to provide 95% control (**Table 5**). Overall, at 21 DAA, a higher rate was required to control the larger Canada fleabane. These results are similar to Keeling *et al.* [17], who determined that higher rates of 2,4-D ester were needed to control larger Canada fleabane plants. At 35 DAA, similar rates of 2,4-D choline/glyphosate DMA provided 50%, 80% and 95% control of the three sizes (10, 20 and 30 cm) of GR Canada fleabane. At 35 DAA, the I50 values for the 10, 20 and 30 cm tall plants were comparable, 638, 702 and 708, respectively (**Tables 3-5**). The dry weight data provided slightly different results than the control ratings at 35 DAA. The I50 values for the dry weight data were diverse for the three sizes of Canada fleabane, 62 for the 10 cm, 1084 for the 20 cm and 2499 when 2,4-D choline/glyphosate DMA was applied to 30 cm tall plants (**Tables 3-5**). According to the dry weight data the 20 and 30 cm tall plants required similar rates to provide 50% control 3305 g·ae·ha⁻¹ and 3933 g·ae·ha⁻¹, respectively, where the 10 cm tall Canada fleabane needed a lower rate of 143 g·ae·ha⁻¹ to provide 50% control (**Tables 3-5**). To provide 80% and 95% control of the Canada fleabane according to the dry weight information a rate could not be determined for the 10 cm tall plants, but a rate higher than 13,760 g·ae·ha⁻¹ was required to provide 80% or greater control of the 20 cm tall plants and for the plants 30 cm tall a rate of 8045 g·ae·ha⁻¹ was needed to achieve 80% and a rate greater than 13,760 g·ae·ha⁻¹ was needed to provide 95% control. According to this dry weight data a lower rate controlled the 10 cm Canada fleabane plants, but the 20 and 30 cm tall plants required similar rates to provide 50%, 80% and 95% control of the GR Canada fleabane.

3.2. Field Studies

Regression analyses indicated that I50 and ED50, 80 and 95 values for the control ratings were similar for 2,4-D choline/glyphosate DMA applied to Canada fleabane at all three plant heights. At 1 week after the application (WAA) of 2,4-D choline/glyphosate DMA, control was similar for the three sizes of GR Canada fleabane. Injury symptoms ranged from slight twisting of the leaves with the lower rates of 2,4-D choline/glyphosate DMA to extreme twisting of the growing point with yellowing/browning of the leaf tips at the high rates. The I50 values 1 WAA of 2,4-D choline/glyphosate DMA on 10, 20 and 30 cm tall plants were 308 - 2411, 352 - 421 and 280 - 1675 g·ae·ha⁻¹, respectively (**Tables 6-8**). The rates of 2,4-D choline/glyphosate DMA that provided 80% control of the GR Canada fleabane 1 WAA of 10, 20 and 30 cm tall GR Canada fleabane plants ranged from 1982 -> 13,760 g·ae·ha⁻¹, 3074 - 11,933 g·ae·ha⁻¹ and 2580 -> 13,760 g·ae·ha⁻¹, respectively (**Tables 6-8**). At 2 WAA, the rates of 2,4-D choline/glyphosate DMA that provided 50% and 80% control of the 10, 20 and 30 cm tall Canada fleabane did not differ. At 2 WAA the rates of 2,4-D choline/glyphosate DMA that provided 50% control of 10, 20 and 30 cm tall GR Canada fleabane were 368 - 584 g·ae·ha⁻¹, 271 - 607 g·ae·ha⁻¹ and 318 - 747 g·ae·ha⁻¹, respectively (**Tables 6-8**). At 4 WAA, the I50 values for the 10, 20 and 30 cm tall Canada fleabane were 359 - 625, 385 - 512 and 101 - 521 g·ae·ha⁻¹, respectively (**Tables 6-8**). The final control ratings, 8 WAA, for the three sizes of Canada fleabane had similar I50, ED50, 80 and 95 values and therefore the weed size at the time of application did not seem to affect the efficacy of 2,4-D choline/glyphosate DMA. At 8 WAA, the rate that provided 95% control of the 10 cm, 20 cm and 30 cm tall Canada fleabane was 952 - 11113 g·ae·ha⁻¹, 1835 - 5737 g·ae·ha⁻¹, and 1232 - 11391 g·ae·ha⁻¹, respectively (**Tables 6-8**). Overall, these rates are very similar and the larger Canada fleabane did not appear to require a higher rate of 2,4-D choline/glyphosate DMA. However, a rate higher than 1720 g·ae·ha⁻¹ of 2,4-D choline/glyphosate DMA in some circumstances may be required or tank mixed with another product to achieve 95% control of GR Canada fleabane.

The I50 value and the ED50, 80 and 95 values for dry weight were lower at 30 cm than at the 10 or 20 cm application timings. For example, I50 value for 2,4-D choline/glyphosate DMA applied to 30 cm plants was 162 compared to 381 and 310 for the 10 and 20 cm tall plants, respectively. The application of 2,4-D choline/glyphosate DMA to GR Canada fleabane that was 30 cm in height had a lower ED50 (306 g·ae·ha⁻¹), ED80 (749 g·ae·ha⁻¹) and ED95 (2908 g·ae·ha⁻¹) dry weight value compared to Canada fleabane that was 10 cm (705, 1623, 5722 g·ae·ha⁻¹) or 20 cm (609, 1976, 13,207 g·ae·ha⁻¹) in height at the time of application (**Tables 6-8**). We speculate the reason for a lower ED50, 80 and 95 value for the dry weight data in the 30 cm timing is that newly emerging Canada fleabane was found in the 10 and 20 cm trial due to 2,4-D's short soil residual activity and they were harvested [10]. The density data reflected the dry weight data showing that the GR Canada fleabane that was 10 cm in height at the time of application required a higher rate to achieve 50% and 80% control compared to Canada fleabane that was 20 or 30 cm tall, we again speculate that this was due to newly emerging Canada fleabane found in the early applications of 2,4-D choline/glyphosate DMA. Overall, applying 2,4-D

Table 6. Rate response of the control of 10 cm Canada fleabane with 2,4-D choline/glyphosate DMA 1, 2, 4, 8 WAA and dry weight and density data 8 WAA for field trials in Ontario, Canada 2012 and 2013.

Rate response ^v	Locations	D ^z	C	B ^y	I50 ^x	ED50 ^w	ED80	ED95
1 WAA	L1 and L3	100 (0)	0.0 (0)	0.9 (0)	993 (31)	993	4302	>13,760
	L4 and L6	85 (2)	0 (0)	0.9 (0.0)	443 (35)	671	10,732	-
	L2	100 (0)	3.4 (2.6)	0.6 (0.0)	2411 (345)	2135	24,822	>13,760
	L5	89 (1)	0 (0)	1.2 (0.1)	308 (15)	380	1982	
2 WAA	L1 and L3	94 (1)	0 (0)	1.2 (0.1)	398 (16)	433	1215	-
	L2 and L4	99 (3)	0.7 (2.0)	0.9 (0.1)	582 (62)	585	2938	20,394
	L5	93 (2)	0.6 (2.2)	1.5 (0.1)	298 (20)	368	1024	-
	L6	89 (2)	0 (0)	2.0 (0.2)	365 (19)	479	1050	-
4 WAA	L1, L3 and L4	97 (1)	0 (0)	1.5 (0.1)	473 (20)	494	1328	6391
	L5 and L6	100 (1)	2.8 (1.4)	1.8 (0.1)	359 (14)	349	760	1836
	L2	100 (0)	5.5(1.6)	1.4 (0.1)	625 (33)	575	1590	4856
8 WAA	L1, L2, L3 and L5	99 (0)	0 (0)	1.0 (0.1)	503 (37)	510	2085	11,113
	L4	100 (0)	0.5 (0)	0.9 (0.1)	256 (28)	253	1169	6502
	L6	98 (0)	0 (0)	2.8 (0.3)	274 (12)	278	467	952
Dry weight	L1, L2, L3, L4, L5 and L6	99 (0)	0 (0)	1.1 (0.1)	381 (45)	705	1623	5722
Density	L1, L2, L3, L4, L5 and L6	86 (0)	0 (0)	1.0 (0.1)	1244 (167)	2131	5340	-

^zD is the upper limit and C is the lower limit; ^yB is the slope of the line; ^xI50 is the rate where there is a 50% response; ^wED = the rate of glyphosate/2,4-D choline where there is 50%, 80% and 95% control; ^vAbbreviations: L1, L6, Windsor; L2, L3 and L5 are harrow; L4 is ridgetown; WAA, weeks after application.

Table 7. Field trials for the rate response of 2,4-D choline/glyphosate DMA for the control of 20 cm tall glyphosate resistant Canada fleabane 1, 2, 4, 8 WAA and the dry weight and density 8 WAA in Ontario, Canada in 2012 and 2013.

Rate response ^v	Locations	D ^z	C	B ^y	I50 ^x	ED50 ^w	ED80	ED95
1 WAA	L1, L6	84 (2)	0 (0)	0.8 (0)	352 (34)	555	11936	-
	L2, L4	92 (1)	0 (0.1)	0.6 (0)	371 (90)	486	7364	-
	L3, L5	87 (1)	0 (0)	1.2 (0)	422 (16)	537	3074	-
2 WAA	L2, L3	91 (2)	0 (0)	1.3 (0.1)	524 (28)	607	2301	-
	L4, L5	92 (0)	0 (0)	0.7 (0)	227 (21)	290	3333	-
	L1	91 (0)	0 (0.1)	1.0 (0.1)	222 (20)	271	1594	-
	L6	100 (0)	0.3 (0.1)	0.8 (0.0)	578 (56)	578	3271	>13,760
4 WAA	L1, L4, L5	100 (0)	0 (0)	1.1 (0)	398 (10)	398	1347	5303
	L2, L6	100 (0)	0.4 (0.1)	1.1 (0.1)	512 (32)	508	1772	7198
	L3	100 (0)	0.2 (0.1)	1.6 (0.1)	385 (20)	384	902	2357
8 WAA	L1, L2, L4	100 (0)	2.0 (1.6)	1.2 (0.1)	502 (27)	485	1561	5737
	L5, L6	100 (0)	0 (0)	1.4 (0.1)	224 (12)	224	603	1835
	L3	100 (0)	0.8 (0.1)	1.8 (0.1)	365 (16)	361	783	1862
Dry weight	L1, L2, L3, L4, L5 and L6	85 (0)	0 (0)	0.8 (0.1)	310 (65)	609	1976	13,207
Density	L1, L2, L3, L4, L5 and L6	94 (5)	0 (0)	0.8 (0.1)	308 (71)	681	2142	12,117

^zD is the upper limit and C is the lower limit; ^yB is the slope of the line; ^xI50 is the rate where there is a 50% response; ^wED = the rate of 2,4-D choline/glyphosate DMA where there is 50%, 80% and 95% control; ^vAbbreviations: L1, L6, windsor; L2, L3 and L5 are harrow; L4 is ridgetown; WAA, weeks after application.

Table 8. Rate response of 2,4-D choline/glyphosate DMA on the control of 30 cm tall Canada fleabane 1, 2, 4, 8 WAA and dry weight and density 8 WAA conducted in field trials in 2012 and 2013 in Ontario, Canada.

Rate response ^v	Locations	D ^z	C	B ^y	I50 ^x	ED50 ^w	ED80	ED95
1 WAA	L1, L2	91 (2)	0 (0)	0.8 (0)	496 (30)	640	6065	-
	L3, L5	88 (1)	0 (0)	1.2 (0)	417 (17)	529	2966	-
	L4	82 (3)	0 (0)	0.8 (0.1)	675 (95)	1190	>13,760	-
	L6	96 (0)	0 (0)	0.7 (0)	280 (27)	312	2580	>13,760
2 WAA	L1, L5	87 (2)	0.9 (1.4)	1.2 (0)	528 (32)	675	4185	-
	L2	93 (0)	0 (0.1)	1.0 (0.1)	274 (29)	318	1754	-
	L3	97 (0)	0 (0.1)	0.9 (0)	475 (49)	510	2688	>13,760
	L4	83 (2)	0 (0)	1.1 (0.1)	513 (37)	747	9855	-
	L6	100 (0)	0.4 (1.6)	1.0 (0.0)	365 (19)	363	1323	5653
4 WAA	L1, L3, L4, L5	100 (0)	0 (0.1)	0.8 (0)	521 (39)	521	2966	>13,760
	L2	100 (0)	0 (0.2)	1.0 (0)	264 (28)	264	1021	4667
	L6	100 (0)	0 (0.1)	1.3 (0.1)	101 (7)	101	289	946
8 WAA	L3, L4, L5	100 (0)	0 (0)	1 (0)	572 (60)	576	2308	11,391
	L2, L6	100 (1)	0.6 (1.4)	2.2 (0.1)	318 (11)	316	599	1232
	L1	98 (2)	0 (0)	1.4 (0.1)	411 (26)	420	1135	4087
Dry weight	L1, L2, L3, L4, L5 and L6	95 (4)	0 (0)	1.0 (0.1)	162 (21)	306	749	2908
Density	L1, L2, L3, L4, L5 and L6	95 (0)	0 (0.4)	1.4 (0.3)	686 (98)	1084	2086	5616

^zD is the upper limit and C is the lower limit; ^yB is the slope of the line; ^xI50 is the rate where there is a 50% response; ^wED = the rate of 2,4-D choline/glyphosate DMA where there is 50%, 80% and 95% control; ^vAbbreviations: L1, L6, windsor; L2, L3 and L5 are harrow; L4 is ridgetown; WAA, weeks after application.

choline/glyphosate DMA to larger Canada fleabane does not reduce the efficacy of this herbicide. These findings are similar to Kruger *et al.* [6], who reported the control of 30 cm tall Canada fleabane plants with 2,4-D ester was the same as when it was applied to rosette sized plants.

4. Conclusion

The efficacy of 2,4-D choline/glyphosate DMA for the control of GR Canada fleabane was not influenced by size (10, 20 or 30 cm tall) of the Canada fleabane at the time of application in the field experiments. There were some differences in control of the three size classes of GR Canada fleabane in the greenhouse experiments, requiring a lower rate of 2,4-D choline/glyphosate DMA to control the 10 cm tall Canada fleabane compared to the 20 and 30 cm tall plants. However, similar rates of 2,4-D choline/glyphosate DMA provided 50%, 80% and 95% control of the 20 and 30 cm tall GR Canada fleabane in the greenhouse, which is comparable to the field experiments.

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