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Response of Soybean Yield Components to 2,4-D

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Soybean plants exposed POST to 2,4-D can have reduced seed yield depending on the dose and time of exposure, but it is unclear how 2,4-D affects specific yield components. Objectives were to quantify soybean injury, characterize changes in seed yield and yield components of soybean plants exposed to 2,4-D, and determine if seed-yield loss can be estimated from visual assessment of crop injury. Ten rates (0, 0.1, 1.1, 11.2, 35, 70, 140, 280, 560, and 2,240 g ae ha^{-1}) of 2,4-D were applied to Becks brand 342 NRR soybean at three soybean growth stages (V2, V5, or R2). The soybeans were planted near Lafayette, IN and Urbana, IL in 2009 and 2010 and near Fowler, IN in 2009. Twenty percent visual soybean injury was caused by 29 to 109 g ha^{-1} 2,4-D at 14 d after treatment (DAT) and 109 to 245 g ha^{-1} at 28 DAT. Nonlinear regression models were fit to describe the effect of 2,4-D on seed yield and yield components of soybean. Seed yield was reduced by 5% from 87 to 116 g ha^{-1} and a 10% reduction was caused by 149 to 202 g ha^{-1} 2,4-D at all application timings. The number of seeds m^{-2}, pods m^{-2}, reproductive nodes m^{-2}, and nodes m^{-2} were the most sensitive yield components. Path analysis indicated that seeds m^{-2}, pods m^{-2}, main stem reproductive nodes m^{-2}, and main stem nodes m^{-2} were the most influential yield components in seed-yield formation. Seed-yield loss was significant ($P < 0.0001$) and highly correlated ($R^2 = 0.95$ to 0.99) to visual soybean injury ratings. A 10% seed-yield loss was caused by 35% soybean injury observed at 14 DAT, whereas a 10% seed-yield loss was a result of 40, 19, and 15% soybean injury observed at 28 DAT when soybean was exposed to 2,4-D at the V2, V5, and R2 growth stages, respectively.

Nomenclature: 2,4-D; soybean, *Glycine max* (L.) Merr.

Key words: 2,4-dichlorophenoxyacetic acid, drift, dose response, soybean injury.

After exposure to 2,4-D, soybean plants can manifest vegetative malformations and lower seed yield; however, the extent of damage will depend on rate, timing of exposure, and cultivar (Andersen et al. 2004; Kelley et al. 2005). It is likely that 2,4-D–resistant crops, including soybean, maize (*Zea mays* L.), and cotton (*Gossypium hirsutum* L.) (Wright et al. 2010) will be commercially available within the next 5 yr. If this occurs, there may be a significant increase in the use of 2,4-D during vegetative and early reproductive growth stages of soybean in current cropping systems. Subsequently, this will increase the likelihood that sensitive soybean plants will be exposed to 2,4-D.

2,4-D mimics indole-3-acetic acid, a naturally occurring plant hormone generally referred to as auxin. Synthetic auxin herbicides activate auxin response genes (Abel and Theologis 1996; Kelley et al. 2004), which leads to the overproduction of ethylene and subsequently abscisic acid (Grossmann 2003, 2010). Increased abscisic acid has been shown to cause stomatal closure, which can limit CO_2 assimilation followed by growth inhibition (Grossmann 2010).

Soybean exposure to 2,4-D primarily happens from particle drift, sprayer contamination, or volatilization. Research has documented that up to 16% of the spray solution can physically drift from the intended application area (Maybank et al. 1978; Wolf et al. 1993), but this can be mitigated in a variety of ways, including adjustments to carrier volume, nozzle selection, pressure, and not spraying when wind is blowing in the direction of sensitive vegetation. Soybean injury from 2,4-D can occur if the spray tank and boom are not properly cleaned. Moreover, under certain environmental conditions 2,4-D could also volatilize and the vapor drift could move from the treated area. Off-target damage from 2,4-D volatilization is primarily found with the ester formulations of 2,4-D (Baskin and Walker 1953).

When soybean plants are exposed to 2,4-D they exhibit injury symptoms such as leaf crinkling, and strapping, and/or twisting and bending of petioles, branches, and stems (Wax et al. 1969), commonly called epinasty. Such injury symptoms have been observed as soon as 4 h after exposure and became most severe at 14 d after treatment (DAT) at rates of 56 to 180 g ha^{-1} (Andersen et al. 2004; Kelley et al. 2005). Visual estimates of injury at 12 to 14 DAT were 5% at 11.2 g ha^{-1}, 8 to 25% at 56 g ha^{-1}, 30 to 35% at 112 g ha^{-1}, and 37 to 52% at 180 g ha^{-1} when 2,4-D was applied at the V3, V7, or R2 growth stage (Andersen et al. 2004; Kelley et al. 2005). However, Sciumbato et al. (2004) reported lower soybean injury at 14 DAT, which ranged from 1.5 to 3.6% when 0.0053 to 53 g ha^{-1} of 2,4-D were applied, but this may be because a more conservative scale was used in visual ratings. Little information exists on the effects 2,4-D rates higher than 180 g ha^{-1} have on soybean injury and how this may be related to seed-yield loss.

Other in-season analyses of 2,4-D rates on soybean have included monitoring of foliar concentration of 2,4-D at various times after exposure. The concentration of 2,4-D in foliage was highly correlated ($R^2 = -0.83$ to -0.91) to soybean seed-yield loss at 0, 6, and 12 DAT, whereas at 24 and 48 DAT the relationship between 2,4-D concentration and yield was not significant (Andersen et al. 2004). Also during the first 12 d after V3 soybean exposure to 2,4-D, plants had a 6 to 37% reduction in plant height with less reduction in height occurring at 11.2 g ha^{-1} and greater loss in height from 112 g ha^{-1} (Andersen 2003). In other work, plant height at harvest was not affected by 2,4-D when 17.5 to 140 g ha^{-1} were applied to soybean at V3 or bloom (Wax et al. 1969). However, higher rates of 2,4-D reduced plant height at harvest by 13 to 25% at 280 g ha^{-1} and from 20 to 38% at 560 g ha^{-1}, with the least reduction occurring from plants treated at 8 to 13 cm and the greatest reduction from plants treated at 76 to 81 cm (Slife 1956). The effects of 2,4-D applications on soybean plants has been correlated with

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Table 1. Sources of material for herbicides.

Common name	Trade name	Rate in active ingredient	Manufacturer
Metribuzin plus	Canopy DF	0.643 g g^{-1}	DuPont Crop Protection, Wilmington, DE (www.dupont.com)
Chlorimuron		0.107 g g^{-1}	
Glyphosate	PowerMax	660 g L^{-1}	Monsanto Company, St. Louis, MO (www.monsanto.com)
Clethodim	Tapout	116 g L^{-1}	Helena Chemical Company, Collierville, TN (www.helenachemical.com)
Trifluralin	Treflan HFP	480 g L^{-1}	Dow AgroSciences, Indianapolis, IN (www.dowagro.com)
Imazethapyr	Pursuit	240 g L^{-1}	BASF Corporation Agricultural Products, Research Triangle Park, NC (www.agro.basf.com)
Sulfentrazone plus	Authority Assist	0.33 g g^{-1}	FMC Corporation, Philadelphia, PA (www.fmc.com)
imazethapyr		0.07 g g^{-1}	
2,4-D	Weedar 64	456 g L^{-1}	Nufarm, Inc., Burr Ridge, IL (www.nufarm.com/US)

foliar 2,4-D concentration and with seed-yield loss (Andersen et al. 2004), but no data have been reported on the relationship visual injury to soybean and seed-yield loss.

Total seed-yield loss tended to be greater when soybean was exposed to 2,4-D during the reproductive growth stages compared to vegetative growth (Slife 1956; Wax et al. 1969). This was observed by Wax et al. (1969), who reported no seed-yield reduction from 2,4-D treatments at the V3 growth stage under rates up to 140 g ha^{-1}, but an 11% seed-yield loss was reported when 70– and 140–g ha^{-1} treatments were applied at soybean bloom stage. Andersen et al. (2004) reported a seed-yield reduction of 32% when 112 g ha^{-1} was applied on V3 soybean. Slife (1956) tested 2,4-D at 280 and 560 g ha^{-1} at four growth stages, which resulted in 9 to 41% and 21 to 62% yield loss, respectively. The causes of seed-yield loss can be described in greater detail by analyzing yield components. Yield components include seed mass, seed number, seeds pod^{-1}, pod number, pods reproductive $node^{-1}$, main stem reproductive node number, main stem node number, and percent reproductive nodes. Characterizing yield components of soybean after exposure to 2,4-D has been reported for seed mass, pod number, and seed number (Andersen et al. 2004; Kelley et al. 2005; Wax et al. 1969). 2,4-D applied during the vegetative growth stage of soybean increased seed mass by 16% at 17.5 g ha^{-1}, but a reduction of up to 5% was noted at 35 to 140 g ha^{-1} (Wax et al. 1969). In addition, Wax et al. (1969) reported that treatments of up to 140 g ha^{-1} had no effect on pod number and seed number. Little is known about the effects of 2,4-D on other soybean yield components.

An analysis of yield components has been useful in quantifying the effect of planting date on yield (Robinson et al. 2009) and comparing old versus new soybean cultivars (Kahlon et al. 2011). These studies showed that the number of nodes, reproductive nodes, pods, and seeds were the main contributors to higher yield. Evaluating yield components is necessary to enhance understanding of how exposure to 2,4-D affects seed yield when applied at different growth stages. The objectives of this research were to characterize the response of soybean growth stage and 2,4-D dose on soybean injury, yield components, and total seed yield of soybean, and to determine if soybean injury ratings can be used to determine seed-yield loss.

Materials and Methods

Field experiments were planted on May 28, 2009 and June 8, 2010 at the Throckmorton-Purdue Agriculture Center located near Lafayette, IN (40°18′00.32″N, 86°54′20.17″W), on May 23, 2009 and May 6, 2010 at the University of Illinois Crop Sciences Research and Education Center near Urbana, IL (40°04′55.27″N, 88°13′37.76″W), and on June 6, 2009 at Dow AgroSciences Midwest Research Center located near in Fowler, IN (40°38′01.05″N, 86°06′36.35″W). Soil type at the Lafayette location was a Throckmorton silt loam (fine–silty, mixed, superactive, mesic Mollic Oxyaquic Hapludalfs). At Urbana, IL and Fowler, IN soil type was a Drummer silty clay loam (fine–silty mixed, superactive, mesic Typic Endoaquolls). Becks brand 342NRR soybean was seeded in 38-cm rows at 430,000 seeds ha^{-1}. Plots measured 3.1 m wide and 9.1 m long. Conventional tillage was implemented for all field sites, except at Lafayette 2009, where no tillage was used.

Plots were kept weed free by applying PRE and POST herbicides and by hand weeding. Weed control at the Lafayette location in 2009 and 2010 consisted of metribuzin (158 g ai ha^{-1}) plus chlorimuron (26 g ai ha^{-1}) applied prior to soybean emergence. POST weed control at the Lafayette location in 2009 consisted of glyphosate (1,060 g ae ha^{-1}) plus ammonium sulfate (2.8 kg ha^{-1}) applied on June 19; in 2010, glyphosate (1,120 g ha^{-1}) plus ammonium sulfate (2.8 kg ha^{-1}) plus clethodim (102 g ai ha^{-1}) were applied on July 11. Clethodim was used to control glyphosate-resistant volunteer maize. At Urbana in 2009 and 2010, sulfentrazone (350 g ai ha^{-1}) plus imazethapyr (70 g ai ha^{-1}) was applied at planting. POST weed control at Urbana consisted of glyphosate (1,060 g ae ha^{-1}) plus ammonium sulfate (2.8 kg ha^{-1}) applied on June 26 and July 14, 2009 and on June 8, 2010. At the Fowler location trifluralin (1,400 g ai ha^{-1}) plus imazethapyr (70 g ai ha^{-1}) were incorporated twice prior to planting. Detailed information of herbicides utilized can be found in Table 1.

The design was a randomized complete block. Treatments were 2,4-D (dimethylamine salt) at rates of 0, 0.1, 1.1, 11.2, 35, 70, 140, 280, 560, and 2,240 g ha^{-1} applied at the V2, V5, or R2 soybean growth stages to simulate drift or overlap rates. Treatments were replicated three times. A plot was considered to have reached a certain stage of development when at least half of the plants reached the desired stage of growth (Fehr and Caviness 1977). All 2,4-D treatments were applied in 140 L ha^{-1} carrier volume with a CO_2-pressurized backpack sprayer at 138 kPa with a 3.1-m-wide boom and XR11002 flat fan nozzles (TeeJet Spraying Systems Company, Wheaton, IL 60189). To avoid cross-contamination, plots received treatments when wind speeds were < 5 km hr^{-1}, and the outside two rows of each plot were not harvested.

Visual estimates of soybean injury from 0 (no injury) to 100% (complete plant death) (Table 2) were collected at 14 and 28 DAT (Figure 1). Plant height was recorded from three arbitrarily selected plants at the R8 growth stage. At maturity, 10 plants from the middle two rows of each treatment were arbitrarily selected to determine the following yield components as outlined by Board and Modali (2005): seed mass (grams per 100 seeds), seeds m^{-2}, seeds pod^{-1}, pods m^{-2},

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Table 2. Rating scale for visual estimate of soybean injury affected by synthetic auxin herbicides.

Rating	Description
0	No injury; plant growth is normal.
10	Slight reduction in height or canopy volume; cupped or bubbled leaves on ≤ upper 10% of the plant; bent petioles; and chlorosis or necrosis.
20	Moderately crinkled leaflets (extended across ≤ upper 20% of the plant); curled petioles; reduced height and canopy volume; cupped terminal leaflets.
30	Moderate to high reduction of height and canopy; compacted internodes and plants begin to have an abnormal appearance; malformation with drawstring, fiddleneck, or cupped effects on ≤ upper 30% of the plant; many petioles curled and main stems may be bent.
40	Highly stunted plants (≤ 40% of the plant); petioles curled and main stems bent and/or starting to curl; upper leaves exhibit severe malformation and expansion of new leaves suppressed; plant may have patches of necrotic tissue.
50	Very high reduction of plant height (≤ 50% of the plant) with little likelihood of recovery from the apical meristem; new growth suppressed; formation of pods reduced or malformed; some leaf and stem tissue becomes necrotic; petioles and stems show severe twisting.
60	Severe height and canopy reduction, including any new growth from axillary buds; leaves severely cupped or fiddlenecked on ≤ 60% of the plant; petioles and stems twisted, swollen, and splitting; more extensive dieback of tissue.
70	Severe to very severe reduction of plants; new growth calloused and inhibited; most leaves severely deformed and mostly necrotic; extensive petiole bending.
80	Very severe soybean injury; ≤ 80% of the plants mainly prostrate; petioles twisted with leaves drooping; leaves are chlorotic or necrotic; stems severely twisted, swollen, and split.
90	Plant dying; ≤ 90% of the plants mainly prostrate; leaves and stems mostly chlorotic or necrotic; all petioles severely twisted, swollen, or split.
100	All plants dead.

main stem reproductive nodes m^{-2}, pods reproductive $node^{-1}$, main stem nodes m^{-2}, and percent reproductive nodes. After counting, plants were threshed and seed weight was added to the machine-harvested weight. Seed yield was adjusted to 130 g kg^{-1} moisture. Oil and protein concentrations were determined from machine harvested seed by near-infrared reflectance spectroscopy with the use of a Infratec 1229 Grain Analyzer (Foss North America, Inc., Eden Prairie, MN 55344) at the Purdue University Grain Quality Laboratory.

Statistical Methods. All data were checked for normality to meet basic assumptions before statistical analysis. Data were subjected to ANOVA with the use of the SAS MIXED procedure (SAS Institute, Cary, NC) to test for significant effects of 2,4-D rate, treatment timing, and the interaction of



Figure 1. Soybean injury at 14 d after 0, 140, and 280 g ha^{-1} 2,4-D treatments at Lafayette, IN in 2009 (V5 growth stage pictured) and Fowler, IN in 2009 (V2 and R2 growth stages pictured). The untreated plants at V2 (a), V5 (b), and R2 (c) had no injury. At 140 g ha^{-1} 2,4-D, soybean injury in each experiment averaged of 43% injury at the V2 growth stage (d), 42% injury at the V5 growth stage (e), and 47% injury at the R2 growth stage (f) and at 280 g ha^{-1} 2,4-D, soybean injury averaged 55% at the V2 growth stage (g), 55% at the V5 growth stage (h), and 53% at the R2 growth stage (i).

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Table 3. Analysis of variance of soybean injury, seed yield, yield components, and seed composition treated with different rates of 2,4-D ranging from 0 to 2,240 g ha^{-1} (except seed composition was analyzed with nine rates, from 0 to 560 g ha^{-1}, because an insufficient amount of seed resulted at the 2,240 g ha^{-1} rate) at the V2, V5, and R2 soybean growth stages. Studies were conducted near Lafayette, IN and Urbana, IL during 2009 and 2010 and near Fowler, IN in 2009.

Attribute	Rate	Timing	Rate × timing
	P value		
Soybean injury			
14 d after treatment (DAT)	< 0.0001	< 0.0001	< 0.0001
28 DAT	< 0.0001	< 0.0001	< 0.0001
Seed yield and plant height			
Seed yield	< 0.0001	0.0001	0.5866
Plant height	< 0.0001	0.0003	< 0.0001
Yield components			
Seeds m^{-2}	< 0.0001	0.1671	0.5848
Seed mass	< 0.0001	< 0.0001	< 0.0001
Pods m^{-2}	< 0.0001	0.4827	0.4487
Seeds pod^{-1}	< 0.0001	0.8649	0.7783
Reproductive nodes m^{-2}	< 0.0001	0.0034	0.0922
Pods reproductive node^{-1}	< 0.0001	0.4798	0.0690
Percent reproductive nodes	< 0.0001	0.0763	0.5209
Nodes m^{-2}	< 0.0001	0.0002	0.0130
Seed composition			
Oil concentration	0.0002	0.1209	0.2202
Protein concentration	0.4414	0.1245	0.2154

2,4-D rate × treatment timing. Location and year were treated as random variables because there was homogeneity of error variances. Seed composition was analyzed in the same manner previously stated with the Tukey pairwise comparison ($P \leq 0.05$), except the 2,240–g ha^{-1} rate was excluded because there was not sufficient seed for the analysis. When the ANOVA did not reveal any differences between treatment timing and 2,4-D rate × treatment timing, the data were pooled for further analysis. However, if a difference was observed, treatment timings were separated according to the analysis. Further analysis was performed with the use of nonlinear regression models (dose–response model) to predict loss from 2,4-D rates using the drc package in R (Knezevic et al. 2007) (R, Version 2.10.1). A lack-of-fit test (nonsignificant P value when $P \geq 0.05$) was used to determine that the data were described by the model, because R^2 values do not adequately measure the fit of nonlinear models (Spiess and Neumeyer 2010). The three-parameter Weibull model was used in two different parameterizations (Equations 1 and 2) to describe the response of 2,4-D (Ritz and Strebig 2011; Seber and Wild 1989).

$$Y = d(1 - \exp\{-\exp[b(\log x - \log e)]\}), \quad [1]$$

$$Y = d(\exp\{-\exp[b(\log x - e)]\}). \quad [2]$$

Y represents the response variable, b the relative slope of the curve, d the upper limit, e the inflection point, and x the rate of 2,4-D. The effective doses (ED) causing 10, 20, and 50% loss were calculated from the dose–response model for visual soybean injury, because these are common values associated with soybean injury from herbicides (Kniss and Lyon 2011). The ED_5, ED_{10}, and ED_{20} were calculated for seed yield and yield components. These ED values were chosen because they have previously been used to estimate herbicide doses causing unacceptable seed-yield loss (Knezevic et al. 1998; Kniss and Lyon 2011).

The SAS GLM procedure was used to correlate soybean injury and seed yield. Quadratic relationships (Equation 3) were developed for visual rating data collected 14 and 28 DAT and were subjected to t tests to determine if similarities existed between DAT and treatment timings. Y represents seed yield, β_0 the intercept, β_1 the linear coefficient, β_2 the quadratic coefficient, and x the value of soybean injury. The correlation coefficient (R^2) values were used to describe the fit of the model because this model was derived as a generalized linear model where each β is linear.

$$\hat{Y} = \hat{\beta}_0 + \hat{\beta}_1 x + \hat{\beta}_2 x^2. \quad [3]$$

Path analysis and correlation of yield components were applied to soybean yield as described by Kahlon et al. (2011). Path analysis is used by plant breeders to distinguish relative importance of yield components and has been used to determine the effect of weeds on yield components of crops (Donald and Khan 1996). Analyses were performed with the primary (seeds m^{-2} and seed mass), secondary (pods m^{-2} and seeds pod^{-1}), tertiary (reproductive nodes m^{-2} and pods reproductive node^{-1}), and quaternary (nodes m^{-2} and percent reproductive nodes) traits for all soybean growth stages combined using the doses between 0 and 560 g ha^{-1} 2,4-D. The 2,4-D rate of 2,240 g ha^{-1} was excluded in the analysis because it was used as a kill rate for dose–response curves and altered linear regression models. Treatment timings were pooled. The SAS IML procedure was utilized to determine the direct path coefficients (Kang 1994). Indirect path coefficients were a result of multiplying the path coefficient with the correlation coefficient. The correlation coefficient and the residual error were calculated according to Kang (1994). A bidirectional path analysis was used because of the simultaneous development of yield components and the ability they have to interact and compensate with one another. The criteria used for identifying an important trait affecting its response variable was when that trait had a positive correlation with the response variable, a large positive direct effect, and a small indirect effect (Kahlon et al. 2011).

Results and Discussion

Visual Symptoms. Injury symptoms typically were bubbling, crinkling, and/or strapping of leaves and/or twisting and bending of petioles, branches, and stems (Figure 1) and a reduction in plant growth. It was observed that 2,4-D rates at the V5 treatment timing showed primarily stunting of plant growth and less epinasty than the V2 and R2 treatment timings. The effect of 2,4-D rate, treatment timing, and 2,4-D rate × treatment timing were significant on soybean injury at 14 DAT (Table 3). Consequently, data were fit to nonlinear regression models to relate 2,4-D rate and soybean injury at 14 DAT for each treatment timing (Figure 2). At 28 DAT data were fit to nonlinear regression models to relate 2,4-D rate and soybean injury for each treatment timing, because 2,4-D rate, treatment timing, and 2,4-D rate × treatment timing had a significant effect on soybean injury at 28 DAT. According to the ED_{20} values, the 2,4-D rate needed to injure soybean plants by 20% at 14 DAT was 77, 29, and 109 g ha^{-1} at the V2, V5, and R2 growth stages, respectively (Table 4). At 28 DAT the ED_{20} values were 109, 145, and 245 g ha^{-1} at the V2, V5, and R2 growth stages, respectively. The 14 and 28 DAT nonlinear regressions predicted similar values of soybean injury at 56 and 180 g ha^{-1}, as was found by Andersen et al. (2004) and

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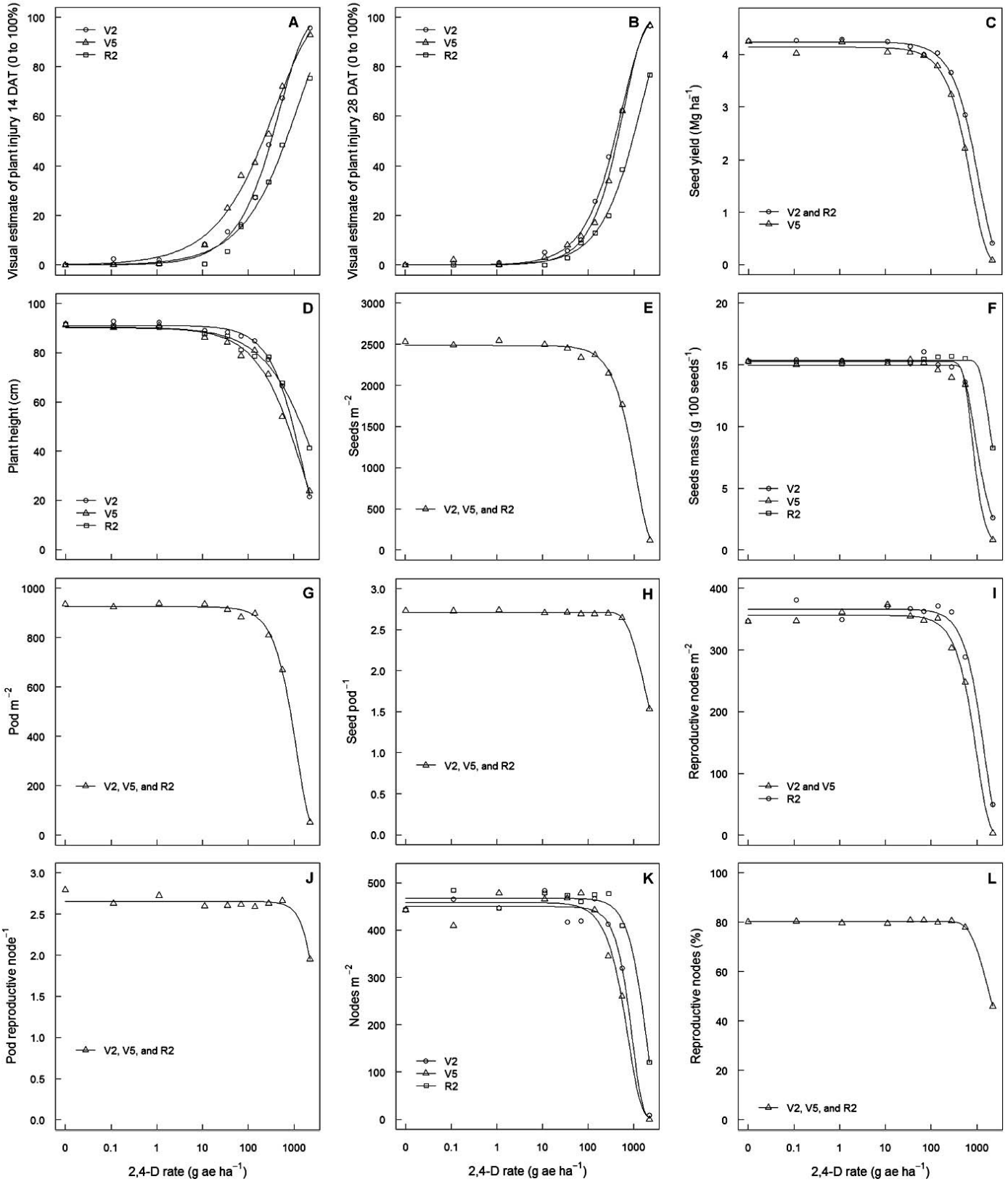


Figure 2. Nonlinear regressions of soybean injury 14 d after treatment (A), 28 d after treatment (B), seed yield (C), plant height (D), seeds m^{-2} (E), seed mass (F), pod m^{-2} (G), seed pod^{-1} (H), reproductive nodes m^{-2} (I), pod reproductive $node^{-1}$ (J), nodes m^{-2} (K), and percent reproductive nodes (L) as affected by 2,4-D treatments applied to soybean at the V2, V5, or R2 growth stages. Studies were conducted near Lafayette, IN and Urbana, IL during 2009 and 2010 and near Fowler, IN in 2009. Regression parameters and effective dose values can be found in Table 5.

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Table 4. Nonlinear curve regression parameters describing visual estimates of soybean injury (0 to 100%) at 14 and 28 DAT used in 2,4-D treatments applied to soybean at the V2, V5, and R2 growth stages. Studies were conducted near Lafayette, IN and Urbana, IL during 2009 and 2010 and near Fowler, IN in 2009.

DAT[b]	Growth stage	Regression parameters[a]			ED_{10} (± SE)[c]	ED_{20} (± SE)	ED_{50} (± SE)
		b	*d*	*e*	g ae ha^{-1}		
14	V2	0.801	100	499	30 (5)	77 (8)	316 (17)
14	V5	0.577	100	395	8 (1)	29 (4)	209 (13)
14	R2	0.629	100	1,180	33 (5)	109 (11)	659 (45)
28	V2	0.923	100	554	48 (7)	109 (10)	372 (22)
28	V5	1.04	100	608	70 (11)	145 (14)	428 (24)
28	R2	0.855	100	1,413	102 (15)	245 (25)	921 (63)

[a] Dose response parameters where *b* represents the relative slope of the curve, *d* the upper limit, and *e* the inflection point of the equation $Y = d\,(1 - \exp\{-\exp[b(\log x - \log e)]\})$.
[b] DAT, days after treatment.
[c] ED (± SE), effective dose causing 10, 20, and 50% visual soybean injury. The standard error is in parentheses.

Kelley et al. (2005) when those authors were expanding previously reported data on soybean injury caused by 2,4-D.

Total Seed-Yield Loss. Soybean seed yield was significantly reduced by 2,4-D rate and treatment timing, but 2,4-D rate × treatment timing did not have a significant effect on seed yield (Table 3). A nonlinear regression model was fit to relate 2,4-D rate and yield loss at the V5 treatment timing, whereas the V2 and R2 treatment timings had a similar effect and data were pooled prior to fitting the regression model (Figure 2). The ED_5 was caused by 116 g ha^{-1}, whereas the ED_{10} was caused by 202 g ha^{-1} at the V2 and R2 treatment timings. At the V5 treatment timing the ED_5 and ED_{10} were caused by 87 and 149 g ha^{-1}, respectively. The effect of 2,4-D on soybean plant height at maturity was similar to what was reported by Wax et al. (1969) and Slife (1956). Seed-yield loss in this experiment from 2,4-D was generally less than what has previously been reported (Andersen et al. 2004; Slife 1956; Wax et al. 1969). These differences may be explained by variations in the natural tolerance of soybean cultivars to 2,4-D (Fribourg and Johnson 1955). The proposed 2,4-D use rate in aryloxyalkanoate dioxygenase-1 and aryloxyalkanoate dioxygenase-12 traited crops (Wright et al. 2010) is 560 to 1,120 g ha^{-1}. To reach the ED_5 levels for yield would require 10 to 20% and 8 to 15% off-target movement from a field application at V2 or R2 and V5 growth stages, respectively.

Characterization of Yield Components. Nonlinear regressions were fit to describe each yield component according to the analysis of variance (Table 3). As 2,4-D rate increased, each yield component decreased in production (Figure 2). The most sensitive yield components were seeds m^{-2}, pods m^{-2}, reproductive nodes m^{-2}, and main stem nodes m^{-2}, which required ≥ 183 g ha^{-1} 2,4-D to cause a 10% reduction. The ED_{10} of seeds m^{-2} was 241 g ha^{-1} 2,4-D and the ED_{10} of pods m^{-2} was 260 g ha^{-1} (Table 5). Reproductive nodes m^{-2} had an ED_{10} of 255 g ha^{-1} 2,4-D at the V2 and V5 treatment timings and 387 g ha^{-1} for the R2 treatment timing. Main stem nodes m^{-2} had ED_{10} values of 314, 183, and 571 g ha^{-1} 2,4-D at the V2, V5, and R2 treatment timings, respectively. Seed mass, seed pod^{-1}, pod reproductive $node^{-1}$, and percent reproductive nodes needed ≥ 548 g ha^{-1} 2,4-D to cause a 10% reduction of these yield components. The ED_{10} of seed mass was 548, 554, and 1,283 g ha^{-1} for the V2, V5, and R2 treatment timings, respectively. Seeds pod^{-1} had an ED_{10} of 867 g ha^{-1} 2,4-D, pod reproductive $node^{-1}$ had an ED_{10} of 1,410 g ha^{-1}, and percent reproductive nodes had an ED_{10} of 859 g ha^{-1}. Similar to what Wax et al. (1969) reported, there was no difference in seeds m^{-2} or pods m^{-2} at 2,4-D doses ranging from 0 to 140 g ha^{-1}. However in this study, seed mass was not reduced from 2,4-D doses ≤ 560 g ha^{-1}; whereas Wax et al. (1969) found at 35 and 140 g ha^{-1} seed mass was reduced. This inconsistency may be attributed to the genetic difference of the soybean cultivars used in the two studies.

Seed Composition. 2,4-D rate had a significant effect on the harvested seed oil concentration (Table 3). Seed oil concentration was reduced from 189 g kg^{-1} for the untreated check to 185 g kg^{-1} at 560 g ha^{-1} 2,4-D, whereas at rates < 560 g ha^{-1} seed oil concentrations were not different from those of the untreated check (data not shown). There were no differences in seed protein concentration from 2,4-D rate (Table 3). Although there was a difference in seed oil concentration, the difference was small (4 g kg^{-1}). At 560 g ha^{-1} 2,4-D can delay the maturity of soybean plants by up to 5 d (Slife 1956). Such a delay can allow the development of soybean at the R5 and R6 growth stages to be at a time where a short period of high temperature can change the oil concentration compared to the other plants with less or no maturity delay (Howell and Cartter 1958). Protein concentration did not change from 2,4-D application at any application timing, which is similar to the results of Wax et al. (1969), who did not find a difference in oil and protein concentration at rates from ≤ 140 g ha^{-1} 2,4-D.

Effect of Yield Components on Total Seed Yield. Path analysis was used to describe the compensation of yield components at the primary, secondary, tertiary, and quaternary levels of soybean reproduction. The direct path effects of seeds m^{-2} on seed yield were 2.5 times greater than seed mass (Figure 3). The indirect path effect of seed mass was strong (−0.32), indicating that the compensation of the yield component reduced the direct path effect of seed mass on seed yield. The indirect path effect of seeds m^{-2} on seed yield through seed mass was −0.13, indicating that little compensation of yield components occurred. As a result the path coefficient and correlation coefficient of seeds m^{-2} on seed yield were large positive values, whereas the indirect effect was moderate. This indicated that seeds m^{-2} had the most influence on seed yield.

The direct effect of the secondary traits (seeds pod^{-1} and pods m^{-2}) on seeds m^{-2} was 3.4 times greater for pods m^{-2}

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Table 5. Nonlinear regression parameters of seed yield, plant height, and yield components as affected by 2,4-D treatments applied to soybean at the V2, V5, or R2 growth stages. Studies were conducted near Lafayette, IN and Urbana, IL during 2009 and 2010 and near Fowler, IN in 2009.

Component	Growth stage	Regression parameters[a]			ED_5 (± SE)[b]	ED_{10} (± SE)	ED_{20} (± SE)
		b	*d*	*e*	g ae ha^{-1}		
Seed yield (Mg ha^{-1})	V2 and R2	1.29	4.24	1,162	116 (22)	202 (30)	363 (38)
	V5	1.34	4.14	794	87 (29)	149 (36)	260 (40)
Plant height (cm)	V2	1.06	91	1,599	97 (21)	191 (49)	389 (69)
	V5	0.776	90	1,519	33 (14)	84 (27)	220 (49)
	R2	0.675	90	3,330	40 (25)	119 (53)	361 (102)
Seeds m^{-2}	V2, V5, and R2	1.48	2,485	1,106	148 (26)	241 (33)	400 (39)
Seed mass	V2	−1.78	15.3	876	472 (51)	548 (53)	670 (56)
	V5	−2.64	15.0	760	502 (39)	554 (39)	635 (43)
	R2	−1.97	15.4	1,958	1,123 (3,513)	1,283 (3,239)	1,538 (2,619)
Pods m^{-2}	V2, V5, and R2	1.52	926	1,144	162 (33)	260 (41)	427 (49)
Seeds pod^{-1}	V2, V5, and R2	−1.07	2.71	1,888	678 (146)	867 (149)	1,211 (142)
Reproductive nodes m^{-2}	V2 and V5	1.65	356	995	165 (50)	255 (57)	401 (61)
	R2	1.69	366	1,462	253 (101)	387 (123)	603 (146)
Pods reproductive $node^{-1}$	V2, V5, and R2	2.31	2.65	3,730	1,033 (612)	1,410 (508)	1,949 (258)
Nodes m^{-2}	V2	2.01	450	963	219 (273)	314 (235)	456 (125)
	V5	1.56	459	776	115 (63)	183 (72)	296 (74)
	R2	1.87	467	1,899	389 (241)	571 (278)	852 (298)
Percent reproductive nodes	V2, V5, and R2	−1.04	80.3	1,918	667 (154)	859 (159)	1,213 (152)

[a] Dose–response parameters where *b* represents the relative slope of the curve, *d* the upper limit, and *e* the inflection point of the equation. The equation $Y = d\,(\exp\{-\exp[b(\log x - e)]\})$ described soybean seed yield, plant height, seeds m^{-2}, pods m^{-2}, main stem reproductive nodes m^{-2}, pods reproductive $node^{-1}$, and main stem nodes m^{-2}; whereas the equation $Y = d\,(1 - \exp\{-\exp[b(\log x - \log e)]\})$ was fit to seed mass, seeds pod^{-1}, and percent reproductive nodes.

[b] ED (± SE), effective dose causing 5, 10, or 20% loss. The standard error is in parentheses.

compared to seeds pod^{-1} (Figure 3). There was a strong negative indirect effect of seeds pod^{-1} on seeds m^{-2} through pods m^{-2} (−0.68). The yield component compensation eliminated any benefit of the direct effect of seeds pod^{-1} on seeds m^{-2}. The indirect effect of pods m^{-2} was small (−0.20), and as a result a high correlation and direct effect of pods m^{-2} on seeds m^{-2} was found. As an outcome, pods m^{-2} had the greatest effect on seeds m^{-2} of the secondary traits.

The tertiary traits, pods reproductive $node^{-1}$ and reproductive nodes m^{-2} on pods m^{-2}, had a direct effect that was 2.3 times greater for the reproductive nodes m^{-2} compared to the pods reproductive $node^{-1}$ when 2,4-D was applied (Figure 3). The indirect effect of pods reproductive $node^{-1}$ was strongly negative (−0.61). The compensation of yield components eliminated the beneficial direct effect of pods reproductive $node^{-1}$ on pods m^{-2} as was seen in the correlation. In contrast, the indirect effect of reproductive nodes m^{-2} on pods m^{-2} through pods reproductive $nodes^{-1}$ was −0.27. As a result the direct effects and the correlation coefficients of reproductive nodes m^{-2} on pods m^{-2} were large positive values. This indicated that the influence of reproductive nodes m^{-2} on pods m^{-2} was the strongest of the tertiary traits.

The direct effect of nodes m^{-2} compared to the percent reproductive nodes (quaternary traits) on reproductive nodes m^{-2} was 7.2 times greater (Figure 3). The indirect path effect of nodes m^{-2} on reproductive nodes m^{-2} through percent reproductive nodes was −0.10, indicating that little seed-yield

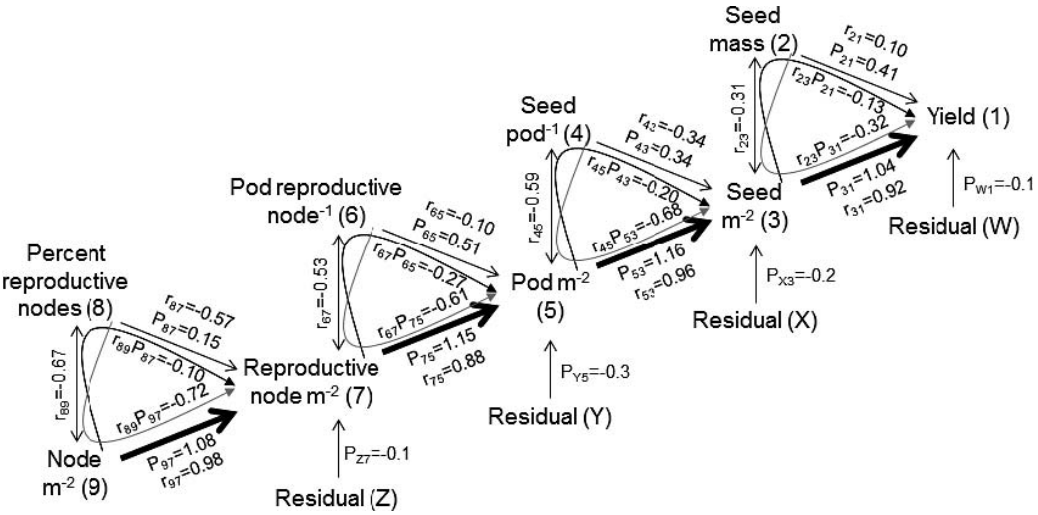


Figure 3. Path analysis of soybean yield components affected by rates of 2,4-D near Lafayette, IN and Urbana, IL in 2009 and 2010 and near Fowler, IN, in 2009. Path coefficient (*P*), correlation coefficient (*r*), and the indirect effect of the path coefficient × correlation coefficient ($r \times P$) values are shown. The bold lines indicate an important trait affecting its response variable whereas the green and blue lines are the indirect effects. The important trait affecting its response variable was determined when there was a large positive correlation, large positive direct effect, and a small negative indirect effect.

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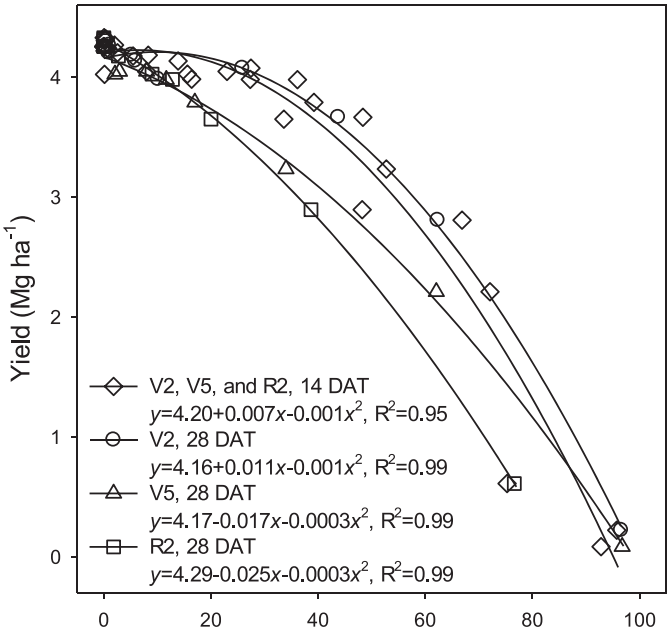


Figure 4. Relationship of visual estimates of soybean injury (0 to 100%) and seed yield (Mg ha^{-1}) of soybean plants treated with 2,4-D rates (0 to 2,240 g ha^{-1}) at the V2, V5, or R2 soybean growth stages. Visual estimates of injury were taken at 14 and 28 days after treatment. Studies were conducted near Lafayette, IN and Urbana, IL in 2009 and 2010 and near Fowler, IN, in 2009. All regressions were significant ($P < 0.0001$).

compensation occurred and the direct path effects and correlations of nodes m^{-2} to reproductive nodes m^{-2} were similar. However, the indirect path effect of percent reproductive nodes on reproductive nodes m^{-2} was −0.72. This yield component compensation negated any direct effect of percent reproductive nodes on reproductive nodes m^{-2} through nodes m^{-2} that received 2,4-D treatments. Of the quaternary traits, nodes m^{-2} had a large positive value for the path coefficient and the correlation coefficient, while the indirect effect was small. As a result, nodes m^{-2} had a greater effect on reproductive nodes m^{-2}.

Implications of Visual Estimates on Seed-Yield Loss. Visual estimates of plant injury (Figure 4) were significant ($P < 0.0001$) and highly correlated to soybean seed-yield loss (R^2 = 0.95 to 0.99). Regression models were combined according to *t* tests; V2, V5, and R2 at 14 DAT; V2 at 28 DAT; V5 at 28 DAT; and R2 at 28 DAT. At 14 DAT a 10% seed-yield loss needed 35% soybean injury. At 28 DAT a 10% seed-yield loss was caused by 40, 19, and 15% soybean injury at the V2, V5, and R2 growth stages, respectively. These data indicate that soybean injury at 14 and 28 days after exposure to 2,4-D can be used to estimate the amount of seed-yield loss. Using soybean injury ratings are likely the easiest, quickest, and most cost-effective method to evaluate potential seed-yield loss from 2,4-D exposure on soybean, but would lack consistency because of human variability in rating soybean injury.

Visual injury on soybean plants from 2,4-D can be subtle and could be difficult to detect in field drift situations because there is not an untreated check available to compare affected with unaffected plants. The slight bending of leaves or petioles may appear to be caused by the heliotropic nature of the soybean, or could be misjudged as a response to another abiotic or a biotic stress. Additionally, when plants are drifted on, the symptoms will gradually with increasing distance from the source that spread the 2,4-D, making it challenging to see any differences in plant height. At nonlethal levels, 2,4-D is likely causing an increase in abscisic acid, which could limit or damage plant growth at the active growth sinks. This may occur for a period of time until the plant can overcome the effects of this hormonal increase and continue normal growth.

A reduction in plant height may be one of the most important symptoms on soybean caused by 2,4-D that causes yield loss. This reduced growth would lessen leaf area, cause fewer main stem nodes, and result in fewer pods or less seed mass because of a smaller source for photosynthesis. A reduction in soybean plant height has been correlated to a reduction in seed number when dicamba treatments were applied (Weidenhamer et al. 1989). Seed number is often highly correlated with soybean yield (De Bruin and Pedersen 2008). Thus, a reduction in plant height can reduce many yield components, causing a seed-yield loss.

No single yield component controls yield; rather, the yield components work together to produce yield. When an herbicide drift occurs on soybean, all yield components are affected. The amount and the timing of the drift incidence will affect the degree of damage sustained. The yield components in this study that were most affected were the number of main stem nodes m^{-2}, main stem reproductive nodes m^{-2}, pods m^{-2}, and seeds m^{-2}. Kahlon et al. (2011) reported a similar response of yield components of old and new soybean cultivars on seed-yield formation, which indicates that these are the primary yield components that can be influenced during plant growth. Soybean seed-yield reduction needs to take an approach to quantify the number of nodes, reproductive nodes, pods, and seed lost to understand the whole effect of herbicide drift on soybean. One of the challenges is soybean plants may compensate for a decrease in one yield component by increasing another, making the analysis of all the important yield components necessary. The other yield components, seed mass, seeds pod^{-1}, pods reproductive node^{-1}, and percent reproductive nodes are more stable because they are likely controlled more by genetics.

Soybean oil and protein concentration have been shown to be correlated with the maximum air temperature during the R6 growth stage (Robinson et al. 2009). A reduction in leaf area from herbicide drift can increase the canopy temperature and can result in an increase in soybean oil and a decrease in protein content, but this situation would likely occur when a misapplication occurs.

In summary, soybean plants were damaged by 2,4-D and as a result the number of nodes, reproductive nodes, pods, and seed were reduced, causing a loss in seed yield. Seed-yield loss could be estimated by observing the visual symptomology, but variability in human ratings, knowing when the drift incident occurred, variable responses of soybean cultivars to 2,4-D, and environmental conditions can make this method less reliable. Counting yield components is laborious and time consuming, but this understanding of how soybean plants respond when affected by 2,4-D shows the complexity of the soybean system and indicates a simple association of visual estimates of plant injury does not always predict yield loss accurately. Although 2,4-D drift on soybean is a concern, greater attention may need to be focused on grapes, flowering bedding plants, vegetables, cotton, and other plants that are sensitive to low rates of 2,4-D (Al-Khatib et al. 1993; Hatterman-Valenti and Mayland 2005; Hemphill and Montgomery 1981; Marple

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et al. 2008). New technology is exploring the use of a 2,4-D choline salt, which has been reported to have reduced drift and volatility. However, these characteristics may be related to the equipment and application methods used (Hillger et al. 2011; Ruen et al. 2011; Scherder et al. 2010). Furthermore, mitigating off-site movement of 2,4-D by using recommended spraying techniques and being aware when sensitive vegetation is downwind will be of utmost importance to avoid 2,4-D drift damage.

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