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Evaluating the vegetative and reproductive response of hemp (*Cannabis sativa*) to simulated off-target events of 2,4-D and dicamba

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Abstract

Introducing soybean cultivars resistant to 2,4-D and dicamba allowed for postemergence applications of these herbicides. These herbicides pose a high risk for off-target movement, and the potential influence on crops such as hemp is unknown. Two studies were conducted from 2020 through 2022 in controlled environments to evaluate hemp response to rates simulating off-target events of 2,4-D and dicamba. The objectives of these studies were to (1) determine the effects of herbicide (2,4-D and dicamba) and rate (1× to 1/100,000× labeled rate) on visible injury, height, and branching, and (2) determine the effect of 2,4-D rate (1× to 1/100,000× labeled rate) on visible injury, height, branching, and reproductive parameters. Herbicides were applied in the early vegetative stage, and evaluations took place 14 and 28 d after treatment (DAT) and at trial termination (42 DAT in the greenhouse trial and at harvest in the growth chamber trial). In the greenhouse study, 2,4-D and dicamba at the 1× rate, and the 1/10× rate of dicamba, caused 68%, 78%, and 20% injury 28 DAT, respectively. At the time of trial termination 42 DAT, plants treated with 1× rates of 2,4-D and dicamba, or 1/10× dicamba, were 19, 25, and 9 cm shorter than the nontreated control, respectively. Simulated off-target rates of 2,4-D and dicamba did not influence branching or plant weight at trial termination. In the growth chamber study, the 1× and 1/10× rates of 2,4-D caused 82% and 2% injury 28 DAT, respectively. Plant height, fresh weight, and cannabidiol (CBD) levels of plants treated with simulated off-target rates of 2,4-D were not different from the nontreated control. These studies suggest that hemp grown for CBD exposed to off-target rates of 2,4-D or dicamba in early vegetative stages may not have distinguishable effects 42 DAT or at harvest.

Introduction

Hemp is a relatively new crop in Ohio agricultural production. Until 2018, hemp was classified along with marijuana (also *C. sativa* L.; collectively referred to as cannabis) as a federally illegal substance and therefore not eligible for production or research purposes. The passage of the 2018 Agriculture Improvement Act (Farm Bill) removed federal restrictions on hemp and allowed for research to take place on this crop (USDA 2021). Although hemp was federally legal at that time, many individual states still had laws in place barring hemp production or research, as was the case in Ohio (Essman 2018). In 2019, the Ohio general assembly passed Senate Bill 57, which differentiated hemp from marijuana and allowed for research to take place (TOLGA 2019). By law, *C. sativa* plants that contain less than 0.3% of the psychoactive compound delta-9-tetrahydrocannabinol (THC) are defined as hemp. By the same law, *C. sativa* plants above the 0.3% threshold are considered marijuana, a federally illegal schedule 1 substance (USDA-AMS 2018).

The use of new herbicide-resistant soybean systems with traits conferring resistance to herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and dicamba (3,6-dichloro-2-methoxybenzoic acid) has increased in recent years, in response to the spread of herbicide-resistant weed biotypes (Gage et al. 2019; Unglesbee 2018). The utilization of these herbicide-resistant soybean trait systems allows for postemergence, in-season applications to growing soybean plants with state-specific application cutoff dates. This can be problematic when a resistant soybean crop is planted adjacent to sensitive soybean or other sensitive crops or trees, as these herbicides present a high risk for off-target movement (Bish et al. 2021). Two main issues arise from in-season applications of these herbicides: physical drift and volatility. Physical drift is a form of primary movement and refers to the off-target movement of an herbicide at the time of application (Bish et al. 2021). Primary movement can also include events such as tank contamination with residue from prior applications (Bish et al. 2021; Hager 2017; Browne et al. 2020). Symptomology from primary movement typically has a distinct pattern, often occurring along a gradient downwind from the application, and is less noticeable farther from the source (Hager 2017; Loux and Johnson 2017). Secondary movement occurs after the time of herbicide application and is often referred to as vapor drift (volatility) or wind erosion of treated soil (Bish et al. 2021; Soltani et al. 2020). Events of secondary movement of growth regulator herbicides are extremely problematic and can be very difficult to predict and diagnose. Even with known temperature and environmental effects, there is not always a distinct injury pattern. The growth regulator herbicides 2,4-D and dicamba are particularly susceptible to volatility because of their chemical makeup and high vapor pressure (Bish et al. 2021). Studies estimate that for dicamba, 0.028 g ae ha^{-1}, as low as 1/20,000× of the labeled rate, can cause visible injury to sensitive soybean (Bish et al. 2019). The proposed dicamba No Observed Adverse Effect Level range for soybean is 0.0003 to 0.033 g ae ha^{-1} (Milosevic et al. 2023). Stunting, leaf cupping, malformed stem growth (epinasty), and apical death are symptoms caused by these herbicides in sensitive weeds and crops (Behrens and Lueschen 1979). Off-target herbicide movement can be highly damaging to nearby sensitive vegetation and can also lead to reduced sensitivity in exposed weed populations over time (Vieira et al. 2020).

In Ohio and much of the Midwest, postemergence applications of dicamba have been of great concern, as soybeans without the resistance trait are highly sensitive to low doses of dicamba and vulnerable to both primary and secondary off-target movement (Egan et al. 2014; Loux and Johnson 2017). In 2017 there were 28 dicamba-related injury claims in Ohio (Bish et al. 2021). Along with sensitive soybean varieties, other sensitive vegetation such as horticultural crops, trees, and landscapes of ornamental plants are at risk from both the physical movement of droplet particles at the time of application and volatility from dicamba applied to resistant soybean varieties. There is a differential response among plant species to off-target movement of 2,4-D and dicamba. In areas with large amounts of land in cotton (*Gossipium hirsutum* L.) production, damage from 2,4-D drift and volatility is a major concern. Cotton is relatively tolerant of dicamba but is very sensitive to 2,4-D (Egan et al. 2014), and an increase in damage was reported following the introduction of 2,4-D-resistant soybeans (McKindra 2018; Unglesbee 2018). Alfalfa (*Medicago sativa* L.), wine grapes (*Vitis vinifera* L.), sweet cherries (*Prunus avium* L.), tomato (*Solanum lycopersicum* L.), watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], garden annuals, and ornamental and fruiting trees have all been reported as being sensitive to drift from these herbicides (Bish et al. 2021; Dintelmann et al. 2020). Aside from the unintended physical damage of nearby plants, producers and applicators must also consider the risk of lawsuits and legal ramifications that may arise from using these products (Rollins 2020). The release of low-volatility herbicide formulations reduced the likelihood of secondary off-target movement, but the risk of injury from volatility persists and can be influenced by tank mix pH and the addition of other herbicides (Mueller and Steckel 2019; Sosnoskie et al. 2015; Striegel et al. 2021). The future use of dicamba-resistant soybeans in Ohio and nationwide is uncertain, as label changes, EPA registration, lawsuits, and herbicide resistance threaten the longevity of these products (Kirk Hall 2024a, 2024b; Unglesbee 2020).

One potential effect of unintended off-target movement of dicamba and 2,4-D from postemergence applications to resistant soybean systems is that on potentially sensitive horticultural and agronomic crops growing nearby. Hemp is a crop with renewed interest in Ohio and has garnered attention from growers across the state seeking to diversify cropping systems and income streams (Essman 2018). The three main outputs of hemp production are fiber, seed, and flower (metabolites or cannabinoids). Much like agronomic crops such as soybean and corn (*Zea mays* L.), genetics plays a large role in the production of hemp, and variation between different cultivars has a considerable effect on their biological and morphological makeup (Campbell et al. 2019). Of these three end products, hemp for metabolite production garnered the most attention from Ohio growers because of the potential for high profit margins, and as a result of more infrastructure in place to support the processing and production of metabolites than grain or fiber (Reese 2021). A great deal of research is needed to understand better the potential influence of growing this crop in outdoor settings adjacent to agronomic crops in Ohio and the United States.

The objectives of these studies were to determine (1) the vegetative response of hemp to rates simulating different off-target events of 2,4-D and dicamba herbicides and (2) the effect of rates simulating different off-target events of 2,4-D on vegetative and reproductive parameters of hemp, including the yield of metabolites such as CBD. The goal of this study was to simulate the potential response of hemp grown in proximity to herbicide-resistant soybean where postemergence applications of 2,4-D or dicamba herbicides might take place, and there is a high risk of physical drift or volatility.

Materials and Methods

To evaluate the response of hemp to simulated off-target events of 2,4-D and dicamba, two studies were conducted, and each replicated twice from 2020 through 2022 in the greenhouse and a growth chamber (Model PGR15; Conviron, Winnipeg, MB, Canada) within Kottman Hall in Columbus, OH. Vegetative cuttings of a cannabinoid hemp variety 'Tangerine' (NY Hemp Source, LLC, New York, NY) were collected from cloned mother plants with sanitized pruning shears. The tips of hemp cuttings were dipped into a rooting hormone (Rootech Indole-3 Butyric Acid Cloning Gel; Technaflora, Vancouver, BC, Canada), and placed under a vented dome in rockwool cubes (Indoor Gardens; Columbus, OH). Rockwool cubes were kept moist with a solution of water and a cloning nutrient solution, mixed to label instructions (Clonex Clone Solution; Hydrodynamics International Inc., Lansing, MI). Once the cuttings had sufficient root mass as visible at the bottom of the rockwool cube, they were placed into 13-cm pots filled with approximately 190 g of a peat-based media (Metro-Mix 830; Sun Gro Horticulture, Agawam, MA). Plants were treated approximately 2 to 4 wk following transplanting, when plants reached 12 to 18 cm in height. This is the growth stage at which hemp plants are most likely to be during the time of concern for potential off-target events. Treatments were applied with a single-nozzle Allen track spray chamber (Allen Machine Works, Midland, MI) set to apply 140 L ha^{-1} with a flat spray tip (8001EVS; TeeJet Technologies®, Springfield, IL). Following application, plants were placed on separate carts by treatment for approximately 2 h. Irrigation resumed 24 h following treatment.

Greenhouse Study

Following successful cutting establishment, a greenhouse trial was initiated in January 2021 and was repeated in time with a second run initiated in August 2021. At 14 DAT, the hemp plants were transplanted into 1.8-L pots. Media was supplemented with a pelleted slow-release fertilizer at a labeled use rate for the duration of the trial (approximately 8 g pot^{-1}; Osmocote; Scotts Miracle-Gro, Marysville, OH). Greenhouse lighting consisted of ambient light supplemented with metal halide lights set to an 18-h day length to keep plants in a vegetative state for the duration of the study. Greenhouse temperature was set at 25.5 C during the day and 21 C at night. Treatments were arranged in a randomized complete block design with three replications of 13 treatments. Treatments consisted of six rates (560, 56, 5.6, 0.56 0.056, 0.0056 g ae ha^{-1}) of dicamba (Xtendimax with VaporGrip; Bayer CropScience, St. Louis, MO), six rates (1,060, 106, 10.6, 1.06, 0.106, 0.0106 g ae ha^{-1}) of 2,4-D (Enlist One; Corteva Agriscience, Indianapolis, IN), and a nontreated control (water). These rates were intended to represent the different types of potential off-target movement as follows: the 1× rate served as the field application rate control; the 1/10× rate represented a sprayer hygiene error such as tank contamination; the 1/100× rate represented particle drift from an adjacent field; and the 1/1,000× rate represented vapor drift (Egan and Mortensen 2012; Egan et al. 2014). Other rates were included to evaluate the potential influence of intermediate and lower rates and to assess the general sensitivity of hemp to these growth regulator herbicides.

Measurements were collected 14 and 28 DAT and at trial termination 42 DAT. These measurements included evaluations of visible injury for growth regulator herbicides on a scale of 0 to 100% (adapted from Behrens and Lueschen 1979 and Egan and Mortensen 2012), plant height, branching (number attached to the main stem), plant fresh weight, and plant dry weight at trial termination 42 DAT. To evaluate weight, plants were clipped at the soil surface and fresh weight was measured. Plants were then placed into paper bags and dried for 3 d at 55 C, after which plant dry weight was measured.

Growth Chamber Study

Hemp plants were transplanted into 3.8-L pots with approximately 400 g of media (Metro-Mix 830; Sungro Horticulture, Agawam, MA) before herbicide application and remained in these pots for the duration of the trial. Because of size constraints within the growth chamber, light was regulated to keep the hemp at a manageable size for the purposes of space and plant health. The media served as the source of nutrients until plants entered the reproductive phase. Once in the reproductive phase, hemp plants in the growth chamber were fertilized using mixtures of silicon, iron, Canna Flores (2-2-4), and Canna Boost (0-1-1) (CANNA, London, UK), per label and growth stage guidelines.

Plants were treated with myclobutanil (Eagle; Dow AgroSciences LLC, Indianapolis, IN), or raw milk dilutions (80% water, 20% raw milk, OSU Waterman dairy farm; Bettiol 1999) as needed for powdery mildew (*Golovinomyces orontii*) management. Thrips (*Thysanoptera* spp.) were managed using predatory beneficial mites (*Amblyseius cucumeris*; Arbico Organics, Oro Valley, AZ) in both studies. Growth chamber lighting was provided via high-pressure sodium lights set to an 18-h day length during the vegetative stage. At 14 DAT, the lighting was adjusted to 12-h day length to induce reproductive growth. Foliage on very small branches at the base of all plants was removed at that time to increase airflow and reduce the risk of disease. As a result, branching at harvest was not evaluated. Growth chamber temperature was set to range from 24.5 to 25.5 C. Temperature, humidity, and lighting were managed according to plant growth stage and to prevent infections from diseases common in hemp production, such as botrytis (*Botrytis cinerea*).

Treatments were arranged in a randomized complete block design with three replications of 11 treatments. Treatments consisted of 10 rates (1,060, 106, 32, 10.6, 3.2, 1.06, 0.32, 0.106, 0.032, 0.0106 g ae ha^{-1}) of 2,4-D (Enlist One, Corteva Agriscience, Indianapolis, IN) and a nontreated control (water). Measurements were collected 14 and 28 DAT and at the time of harvest. These measurements consisted of evaluations of visible injury for growth regulator herbicides on a scale of 0 to 100% (adapted from Behrens and Lueschen 1979 and Egan and Mortensen 2012), plant height, and branching (number attached to the main stem). Flower maturity was determined using a hand lens with a light to evaluate trichome development and color. Harvest was initiated when trichomes became cloudy and were just turning amber (Mahlberg and Kim 2004). At harvest, plants were clipped at the soil surface and fresh weight was measured. Plants were then hung upside down in the growth chamber with the lights off, humidity at 50%, and a temperature range of 20 to 24 C until buds were sufficiently dry to process (10 to 23 d). Bud (flower) weight and metabolite levels within the dried buds were also measured to evaluate the potential influence on yield. Extraneous branches containing buds were included in the harvest, and all buds were collected and pooled for weight and metabolite analysis. Liquid chromatography–mass spectrometry was performed (Rodriguez-Saona Lab; Department of Food Science and Technology, The Ohio State University, Columbus, OH) to quantify % (w/w) of CBD and delta-9 THC as well as the acidic precursors CBDA and THCA. These precursors are converted to CBD and THC by decarboxylation, a process that is regulated by temperature and occurs naturally over time, or more rapidly via heat exposure (Wang et al. 2016).

Data Analysis

Data were analyzed as a randomized complete block design using PROC GLIMMIX in SAS 9.4 (SAS Institute, Cary, NC). Analytical procedures were like those performed in Dintelmann et al. (2020), in terms of combining herbicide and rate for comparison with the nontreated control for measurements of height, branching, and weight. For the evaluation of injury ratings, herbicide, rate, and the interaction between herbicide and rate were fixed effects, and trial run and replication nested within trial run were random effects. For plant height, branching, fresh plant weight, dry bud weight, and metabolite levels, herbicide and rate were combined for comparison with the nontreated control. The global F-test was used to evaluate significance, and treatment means were separated using the P values for differences of the least significant means at an alpha value of ≤0.05. Visual evaluations of herbicide injury at very low levels can be difficult to detect. Some injury ratings at the 14-DAT evaluation were suspected to be false positives because of leaf crinkling or symptomology that was present but not necessarily associated with herbicide injury. These ratings were removed from the data if they were both (1) equal to or less than 5% injury and (2) recorded as no injury present at the 28 DAT evaluation (Egan and Mortensen 2012).

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Results and Discussion

Greenhouse Study

The interaction between herbicide and rate was significant for injury ratings at both evaluation timings following herbicide application. The 1× rates of dicamba and 2,4-D were not different from one another, and caused 72% and 70% injury at 14 DAT, respectively. Symptomology associated with this range included epinasty, leaf cupping, and strongly malformed terminal growth. The 1/10× rate of dicamba caused 30% injury or cupping of the terminal leaflets and crinkles on secondary leaves. This was greater than the injury caused by the 1/10× rate of 2,4-D, which was 4% 14 DAT. There was no difference between the 1/10× rate of 2,4-D and the lower rates of either herbicide at that time (Table 1). At 28 DAT, the 1× rate of dicamba caused greater injury than the 1× rate of 2,4-D. Injury symptoms at 1× rates for this evaluation included those observed 14 DAT, swelling of stem tissue, and limited shoot growth. The 1/10× rate of dicamba caused cupping and crinkling of affected leaves, but there was little to no visible injury from the lower rates of either herbicide at the 14 or 28 DAT evaluations (Table 1).

The greatest reduction in the hemp height occurred in treatments where 2,4-D and dicamba were applied at the 1× rate (Table 2). Height at the 1/10× rate of dicamba was reduced relative to the nontreated control 14 and 28 DAT but was not always different from the 1/10× rate of 2,4-D or other rates of dicamba, except for the 1× and 1/100,000× rates. The height of plants treated with the 1/10× rate of 2,4-D was not different from the nontreated control. At 42 DAT, plants treated with 1× rates of either herbicide or the 1/10× rate of dicamba were 9.4 to 24.7 cm shorter than the nontreated control (Table 2). Growth regulator herbicides at sublethal rates may cause stunting and yield loss in sensitive crops at certain rates, growth stages, and environmental conditions (Johnson et al. 2012; Kelley et al. 2005; Marple et al. 2008). Sublethal rates of growth regulator herbicides can also stimulate plant growth, enlarge fruit size, and increase yield (Auch and Arnold 1978; Belz and Duke 2017). These effects are dependent upon rate, environment, and species. The number of plant branches was unaffected by herbicide and sublethal rates 14 DAT (Table 3). At 42 DAT, none of the rates lower than 1× of both herbicides influenced plant branching (Table 3). In this study, rates of 2,4-D and dicamba lower than the labeled field use rate (1×) did not influence fresh or dry plant weight relative to the nontreated control. Only when hemp was treated with 1× rates of either herbicide were fresh and dry weights reduced (Supplementary Materials Table 1).

Table 1. Injury to hemp from simulated off-target rates of 2,4-D and dicamba based on visual evaluations 14 and 28 d after treatment (DAT) in a greenhouse study evaluating vegetative effects of growth regulator herbicides in Columbus, OH, from 2020 to 2022.[a,b]

Herbicide	Rate	Hemp injury 14 DAT		Hemp injury 28 DAT	
		%			
Dicamba	1×	72	a	78	a
Dicamba	1/10×	30	b	20	c
Dicamba	1/100×	3	c	1	d
Dicamba	1/1000×	0	c	0	d
Dicamba	1/10,000×	0	c	0	d
Dicamba	1/100,000×	0	c	0	d
2,4-D	1×	70	a	68	b
2,4-D	1/10×	4	c	3	d
2,4-D	1/100×	0	c	0	d
2,4-D	1/1000×	0	c	0	d
2,4-D	1/10,000×	0	c	0	d
2,4-D	1/100,000×	0	c	0	d

[a]Means within a column followed by a different letter are significantly different based on the *lsmeans* function at $\alpha = 0.05$.
[b]Herbicide rate fractions are relative to the full labeled field use rates in resistant soybean (1×) of 560 g ae ha^{-1} of dicamba and 1,060 g ae ha^{-1} of 2,4-D.

Table 2. Hemp height 14, 28, and 42 d after treatment (DAT) from simulated off-target rates of 2,4-D and dicamba in a greenhouse study evaluating vegetative effects of growth regulator herbicides in Columbus, OH from 2020 to 2022.[a,b]

Herbicide	Rate	Hemp height 14 DAT		Hemp height 28 DAT		Hemp height 42 DAT	
		cm					
Nontreated	0	30.0	abc	41.7	abc	55.4	ab
Dicamba	1×	19.6	e	19.8	e	30.7	e
Dicamba	1/10×	25.6	d	35.8	d	46.0	d
Dicamba	1/100×	26.8	bcd	39.5	bcd	52.1	abcd
Dicamba	1/1,000×	27.0	bc	35.8	d	46.8	dc
Dicamba	1/10,000×	28.2	bcd	39.9	bcd	51.0	bcd
Dicamba	1/100,000×	30.4	ab	44.0	ab	56.3	ab
2,4-D	1×	18.2	e	22.0	e	36.5	e
2,4-D	1/10×	25.8	cd	38.0	dc	53.0	ab
2,4-D	1/100×	30.3	ab	44.6	ab	54.9	ab
2,4-D	1/1,000×	32.6	a	45.7	a	57.8	a
2,4-D	1/10,000×	28.5	abcd	39.7	bcd	52.7	abc
2,4-D	1/100,000×	29.0	abcd	39.9	bcd	52.4	abc

[a]Means within a column followed by a different letter are significantly different based on the *lsmeans* function at $\alpha = 0.05$.
[b]Herbicide rate fractions are relative to the full labeled field use rates (1×) of 560 g ae ha^{-1} of dicamba and 1,060 g ae ha^{-1} of 2,4-D.

Growth Chamber Study

The 1× and 1/10× rates of 2,4-D caused visible injury of hemp 14 and 28 DAT in the growth chamber study. The 1× rate caused substantial visible injury, ranging from 82% to 83% (Table 4). Symptomology was consistent with growth regulator herbicide injury and included leaf cupping, stem swelling, epinasty, and necrosis. The 1/10× rate caused 10% injury 14 DAT, comprised of crinkles present in terminal leaves to mild cupping of leaflets. This effect was not as visible at the 28 DAT evaluation, and only slight crinkles were detected on some plants. The greatest influence on height occurred in plants that had been treated with the 1× rate of 2,4-D (Table 5). Plants treated with the 1/10× rate of 2,4-D were shorter than some lower rates 14 DAT but were not different from the nontreated control, and there was no difference 28 DAT or at harvest. There was no difference in branching 14 or 28 DAT (data not shown). Fresh plant weight did not differ between the rates lower than 1× and the nontreated control (Supplementary Materials Table 2). Metabolite content was not quantified for plants treated with the 1× rate because of a lack of bud development or plant death. There was no difference in CBD between the nontreated plants and those that received an application of a rate lower than 1× of 2,4-D in this study (Supplementary Materials Table 2).

Practical Implications

The 1× rates of 2,4-D and dicamba did not always cause the death of all hemp plants in these studies. Plants exposed to labeled use rates (1×) had severe symptomology, including leaf cupping,

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Table 3. Average number of hemp branches on the primary stem 14, 28, and 42 d after treatment (DAT) of simulated off-target rates of 2,4-D and dicamba in the greenhouse study evaluating vegetative effects of growth regulator herbicides in Columbus, OH from 2020 to 2022.[a,b]

Herbicide	Rate	Hemp branches 14 DAT	28 DAT		42 DAT	
		No.				
Nontreated	0	3.8	11.8	ab	14.3	a
Dicamba	1×	2.7	4.3	c	7.2	b
Dicamba	1/10×	3.8	9.8	b	13.2	a
Dicamba	1/100×	3.3	11.5	ab	13.7	a
Dicamba	1/1,000×	3.2	11.2	ab	14.0	a
Dicamba	1/10,000×	4.5	12.5	a	14.5	a
Dicamba	1/100,000×	3.7	11.8	ab	14.3	a
2,4-D	1×	2.0	3.7	c	6.2	b
2,4-D	1/10×	3.7	11.8	ab	14.0	a
2,4-D	1/100×	4.5	13.2	a	14.7	a
2,4-D	1/1,000×	4.3	12.2	a	14.5	a
2,4-D	1/10,000×	4.2	12.2	a	15.5	a
2,4-D	1/100,000×	4.0	11.3	ab	14.3	a

[a]Means within a column followed by a different letter are significantly different based on the *lsmeans* function at $\alpha = 0.05$.
[b]Herbicide rate fractions are relative to the full labeled field use rates (1×) of 560 g ae ha^{-1} of dicamba and 1,060 g ae ha^{-1} of 2,4-D.

Table 4. Visual evaluations hemp injury resulting from applications of simulated off-target rates of 2,4-D taken 14 and 28 d after treatment (DAT) from hemp grown in a growth chamber study evaluating vegetative and reproductive effects of growth regulator herbicide 2,4-D in Columbus, OH from 2020 to 2022.[a,b]

Herbicide	Rate	Hemp injury 14 DAT		28 DAT	
		%			
2,4-D	1×	83	a	82	a
2,4-D	1/10×	10	b	2	b
2,4-D	3/100×	2	c	0	c
2,4-D	1/100×	0	c	0	c
2,4-D	3/1,000×	0	c	0	c
2,4-D	1/1,000×	0	c	0	c
2,4-D	3/10,000×	0	c	0	c
2,4-D	1/10,000×	0	c	0	c
2,4-D	3/100,000×	0	c	0	c
2,4-D	1/100,000×	0	c	0	c

[a]Means within a column followed by a different letter are significantly different based on the *lsmeans* function at $\alpha = 0.05$.
[b]Herbicide rate fractions are relative to the full labeled field use rate (1×) 1,060 g ae ha^{-1} of 2,4-D.

epinasty, malformed growth, stem swelling and callusing, and death of terminal buds with occasional regrowth of secondary growing points. Some plants remained in this state, growing little, if at all, relative to the other plants for the duration of both trials. In general, plant responses to growth regulator herbicides can be difficult to quantify (Bobadilla et al. 2021), which makes detecting herbicide susceptibility difficult. Since the initiation of this work, other studies have been published evaluating hemp response to herbicides. Ortmeier-Clarke et al. (2022) found that 1× and 1/8× label rates of 2,4-D and dicamba applied postemergence resulted in biomass reduction relative to nontreated controls. In that trial, hemp was grown from seed, and plants were treated from 5 cm to 10 cm in height. A preliminary study in Nebraska found that 157 g ae ha^{-1} of clopyralid (3,6-dichloro-2-pyridinecarboxylic acid; Group 4) was among the safest herbicides tested on industrial hemp, causing only temporary injury up to 10% (Knezevic et al. 2020). A second preliminary study in Nebraska found that among

Table 5. Height of hemp in response to application of simulated off-target rates of 2,4-D measured 14 and 28 d after treatment (DAT) and at harvest from hemp grown in a growth chamber study evaluating vegetative effects and reproductive effects of growth regulator herbicide 2,4-D in Columbus, OH from 2020 to 2022.[a,b]

Herbicide	Rate	Hemp height 14 DAT		28 DAT		At harvest	
		cm					
Nontreated	0	26.9	ab	46.1	a	51.4	a
2,4-D	1×	15.2	c	16.4	b	17.5	b
2,4-D	1/10×	23.0	b	41.2	a	44.9	a
2,4-D	3/100×	29.4	a	45.7	a	51.3	a
2,4-D	1/100×	27.8	a	45.9	a	51.4	a
2,4-D	3/1,000×	27.5	ab	45.6	a	50.4	a
2,4-D	1/1,000×	25.9	ab	41.9	a	46.9	a
2,4-D	3/10,000×	29.2	a	46.3	a	50.8	a
2,4-D	1/10,000×	26.7	ab	46.3	a	51.5	a
2,4-D	3/100,000×	27.1	ab	46.6	a	51.2	a
2,4-D	1/100,000×	27.4	ab	45.0	a	50.0	a

[a]Means within a column followed by a different letter are significantly different based on the *lsmeans* function at $\alpha = 0.05$.
[b]Herbicide rate fractions are relative to the full labeled field use rate (1×) 1,060 g ae ha^{-1}.

Group 4 herbicides, both clopyralid and quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) caused initial injury followed by regrowth, but that fluroxypyr (4-amino-3,5-dichloro-6-fluoro-2-pyridyloxyacetic acid) was highly injurious (Cuvaca et al. 2020). Both studies tested industrial hemp grown for fiber production in outdoor settings, and results indicated that hemp may possess some natural tolerance to some of the Group 4 herbicides. Studies published since the initiation of this work have also shown variability in hemp response to herbicides between different hemp varieties (Ortmeier-Clarke et al. 2022).

Severe stunting and malformed growth occurred when dicamba and 2,4-D were applied at the 1× rate, which did not always result in death of all plants, but symptoms consistent with severe damage and a general inability to regrow. The 1/10× and 1/100× rates of dicamba initially caused more visible injury than the same rates of 2,4-D. Height was initially reduced in hemp plants exposed to some simulated off-target rates of dicamba, but differences were not evident at the time of termination. These studies suggest that off-target movement of growth regulator herbicides 2,4-D and dicamba may cause visible injury to nearby hemp plants. In these controlled-environment studies, rates of 2,4-D or dicamba lower than 1× did not influence branching or plant weight at the time of termination. Herbicides in these trials were applied when hemp was in the early vegetative stage, and it remains unknown at this time how simulated off-target rates may influence vegetative and reproductive parameters if hemp was exposed at later growth stages, or when exposure occurs to plants growing under field conditions. Further studies evaluating different hemp varieties and in-field settings would be required to effectively quantify the potential damage caused by the off-target movement of 2,4-D and dicamba on hemp. Other areas to be explored include residues of these and other herbicides that exist in hemp vegetative tissues, flowers, fibers, and seed following exposure.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wet.2024.38

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