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Influence of Application Timings and Sublethal Rates of Synthetic Auxin Herbicides on Soybean

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Synthetic auxin herbicides have long been utilized for the selective control of broadleaf weeds in a variety of crop and noncrop environments. Recently, two agrochemical companies have begun to develop soybean with resistance to 2,4-D and dicamba which might lead to an increase in the application of these herbicides in soybean production areas in the near future. Additionally, little research has been published pertaining to the effects of a newly-discovered synthetic auxin herbicide, aminocyclopyrachlor, on soybean phytotoxicity. Two field trials were conducted in 2011 and 2012 to evaluate the effects of sublethal rates of 2,4-D amine, aminocyclopyrachlor, aminopyralid, clopyralid, dicamba, fluroxypyr, picloram, and triclopyr on visible estimates of soybean injury, height reduction, maturity, yield, and yield components. Each of these herbicides was applied to soybean at the V3 and R2 stages of growth at 0.028, 0.28, 2.8, and 28 g ae ha^{-1}. Greater height reductions occurred with all herbicides, except 2,4-D amine and triclopyr when applied at the V3 compared to the R2 stage of growth. Greater soybean yield loss occurred with all herbicides except 2,4-D amine when applied at the R2 compared to the V3 stage of growth. The only herbicide applied that resulted in no yield loss at either stage was 2,4-D amine. When applied at 28 g ae ha^{-1} at the V3 stage of growth, the general order of herbicide-induced yield reductions to soybean from greatest to least was aminopyralid > aminocyclopyrachlor = clopyralid = picloram > fluroxypyr > triclopyr > dicamba > 2,4-D amine. At the R2 stage of growth, the general order of herbicide-induced yield reductions from greatest to least was aminopyralid > aminocyclopyrachlor = picloram > clopyralid > dicamba > fluroxypyr = triclopyr > 2,4-D amine. Yield reductions appeared to be more correlated with seeds per pod than to pods per plant and seed weight. An 18- to 26-d delay in soybean maturity also occurred with R2 applications of all synthetic auxin herbicides at 28 g ae ha^{-1} except 2,4-D. Results from this research indicate that there are vast differences in the relative phytotoxicity of these synthetic auxin herbicides to soybean, and that the timing of the synthetic auxin herbicide exposure will have a significant impact on the severity of soybean height and/or yield reductions.

Nomenclature: Aminocyclopyrachlor; aminopyralid; clopyralid; dicamba; fluroxypyr; picloram; triclopyr; 2,4-D; soybean, *Glycine max* (L.) Merr.

Key words: Growth regulator herbicides, herbicide-resistant crops, off-target spray, spray drift, tank contamination.

Los herbicidas auxinas-sintéticas han sido utilizados por un largo tiempo para el control selectivo de malezas de hoja ancha en una variedad de situaciones con y sin cultivos. Recientemente, dos compañías de agroquímicos iniciaron el desarrollo de soya con resistencia a 2,4-D y dicamba, lo que podría llevar a un incremento en la aplicación de estos herbicidas en zonas productoras de soya en un futuro cercano. Adicionalmente, pocas investigaciones han sido publicadas en relación a los efectos de aminocyclopyrachlor, un herbicida auxina-sintética recientemente descubierto, sobre la fitotoxicidad en soya. Se realizaron dos experimentos de campo en 2011 y 2012 para evaluar los efectos de dosis subletales de 2,4-D amine, aminocyclopyrachlor, aminopyralid, clopyralid, dicamba, fluroxypyr, picloram, y triclopyr sobre los estimados visuales de daño en soya, la reducción en la altura, la madurez, el rendimiento, y los componentes de rendimiento. Cada uno de estos herbicidas fue aplicado a soya en los estadios de desarrollo V3 y R2 a 0.028, 0.28, 2.8, y 28 g ae ha^{-1}. Las mayores reducciones en altura ocurrieron con todos los herbicidas, excepto 2,4-D amine y triclopyr cuando se aplicó en el estadio de desarrollo V3 en comparación con R2. Las mayores pérdidas en el rendimiento de la soya ocurrieron con todos los herbicidas excepto 2,4-D amine cuando se aplicó en el estadio R2 en comparación con V3. El único herbicida aplicado que no resultó en pérdidas de rendimiento en ninguno de los estadios de desarrollo fue 2,4-D amine. Cuando se aplicó a 28 g ae ha^{-1} en el estadio V3, el orden general de mayor a menor, de reducciones en el rendimiento de la soya inducidas por el herbicida fue: aminopyralid > aminocyclopyrachlor = clopyralid = picloram > fluroxypyr > triclopyr > dicamba > 2,4-

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D amine. En el estadio de desarrollo R2, el orden general, de mayor a menor, de reducciones en el rendimiento de la soya inducidas por el herbicida fue: aminopyralid > aminocyclopyrachlor = picloram > clopyralid > dicamba > fluroxypyr = triclopyr > 2,4-D amine. Las reducciones en el rendimiento parecieron estar más correlacionadas con el número de semillas por vaina que el número de vainas por planta o el peso de la semilla. Un retraso de 18 a 26 d en la madurez de la soya también ocurrió con aplicaciones en R2 de todos los herbicidas auxinas-sintéticas a 28 g ae ha^{-1} excepto 2,4-D. Los resultados de esta investigación indican que existen amplias diferencias en la fitotoxicidad relativa de esos herbicidas auxinas-sintéticas en soya, y que el momento de exposición a estos herbicidas tendrá un impacto significativo en la severidad de las reducciones en altura y/o rendimiento de la soya.

As of 2012, 93% of soybean hectares planted in the United States were genetically engineered, herbicide-resistant varieties (USDA 2012). Due to the increase in the occurrence of glyphosate-, protoporphyrinogen oxidase- (PPO) and acetolactate synthase/acetohydroxyacid synthase- (ALS/AHAS) resistant weed populations, several new herbicide-resistant crop offerings are expected to be introduced onto the marketplace in the near future. Among these are soybean that have been genetically modified to withstand applications of either 2,4-D (Wright et al. 2010) or dicamba (Behrens et al. 2007). Although 2,4-D was first introduced in 1945 (Troyer 2001) and dicamba in 1967 (CCME 1999), weeds with resistance to these herbicides have been relatively slow to evolve. To date, only 30 weed species in the world have been characterized with resistance to at least one of the members of the synthetic auxin herbicide family (Heap 2013). Specifically, there have been 18 species characterized with resistance to 2,4-D, and six with resistance to dicamba (Heap 2013). In these instances, resistance to synthetic auxin herbicides was associated with continuous applications of a single active ingredient over many years (Cranston et al. 2001; Heap and Morrison 1992; Holt and LeBaron 1990).

Common symptoms of off-target movement of synthetic auxin herbicides include leaf cupping, stem and leaf epinasty, and cracked and swollen stems, as well as chlorosis and necrosis (Al-Khatib and Peterson 1999; Andersen et al. 2004; Auch and Arnold 1978; Kelley et al. 2005; Sciumbato et al. 2004; Wax et al. 1969). Kelley et al. (2005) described that dicamba applications to soybean resulted in new trifoliate leaves being cupped and crinkled, with higher rates resulting in smaller leaves and reduced overall growth compared to lower rates. Symptoms associated with 2,4-D include leaf and stem epinasty, leaf elongation (often known as "strapping"), as well as swollen and cracked stems (Kelley et al. 2005; Wax et al. 1969). Clopyralid injury has been described as similar to dicamba, but with more thin, elongated leaves with parallel venation and less leaf cupping (Kelley et al. 2005). Due to the diversity of cropping systems in the United States, it is not uncommon for crops that are tolerant of synthetic auxin herbicides to be grown in close proximity to crops that are more susceptible to these herbicides, and often in rotation with one another (Wax et al. 1969). Thus, off-target movement can become a major concern due to the widespread use of 2,4-D, dicamba, picloram, triclopyr, and clopyralid in controlling emerged broadleaf weeds in corn (*Zea mays* L.), sorghum (*Sorghum bicolor* L. Moench), small grains, fallow land, turfgrasses, pastures, and rangelands. Injury to susceptible plants from off-target movement of synthetic auxins has been well documented in many crops, including cotton (*Gossypium hirsutum* L.) (Everitt and Keeling 2009; Johnson et al. 2012; Marple et al. 2007), alfalfa (*Medicago sativa* L.) (Al-Khatib et al. 1992), common sunflower (*Helianthus annuus* L.) (Derksen 1989; Lanini 2000), peanut (*Arachis hypogaea* L.) (Johnson et al. 2012), wine grape (*Vitis vinifera* L.) (Al-Khatib et al. 1993), and many other crops (Derksen 1989; Hemphill and Montgomery 1981; Lanini 2000). As a result, certain states have laws that dictate which synthetic auxin herbicides may be applied, the chemical formulation, and at what time of year the herbicide may be applied (ASPB 2012; Texas Agriculture Code 1984).

Soybean are especially at risk of injury from off-target movement of synthetic auxin herbicides due to their similar geographic vicinity and rotation with monocot crops (Wax et al. 1969). Al-Khatib and Peterson (1999) evaluated the response of soybean to reduced rates of dicamba and other herbicides when applied at the V2 to V3 stage of growth. In their research, they found that 187 g ae ha^{-1} of dicamba (33% of the labeled use rate in corn) resulted in yield reductions of 92 and 80%, respectively. In the same study, 56 g ae ha^{-1} of dicamba (10% of the labeled use rate in corn) resulted in yields 45% lower than the control (Al-Khatib and Peterson 1999). Andersen et al. (2004) found that when 5.6 g ae ha^{-1} of dicamba (1% of the labeled use rate in corn) was applied to soybean at the V3 stage of growth, yield reductions of 14 to 34% occurred. The same study reported that it took applications of 112 g ae ha^{-1} of 2,4-D (20% of the labeled use rate in corn) to provide similar yield reductions (Andersen et al. 2004). In a similar study, Kelley et al. (2005) observed that applications of 5.6 g ae ha^{-1} dicamba to V3 soybean resulted in yield reductions of 6%, whereas applications of 2,4-D at 180 g ae ha^{-1} resulted in a 25% yield reduction. Dicamba applications of 0.56 and 5.6 g ae ha^{-1} to soybean in the R2 stage of growth resulted in yield reductions of 0 and 7%, respectively, and 2 and 15% for 56 and 180 g ae ha^{-1} of 2,4-D, respectively (Kelley et al. 2005). In the same study, clopyralid was applied at 2.1 and 6.6 g ae ha^{-1} to both V3 and R2 soybean, respectively, resulting in yield reductions of 9 and 15%, respectively, for the V3 applications, and 0 and 12%, respectively, for the R2 applications (Kelley et al. 2005). With the exception of 5.6 g ae ha^{-1} dicamba, all treatments resulted in lower yields when applied at the V3 compared to the R2 stage of growth (Kelley et al. 2005). This is in contrast to previous research, which reported greater injury and yield reductions when dicamba was applied at later soybean growth stages (Auch and Arnold 1978; Slife 1956; Wax et al. 1969). Wax et al. (1969) determined that approximately 16.7 g ae ha^{-1} of dicamba applied to soybean at the prebloom and bloom growth stages resulted in yield reductions of 11 and 49%, respectively, with 2,4-D applications at these stages resulting in no yield losses. In the same study, 8.75 g ae ha^{-1} of picloram resulted in soybean yield reductions of 18 and 98% when applied at the prebloom and bloom stages, respectively (Wax et al. 1969).

Delayed maturity of soybean following exposure to synthetic auxin herbicides has also been documented in a number of previous experiments (Auch and Arnold 1978; Kelley et al. 2005; Wax et al. 1969). Wax et al. (1969) observed greater maturity delay when dicamba and picloram were applied during the reproductive stages compared to earlier vegetative stages. When picloram was applied at 8.75 g ae ha^{-1} to soybean in the prebloom and bloom growth stages, soybean maturity was delayed 2 and 27 d, respectively (Wax et al. 1969). Dicamba applied at 16.7 g ae ha^{-1} to soybean in the prebloom and bloom growth stages resulted in delays in maturity of 4 and 14 d, respectively (Wax et al. 1969). Auch and Arnold (1978) also observed a delay in soybean maturity from foliar applications of dicamba throughout the reproductive growth stages. When comparing early-bloom, midbloom, early-pod, and late-pod dicamba applications, most rates and applications resulted in additional delays in maturity as soybean further developed (Auch and Arnold 1978).

A variety of research has been conducted to determine the effects of synthetic auxin herbicides on soybean phytotoxicity and yield loss. However, few of these studies have provided results pertaining to aminocyclopyrachlor and aminopyralid, which are two of the newest synthetic auxin herbicides introduced onto the marketplace. Some authors have evaluated the response of soybean to different rates of synthetic auxin herbicides and the rates selected were based on fractions of the recommended use rate of these herbicides in other cropping systems (Andersen et al. 2004; Sciumbato et al. 2004; Weidenhamer et al. 1989), whereas other authors (Everitt and Keeling 2009; Marple et al. 2007; Thompson et al. 2007) have conducted this research with equivalent rates of the synthetic auxin herbicides to determine the relative response of all synthetic auxin herbicides to each other. The objective of this research was to determine the relative effects of sublethal rates of 2,4-D amine, aminocyclopyrachlor, aminopyralid, clopyralid, dicamba, fluroxypyr, picloram, and triclopyr on visible soybean injury, height reduction, yield, and yield components when applied to plants in the V3 and R2 stages of growth.

Materials and Methods

General Trial Information. Duplicate field trials were conducted during 2011 and 2012 in Boone County, Missouri at the University of Missouri Bradford Research Center (38.9089°N, 92.20°W). The soil was a Mexico silt loam (fine, smectic, mesic Aeric Vertic Epiaqualfs) with 2.3% organic matter and pH of 6.0 in 2011 and a pH of 6.3 and organic matter content of 2.4% in 2012. On June 6, 2011

Table 1. Sources of materials used in the experiment.

Common name[a]	Trade name	Formulation[b]	Manufacturer
2,4-D amine	Weedar 64	456 g L^{-1} EC	Nufarm, Inc., Burr Ridge, IL (www.nufarm.com/US)
Dicamba	Clarity	480 g L^{-1} EC	BASF Crop Research Triangle Park, NC (www.agro.basf.com)
Clopyralid	Transline	360 g L^{-1} EC	Dow Agrosciences, Indianapolis, IN (www.dowagro.com)
Picloram	Tordon 22K	240 g L^{-1} EC	Dow Agrosciences
Triclopyr	Remedy Ultra	480 g L^{-1} EC	Dow Agrosciences
Aminopyralid	Milestone	240 g L^{-1} EC	Dow Agrosciences
Aminocyclopyrachlor	MAT28	0.50 g g^{-1} SG	DuPont Corporation, Wilmington, DE (www.dupont.com)
Fluroxypyr	Starane	180 g L^{-1} EC	Dow Agrosciences

[a] InterLock® at 0.208% v/v was added to each herbicide solution.

[b] Abbreviations: EC, emulsifiable concentrate; SG, soluble granule.

and May 22, 2012, Asgrow 3803 glyphosate-resistant soybean were planted into a conventionally-tilled seedbed in rows spaced 76 cm apart at a rate of 432,000 seeds ha^{-1}. All treatments were arranged in a randomized complete block (RCB) design with six replications. Individual plots were 2 by 8 m in size. In both years, the entire trial was maintained weed-free with a PRE application of sulfentrazone plus cloransulam plus pendimethalin (139 + 18 + 780 g ae ha^{-1}) followed by POST applications of glyphosate (1,121 g ae ha^{-1}). Treatments included the eight synthetic auxin herbicides listed in Table 1. Each of these herbicides was applied at the V3 and R2 stages of soybean growth at 0.028, 0.28, 2.8, and 28 g ae or ai ha^{-1}. In 2011, V3 and R2 applications were made on July 1 and August 3, respectively, whereas in 2012, V3 and R2 applications were made on June 18 and July 13, respectively. All treatments were applied with a CO_2-pressurized backpack sprayer equipped with 80025 air induction nozzles that delivered coarse to extremely coarse droplets at 140 L ha^{-1} and 117 kPa. In an effort to minimize spray drift and/or contamination between plots: (1) drift shields were established on three sides of the spray boom during treatment; (2) all treatments included a drift reduction agent (InterLock®, 0.2% v/v; Winfield Solutions LLC, P.O. Box 64589, St. Paul, MN 55164); and (3) each herbicide was applied using a specific boom that had never been used before and was designated for that active ingredient only. Monthly rainfall totals and average monthly temperatures for each year are presented in Table 2.

Table 2. Monthly rainfall (mm) and average monthly temperatures (C) from April through October in 2011 and 2012 in comparison to the 30-yr average in Boone County, Missouri.

	Rainfall			Temperature		
Month	2011	2012	30-yr average[a]	2011	2012	30-yr average[a]
	mm			C		
April	72	171	121	13.6	13.9	13.6
May	130	25	127	16.5	21.0	18.9
June	77	39	94	24.0	24.1	23.8
July	59	18	101	27.6	28.5	25.7
August	61	5	75	24.6	24.7	24.8
September	46	46	78	17.4	18.6	20.4
October	26	68	99	13.8	11.7	14.0
Total	471	372	695	—	—	—

[a] 30-yr averages (1981–2010) obtained from National Climatic Data Center (2011).

Treatment Evaluation and Data Collection. Visible herbicide injury and soybean height were evaluated at 2 and 4 wk after treatment (WAT). Visible estimates of injury were evaluated on a scale from 0 to 100%, where 0 equals no injury and 100 was equivalent to complete crop death. Soybean height was evaluated by measuring six random soybean plants per plot (three from each row) from the soil surface to the top of the central stem. Delayed maturity was measured by recording the day on which 95% of the soybean pods in each plot reached a mature color and then comparing that with the day when the nontreated control plots reached maturity. Before harvest, a sample of six random soybean plants from the center of each plot were collected and used for yield component analysis. Each sample was evaluated by counting the number of seeds per pod and pods per plant to determine an average value for each respective treatment. Soybean were harvested from the center two rows of each plot with a small plot combine, and seed yields were adjusted to 13% moisture

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content. A 100-count seed subsample was collected from each plot to determine seed weight.

Statistical Analysis. All data were checked for normality to meet basic assumptions prior to statistical analysis. Visible estimates of injury, soybean height, yield component analyses, and soybean yield were subjected to ANOVA using the PROC MIXED procedure in SAS (SAS 9.2, SAS® Institute Inc.) and tested for appropriate interactions. Year–location combinations were considered an environment sampled at random, as suggested by Carmer et al. (1989) and Blouin et al. (2011). Herbicide, herbicide rate, and application timing were considered fixed effects in the model, whereas environment, replications, subsamples, and interactions within environment were considered random effects. Analyses were performed on the means and least squares means and detected using Fisher's protected LSD at $\alpha = 0.05$.

Results and Discussion

Visible Estimates of Injury. At 2 WAT, injury symptoms were dependent on herbicide and rate, regardless of growth stage (Table 3). In general, injury intensity increased with increasing herbicide rates. No significant injury was noted following any application of 2,4-D amine. Soybean injury was greatest in response to aminopyralid, aminocyclopyrachlor, picloram, clopyralid, and dicamba, and least with triclopyr and 2,4-D amine (Table 3).

By 2 WAT, 28 g ha^{-1} aminocyclopyrachlor and picloram applied at the V3 stage of growth resulted in terminal clusters of undeveloped buds, moderate epinasty, and chlorosis, with noticeable cupping of leaves. Applications of aminopyralid and clopyralid at the same rate resulted in more necrotic buds and bleached tissues, but less cupping than many of the other synthetic auxin herbicides. Although there were varying degrees of symptomology observed, by 2 WAT of the V3 application timing, 28 g ha^{-1} aminopyralid, aminocyclopyrachlor, picloram, clopyralid, and fluroxypyr resulted in 56 to 73% visible soybean injury, which was the highest observed in these trials (Table 3). Dicamba and triclopyr at 28 g ha^{-1} resulted in intermediate levels of soybean injury at 44 and 29%, respectively, with soybean exhibiting fewer necrotic buds and overall leaf cupping in response to these herbicides. Although leaf cupping is more characteristic of dicamba exposure to soybean, at 28 g ha^{-1} leaves that developed following herbicide treatment did not expand further than bud clusters; thus, visible leaf cupping was minimal. Similar symptoms have been described previously (Al-Khatib and Peterson 1999; Andersen et al. 2004; Auch and Arnold 1978; Kelley et al. 2005; Wax et al. 1969; Weidenhamer et al. 1989). When applied at the V3 stage of growth, 28 g ha^{-1} 2,4-D amine resulted in only 3% soybean injury, which was the lowest level of injury observed in these experiments. There were no leaf or stem epinastic symptoms observed following treatment with triclopyr or 2,4-D amine at any rate.

Applications of aminopyralid, picloram, clopyralid, aminocyclopyrachlor, and dicamba at 2.8 and 0.28 g ha^{-1} to soybean in the V3 stage of growth caused noticeable leaf cupping and leaf mottling/puckering, as well as chlorotic, undeveloped bud clusters 2 WAT. Due to fewer necrotic buds and stems, visible injury values were overall lower compared to the 28 g ha^{-1} rate of these same herbicides. In response to V3 applications of 0.028 g ha^{-1} aminopyralid and dicamba, soybean exhibited a moderate degree of leaf cupping and chlorosis of leaf edges, with dicamba displaying more cupped bud clusters than the other synthetic auxin herbicides. No significant soybean injury was noted 2 WAT of the V3 applications of 0.028 g ha^{-1} aminocyclopyrachlor and 0.028, 0.28, and 2.8 g ha^{-1} 2,4-D, triclopyr, and fluroxypyr (Table 3).

Aminopyralid, clopyralid, picloram, and aminocyclopyrachlor applied at 28 g ha^{-1} to R2 soybean resulted in the greatest injury (30 to 39%) 2 WAT (Table 3). These treatments resulted in terminal bud death, loss of apical dominance/expansion, and severe stem chlorosis and epinasty. Soybean stems had splits, callouses, and angles of 45 to 120 degrees. These symptoms predominantly occurred on newer plant tissues, and therefore visible injury ratings were overall much lower than V3 applications. Equivalent applications of dicamba and triclopyr to R2 soybean resulted in similar bud necrosis/death, but less epinasty and chlorosis. Overall injury was 15 and 18% in response to 28 g ha^{-1} triclopyr and dicamba, respectively (Table 3). R2 applications of 0.028, 0.28, and 2.8 g ha^{-1} dicamba all resulted in similar levels of leaf cupping/mottling. At the same timing, 0.028, 0.28, and 2.8 g ae ha^{-1} of aminopyralid and clopyralid resulted in terminal leaf cupping/chlorosis and bud abortions,

Table 3. Soybean injury, rate of maturity, and height in response to eight synthetic auxin herbicides applied at the V3 and R2 stages of soybean growth combined across 2011 and 2012.

		Injury[a]				Soybean height				Maturity delay[b]	
		2 WAT		4 WAT		2 WAT		4 WAT			
Herbicide	Rate	V3	R2	V3	R2	V3	R2	V3	R2	V3	R2
	g ae ha^{-1}	%[cd]				% of nontreated control[cd]				No. days[cd]	
2,4-D amine	0.028	2	0	1	0	96	102	103	103	0	0
	0.28	1	0	1	1	102	100	101	100	0	0
	2.8	1	0	0	0	99	101	101	101	0	0
	28	3	0	0	0	94	95	99	98	0	0
Aminocyclopyrachlor	0.028	5	3	2	3	103	100	104	101	0	0
	0.28	11	9	4	8	95	97	99	99	0	0
	2.8	32*	13	11	14	78	85*	83*	76	4	10*
	28	70*	33	63*	29	52	68*	47	59*	8	23*
Aminopyralid	0.028	31*	12	7	9	87	91	92*	86	1	1
	0.28	41*	11	14	11	84	91*	88	84	1	1
	2.8	48*	14	43*	13	74	80*	66	71	3	16*
	28	73*	39	65*	34	44	59*	26	53*	21	23*
Clopyralid	0.028	7	10	1	7*	93	102*	97	101	0	0
	0.28	11	12	2	8*	92	96	95	93	0	0
	2.8	41*	14	7	14*	83	86	83	80	2	1
	28	60*	30	68*	21	52	56	35	57*	8	26*
Dicamba	0.028	21	15	10	17*	89	94	94	89	0	0
	0.28	28	17	9	16*	85	93*	90	85	3	0
	2.8	32*	14	9	15*	79	86*	75	77	3	1
	28	44*	18	12	14	80*	74	74*	62	5	24*
Fluroxypyr	0.028	1	0	0	1	102	102	101	102	0	0
	0.28	1	1	0	2	101	99	101	100	0	0
	2.8	4	1	1	2	93	97	96	99	0	0
	28	56*	15	36*	8	58	74*	59	72*	4	18*
Picloram	0.028	10	5	2	4	98	98	99	101	0	0
	0.28	11	7	2	6	98	96	99	98	0	0
	2.8	30*	10	5	12*	85	85	90	84*	1	10*
	28	69*	32	66*	25	52	64*	46	56*	8	26*
Triclopyr	0.028	1	0	0	0	97	99	100	101	0	0
	0.28	3	1	1	1	98	98	98	100	0	0
	2.8	2	0	0	1	98*	92	99	96	0	0
	28	29	15	7	10	71	76	78*	62	0	18*
Nontreated	—	1	0	0	0	100	100	100	100	0	0
LSD (0.05)[d]	—	18	9	5	3	6	4	6	4	1	1

[a] Injury ratings on a scale of 0 (no injury) to 100% (complete kill).

[b] Measured by recording the day when 95% of the soybean pods in each plot reached maturity compared to the nontreated control.

[c] Values followed by an asterisk indicate a significantly higher level of visible injury, soybean height reduction, and maturity delay between the V3 and R2 applications of a given active ingredient and rate, LSD (0.05).

[d] LSD (0.05) within a column between herbicide treatments applied at the same soybean growth stage.

with 0.28 and 2.8 g ha^{-1} of aminopyralid displaying unexpanded/undeveloped bud clusters and stem epinasty. Aminocyclopyrachlor at 2.8 g ha^{-1} exhibited chlorotic terminal leaf cupping and mottling, as well as undeveloped bud clusters similar to aminopyralid. The 0.028, 0.28, and 2.8 g ha^{-1} rates of picloram applied at R2 resulted in slight cupping of the newest trifoliates. This differential response to the eight synthetic auxin herbicides was not surprising because plants absorb, translocate, and metabolize herbicides at different rates.

By 4 WAT, all soybean exposed to synthetic auxin herbicides at the V3 growth stage, except for

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28 g ha^{-1} clopyralid, picloram, aminocyclopyrachlor, and 2.8 and 28 g ha^{-1} of aminopyralid, had recovered from 2 wk prior (Table 3). Conversely, soybean treated with synthetic auxin herbicides at the R2 stage of growth did not recover as well, and in many instances exhibited similar levels of injury as 2 WAT.

Soybean Height. Previous research has correlated soybean yield loss with reductions in plant height following an application of dicamba (Weidenhamer et al. 1989). In this research, reductions in plant height were generally correlated with, but less severe than, visible injury estimates. Greater height reductions occurred with all herbicides except for 2,4-D amine and triclopyr when applied at the V3 compared to the R2 stage of growth (Table 3). Auch and Arnold (1978) observed that the greatest soybean height reductions from dicamba applications were made at the early-bloom stage, as compared to applications made at vegetative growth stages or from midbloom through late-pod. At 2 WAT, soybean height was not reduced following V3 or R2 applications of 2,4-D and triclopyr at 0.028, 0.28, and 2.8 g ha^{-1}, and for aminocyclopyrachlor, fluroxypyr, and picloram at 0.028 and 0.28 g ha^{-1}, but was reduced for all rates of aminopyralid, clopyralid, and dicamba (Table 3). At 2 WAT when herbicides were applied at 28 g ae ha^{-1}, soybean height expressed as a percent of the nontreated was equal for V3 and R2 applications of 2,4-D (94 and 95% of the nontreated) and clopyralid (52 and 56%), but height reduction for 28 g ha^{-1} was greater for R2 compared to V3 applications for aminocyclopyrachlor (52 and 68%), aminopyralid (44 and 59%), dicamba (80 and 74%), fluroxypyr (58 and 74%), picloram (52 and 64%), and triclopyr (71 and 76%). At 4 WAT soybean height compared with the nontreated control was reduced with V3 and R2 applications of aminocyclopyrachlor, aminopyralid, clopyralid, dicamba, and picloram at 2.8 and 28 g ha^{-1} and with fluroxypyr and triclopyr at 28 g ha^{-1}.

Soybean Maturity. The specific herbicide, herbicide rate, and timing of herbicide application had significant effects on the delay in soybean maturity (Table 3). In general, applications made to soybean in the R2 stage of growth resulted in greater delays in soybean maturity compared to V3 herbicide applications. Wax et al. (1969) also observed greater maturity delays following dicamba and picloram applications to soybean in the reproductive stages of growth compared to the prebloom stages of growth. Applications of aminocyclopyrachlor, clopyralid, dicamba, and picloram at 28 g ha^{-1} delayed maturity 5 to 8 d when applied at the V3 stage of growth and 23 to 26 d when applied at the R2 stage of growth (Table 3). V3 and R2 applications of 28 g ha^{-1} aminopyralid delayed maturity 21 and 23 d, respectively. Applications of aminocyclopyrachlor, aminopyralid, dicamba, and picloram at 2.8 g ha^{-1} delayed soybean maturity 1 to 4 d when applied at the V3 stage of growth and 1 to 16 d when applied at the R2 stage of growth. Soybean maturity was not delayed for 2,4-D regardless of application timing or for triclopyr at all rates at V3. Wax et al. (1969) also reported that dicamba delayed soybean maturity more than 2,4-D. Triclopyr applied at R2 delayed maturity 18 d for only the 28 g ha^{-1} rate.

Soybean Yield. In general, herbicide treatments and rates resulting in less than 10% injury 2 WAT did not reduce yield (Tables 3 and 4). Except for either application timing of 2,4-D amine and V3 applications of dicamba, all herbicides resulted in greater soybean yield loss with increasing herbicide rates (Table 4). Additionally, greater soybean yield loss occurred with applications made to R2 compared to V3 soybean, except for 2,4-D amine, which did not reduce soybean yield compared to the nontreated control at either application timing. This result is consistent with previous research; Slife (1956) and Wax et al. (1969) reported less yield reduction from early compared to later 2,4-D treatments, and Robinson et al. (2013) reported soybean yield losses of 5% with V2 or R2 applications of 2,4-D at rates up to 116 g ha^{-1}. Soybean yield after R2 applications of dicamba ranged from 2 to 67% less than the nontreated control, but V3 applications of dicamba did not result in any soybean yield loss. This result is in agreement with previous research, where 9 to 11 g ha^{-1} dicamba reduced yields in the flowering stage, compared with prebloom applications that required rates of 56 to 70 g ha^{-1} to reduce yields (Auch and Arnold 1978; Wax et al. 1969). In relation to the significant injury following early-season dicamba applications, Behrens and Leuschen (1979) determined yield reductions following dicamba drift injury to soybean at the first trifoliate stage were associated with injury ratings of 60 to 70 or more.

Table 4. Soybean yield and yield components in response to eight synthetic auxin herbicides applied at the V3 and R2 stages of soybean growth combined across 2011 and 2012.

Herbicide	Rate	Soybean yield[ab]		Seeds per pod[ab]		Pods per plant[ab]		Seed weight[ab]	
		V3	R2	V3	R2	V3	R2	V3	R2
	g ae ha^{-1}	——kg ha^{-1}——		——————No.——————				—g 100 seeds^{-1}—	
2,4-D amine	0.028	4,345	4,340	2.22	2.33	45	55*	16.77	16.62
	0.28	4,306	4,395	2.27	2.22	45	53*	16.68	16.83
	2.8	4,462	4,354	2.26	2.20	49	48	16.63	16.66
	28	4,306	4,373	2.23	2.20	51	45	16.88	17.25
Aminocyclopyrachlor	0.028	4,513	4,466	2.28	2.24	46	48	16.72	17.11
	0.28	4,440	4,594	2.20	2.18	46	45	16.40	17.18
	2.8	4,222*	3,823	2.27*	2.02	48*	37	16.24	19.37*
	28	1,927*	435	2.23	0.19*	45*	7	16.42	17.16*
Aminopyralid	0.028	4,141	4,016	2.27	2.17	45	40	16.37	17.99*
	0.28	4,086	3,898	2.26*	2.07	49*	40	16.25	17.54*
	2.8	3,329*	2,752	2.10*	1.93	44	41	16.24	18.79*
	28	423*	135	0.76*	0.01	16*	1	16.61	15.87
Clopyralid	0.028	4,369	4,640*	2.25	2.20	44	48	16.52	17.50
	0.28	4,015	4,073	2.19	2.15	47	46	16.08	17.27*
	2.8	3,944	3,795	2.24	2.00*	48*	40	16.14	18.01*
	28	1,838*	622	2.28*	0.08	49*	9	16.33	17.87*
Dicamba	0.028	4,147	4,222	2.17	2.06	45	42	16.23	18.11*
	0.28	4,260	4,052	2.17	2.07	50	43	16.35	18.35*
	2.8	4,178*	3,730	2.16*	2.00	45	39	16.44	17.73*
	28	4,128*	1,427	2.20*	0.64	50*	13	16.35	18.99*
Fluroxypyr	0.028	4,463	4,671	2.29	2.17	50	46	16.47	17.02
	0.28	4,447	4,425	2.23	2.22	45	48	16.60	16.99
	2.8	4,289	4,530	2.28	2.30	49*	40	16.80	17.35
	28	3,079*	2,306	2.30*	1.07	50*	15	16.45	18.98*
Picloram	0.028	4,464	4,511	2.27	2.27	47	44	16.79	17.11
	0.28	4,401	4,242	2.22	2.18	45	44	16.53	17.10
	2.8	4,088*	3,653	2.28	2.15	44	42	16.39	18.38*
	28	2,070*	480	2.29*	0.12	53*	10	16.34	16.67
Triclopyr	0.028	4,446	4,464	2.13	2.20	51	53	16.78	16.67
	0.28	4,360	4,550	2.25	2.23	50	49	16.67	17.07
	2.8	4,543	4,513	2.35	2.33	47	45	16.87	17.69*
	28	3,832*	2,468	2.31*	1.07	49*	11	16.45	20.41*
Nontreated	—	4327	4,327	2.27	2.27	48	48	16.70	16.70
LSD (0.05)[b]	—	267	234	0.12	0.14	8	6	0.37	0.89

[a] Values followed by an asterisk indicate a significantly higher level of soybean yield, seeds per pod, pods per plant, and seed weight between the V3 and R2 applications of a given active ingredient and rate, LSD (0.05).

[b] LSD (0.05) within a column between herbicide treatments applied at the same soybean growth stage.

Other authors (Auch and Arnold 1978; Slife 1956; Wax et al. 1969) have also noted greater yield reductions following dicamba applications to soybean in the reproductive rather than vegetative stages of growth. Conversely, Kelley et al. (2005) reported equivalent or greater yield reductions from V3 applications of dicamba, 2,4-D, and clopyralid, compared to R2 applications of these same herbicides.

Regardless of growth stage, yields were significantly reduced following 0.28, 2.8, and 28 g ha^{-1} clopyralid and 2.8 and 28 g ha^{-1} picloram. Only 2.8 and 28 g ha^{-1} aminopyralid applied to V3 soybean reduced yield, while all aminopyralid rates applied to R2 soybean resulted in yields 7 to 97% less than the nontreated control. Similarly, only 28 g ha^{-1} aminocyclopyrachlor applied to V3 soybean reduced yield, while the 2.8 and 28 g ha^{-1} rates applied at the R2 stage reduced yield 12 and 90%, respectively. Lastly, only 28 g ha^{-1} of triclopyr and fluroxypyr applied at either growth stage resulted in yields less than the nontreated control. When

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applied at 28 g ha^{-1} at the V3 stage of growth, the general order of herbicide-induced yield reductions to soybean from greatest to least was aminopyralid > aminocyclopyrachlor = clopyralid = picloram > fluroxypyr > triclopyr > dicamba - 2,4-D amine. At the R2 stage of growth, the general order of herbicide-induced yield reductions from greatest to least was aminopyralid > aminocyclopyrachlor = picloram > clopyralid > dicamba > fluroxypyr = triclopyr > 2,4-D amine.

Interestingly, certain synthetic auxin treatments resulted in yields higher than the nontreated control (Table 4). When applied at the R2 stage of growth, 0.028 g ha^{-1} clopyralid and fluroxypyr resulted in yields 313 and 344 kg ha^{-1} greater than the nontreated control. This response can be explained by a phenomenon known as herbicide hormesis (Southman and Ehrlich 1943), or the Arndt-Schultz law (Thimann 1956), which states that every toxicant is a stimulant at low levels (Schabenberger et al. 1999). Several other authors have reported stimulatory effects on field crops from low concentrations of 2,4-D and other synthetic auxin herbicides (Miller et al. 1962; Taylor 1946; Wiedman and Appleby 1972).

Soybean Yield Components. Generally, all synthetic auxin herbicides other than 2,4-D amine reduced soybean seeds per pod in response to increasing herbicide rates. All rates of 2,4-D amine resulted in seeds per pod equivalent to the nontreated control. In general, R2 applications of synthetic auxin herbicides influenced seeds per pod more than V3 applications, but the response varied by herbicide and rate (Table 4). Kelley et al. (2005) found that 5.6 g ha^{-1} dicamba reduced seeds per pod more when applied to soybean at V7 compared to V3 in 1 of 2 yr. Dicamba was the only herbicide where all rates applied to R2 soybean resulted in fewer seeds per pod than the nontreated control (Table 4). Following V3 applications, all herbicides except triclopyr and aminopyralid resulted in similar numbers of seeds per pod, regardless of herbicide rate. When compared to the nontreated control, 2.8 and 28 g ha^{-1} aminopyralid and 0.028 g ae ha^{-1} triclopyr were the only herbicides applied at the V3 timing that reduced soybean seeds per pod. Overall, seeds per pod were most affected by aminopyralid and least by 2,4-D amine; therefore, the number of soybean seeds per pod were strongly correlated with the soybean yield losses observed.

Following V3 applications, the number of pods per plant was only reduced in response to the highest rate of aminopyralid; all other synthetic auxin herbicides and rates resulted in a similar number of pods per plant as the nontreated control (Table 4). Kelley et al. (2005) reported that soybean treated at the V3 and V7 stages with 5.6 g ha^{-1} dicamba resulted in a similar number of pods per plant as the nontreated control. In contrast, following R2 applications, the number of pods per plant was highly influenced by herbicide rate. All synthetic auxin herbicides applied at the R2 stage of soybean growth resulted in significant differences in pods per plant in response to rate, with higher rates reducing pods per plant more than lower rates (Table 4). The lowest rate of 2,4-D applied to R2 soybean was the only treatment that resulted in more pods per plant than the nontreated control. All rates of aminopyralid, 2.8 and 28 g ha^{-1} dicamba, clopyralid, aminocyclopyrachlor, and fluroxypyr, and 28 g ha^{-1}picloram and triclopyr applied to R2 soybean reduced pods per plant in comparison to the nontreated control. As with seeds per pod, the differences in pods per plant was greatest with aminopyralid and least with 2,4-D.

Soybean seed weight was variable, with no consistent trend in response to either application timing. When applied at the V3 growth stage, there were no treatments that resulted in soybean seed weight greater than the nontreated control, whereas the same treatments applied to the R2 growth stage resulted in no seed weights less than the nontreated control (Table 4). Applications of 2,4-D at either soybean growth stage resulted in similar soybean seed weight as the nontreated control. Robinson et al. (2013) observed similar seed weight as the nontreated control with doses $\leq$ 560 g ha^{-1} 2,4-D. Only 0.028 g ha^{-1} dicamba, 2.8 g ha^{-1} aminocyclopyrachlor, and 0.28 and 2.8 g ha^{-1} clopyralid and aminopyralid applied to V3 soybean resulted in seed weight less than the nontreated control. Wax et al. (1969) reported > 1 g reductions in seed weight per 100 seeds following prebloom applications of 1 to 33 g ha^{-1} dicamba. Following R2 applications, all rates of dicamba, and several rates of all other synthetic auxin herbicides other than 2,4-D resulted in seed weight greater than the nontreated control (Table 4). Weidenhamer et al. (1989) also observed increases in seed weight following later applications of dicamba, whereas earlier dicamba applications reduced seed weight. Wax et al. (1969) also reported greater soybean seed weight from late- compared to early-season treatments of dicamba and picloram, noting that the increased seed size did not counteract the reduction in seed number and thus resulted in lower yields. The increase in seed weight was likely due to the reduction in the number of seeds produced.

The results from this research indicate that the risk to soybean from herbicide drift and/or tank contamination is dependent on herbicide, herbicide rate, and maturity of soybean following exposure. Overall, soybean are more likely to recover from misapplications of synthetic auxin herbicides made earlier, rather than later in the growing season. In this research, soybean exposed to synthetic auxin herbicides in early vegetative stages were able to maintain seed and pod set more efficiently than equivalent exposure to these herbicides at reproductive stages. In general, herbicide-induced injury increased with increasing herbicide rate, with aminopyralid, clopyralid, aminocyclopyrachlor, and dicamba resulting in more phytotoxicity to soybean than 2,4-D amine, triclopyr, and fluroxypyr. In this study, yield reductions were correlated with seeds per pod and pods per plant more so than seed weight.

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