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EFFECTS OF MICRO-RATES OF 2,4-D AND DICAMBA ON LETTUCE AND PUMPKIN IN NEBRASKA

By

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EFFECTS OF MICRO-RATES OF 2,4-D AND DICAMBA ON LETTUCE AND PUMPKIN IN NEBRASKA

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Off-target herbicide injury from dicamba and 2,4-D is an increasingly common problem for specialty crop growers in the Midwestern United States. Both lettuce (*Lactuca sativa L*.) and pumpkin (*Cucurbita spp*.) are common specialty crops grown in Nebraska, and their proximity to corn and soybean production makes these crops susceptible to herbicide drift injury and yield loss. The objectives of this thesis research was to quantify crop injury and yield loss in greenhouse- and field-grown lettuce and field-grown pumpkins at different growth stages after exposure to sub-lethal doses of dicamba or 2,4-D. Dose response curves were generated to determine effective dose (ED) values and to relate drift rates with crop injury and yield loss. In addition, a dicamba residue test was conducted in lettuce to relate residue levels, drift rates, crop injury, and yield loss. Our study found out all modern lettuce varieties ‘Green Forest’, ‘Vulcan’, and ‘Allstar’ were highly susceptible to dicamba and 2,4-D. Mature stage lettuce had higher tolerance for both herbicide but with observed high variation on yield. Some increase in yield was observed in mature stage lettuce but the benefits of the small increase in biomass was offset by visual injury and reduced marketability. 2,4-D choline caused yield reduction on seedling stage ‘Green Forest’ and ‘Vulcan’ at the rate above 21.3 g ae ha^{-1} with 50% yield loss at the rate of 33.6 g ae ha^{-1}. ‘Green Forest’ at seedling stage were highly susceptible to dicamba with 50% yield loss when treated at the rate of 16.8 g ae ha^{-1}. Pumpkins studies showed less susceptibility to dicamba and 2,4-D at flowering stage with high variability on yield that caused poor lack of fit

on the dose-response model. Dicamba at the rate of 139.8 g ae ha^{-1} and 2,4-D at the rate of 266.6 g ae ha^{-1} caused significant yield reduction on vegetative pumpkins compared with the control. The results provided information to Nebraska growers and aid to quantify economic loss from off-target herbicide drift events and highlight the need for communication between commercial herbicide applicators and specialty crop growers.

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CHAPTER 1, IMPACT OF MICRO-RATES OF 2,4-D CHOLINE ON LETTUCE INJURY AND YIELD LOSS

Off-target herbicide injury from dicamba and 2,4-D is an increasingly common problem for specialty crop growers in the Midwestern United States. Lettuce (*Lactuca sativa L.*) is a common specialty crop that is grown in Nebraska, but its proximity to corn and soybean production leaves growers vulnerable to crop injury and significant economic loss. The goal of this study was to quantify crop injury and yield loss in greenhouse- and field-grown lettuce after exposure to simulated sub-lethal doses of 2,4-D. Sublethal doses were determined based on a percentage of the maximum labeled rate and ranged from 1/10000 to 1/4. The lettuce varieties of ‘Green Forest,’ ‘Vulcan,’ and ‘Allstar,’ were tested and each received herbicide doses at the seedling and mature growth stage. Plant injury ratings were recorded every 4 days after herbicide application until mature for harvest. Lettuce was harvested and dry (greenhouse) or fresh (field) weights were recorded. Dose response curves were generated to determine effective dose (ED) values and to relate drift rates with crop injury and yield loss. All lettuce varieties were more tolerant of herbicides at the mature growth stage than at the seedling growth stage. For greenhouse lettuce, dose response curves for injury and yield loss were similar at the seedling and mature stage. The effective dose value (ED) for percentage yield loss matched well across years for all three lettuce varieties. For all three lettuce varieties, rates above 21.3 g ae ha^{-1} caused yield reduction. Hormesis was observed in our mature stage lettuce but any growth stimulus would be negated by injury symptoms and reduced marketability of the crop. In 2020, we observed yield reduction on both ‘Green Forest’ and ‘Vulcan’ lettuce even with no visually observed injuries. Study results confirmed the susceptibility of lettuce to relatively low rates of

2,4-D, which highlights the importance of drift mitigation efforts in the Midwestern United States.

Keywords: Crop injury, yields loss, herbicide drift

1.1 INTRODUCTION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a synthetic auxinic herbicide belong to phenoxyalkanoic acids group that selectively controls broadleaf weeds in agriculture and nonagricultural settings. In 1944, the American Chemical Paint Company commercialized and marketed 2,4-D as the first systemic herbicide under the brand name “Weedone” (Peterson 1967). Until recently, 2,4-D has been registered as different kinds of salts. Amine and ester formulations are the most common kind that can be applied in corn (*Zea mays*) up to 20 cm and soybean (*Glycine max*) as preplant herbicide (Nebraska Weed Guide, 2020). 2,4-D is registered for use in both terrestrial and aquatic environments including turf, lawns, rights-of-way, aquatic sites, forestry sites, cropping field, and fruit and vegetable crops. Formulations of 2,4-D are emulsifiable, concentrate, granules, soluble concentrate, water dispersed granules, and wettable powder. 2,4-D choline is a new formulation marketed as Enlist^{TM} with Colex-D^{TM} (Dow AgroSciences LLC, Indianapolis) for use in 2,4-D tolerant corn, soybeans, and cotton (*Gossypium hirsutum* L.) (known as Enlist corn, Enlist E3 soybeans, and Enlist cotton). This technology is popular among Midwest farmers because it can contribute to a chemical-based no-till cropping system. 2,4-D choline has been shown to have lower volatility when compared to 2,4-D ester, 2,4-D amine, and dicamba acid in a drift experiment on cotton and soybean (Eytcheson et al. 2012). Similar research in cotton demonstrated that the choline formulation had the lowest off-target movement compared to the ester formulation and was less damaging than the amine formula (Sosnoskie et al. 2015). It is known that the formulation of 2,4-D can influence the uptake and translocation by plants (Peterson et al. 2016). Generally, the uptake for 2,4-D ester formulation is greater than the amine formulation (Peterson et al. 2016). On Bigleaf maple (*Acer macrophyllum*), 2,4-D ester had the absorption rate of 20.8% with 95.4%

translocation compared with 2,4-D amine with only has 1.7% absorption with 68.8% translocation (Norris and Freed 1966). However, there is considerably less information about how 2,4-D choline drift might influence specialty crops like lettuce (*Lactuca sativa*).

In the Midwest, 2,4-D has historically been used in corn as a post emergence herbicide to control broadleaf weeds. The Pesticide National Synthesis Project of the U.S. Geological Survey found that the use of 2,4-D is prevalent across agricultural land in the whole Midwest region. Total applications in the Midwest were estimated in 2017 at more than 28 pounds per square mile, which is two to three times higher than the average of 3 to 11 pounds per square mile in the north and northeastern regions of the U.S. (USGS, 2017). Many specialty crops are broadleaf dicots that have vascular bundles arranged in rings, which makes them particularly susceptible to growth regulator herbicides. Upon drift by auxinic herbicides, affected plants will grow uncontrolled and crush the vascular cambium, which leads to mortality. In 1983 and 1984, almost $20 million of conventional cotton was destroyed by a 2,4-D drift event in southwest Texas when the adjacent wheat field was aerially sprayed with the herbicide (Hanner, 1984). In Nebraska, Driftwatch has registered 140 grapes farms with the total of 990 acres and 74 vegetables farms with a total of 955 acres. Most of those farms are located in close proximity to corn and soybean farms where herbicide drift and off-target injury are most likely to occur. With the recent release of 2,4-D-tolerant seed traits (Enlist) in soybean, cotton, and corn, 2,4-D use will likely increase throughout the Midwest. It is likely that off-target drift events will increase proportionally. From 2011 to 2014, there were a total of 38 off-target herbicide injury complaints reported to Nebraska Department of Agriculture (NDA), 28 of which were related to 2,4-D off-target drift injury in tree nursery, fruit trees, grapes, vegetables, organic crops, and vegetables (personal communication with Craig Romary, Environmental Programs Specialist).

Lettuce is a high value leafy crop that is in high demand in local, direct-to-consumer markets. There were 57 farms growing and selling lettuce across 12 acres in Nebraska in 2017 (Nebraska 2017 Census, USDA NASS). In 2020, both head lettuce, Romaine lettuce, and tomatoes were the highest value vegetable crops in the US accounting for 30% of the total value when combined (USDA, National Agricultural Statistics Service, Vegetables 2020 Summary). Although lettuce is known to be susceptible to growth regulator herbicides, to the best of our knowledge, there has been only a few research studies on the effects of sublethal 2,4-D rates on lettuce injury and yield loss. Hemphill and Montgomery (1981) tested 3 lettuce varieties, 'Buttercrunch' butterhead, 'Ithaca' crisphead, and 'Grand Rapids' leaf with 2,4-D at rates of 2.1, 20.8, 104, and 208 g ha^{-1} when lettuce were nearly heading. Exposure to 20.8 g ha^{-1} had no effect on yield, but 104 g ha^{-1} increased lettuce bolting and reduced quality. The rate of 208 g ha^{-1} showed significant reduction in yield when compared with the nontreated control (Hemphill and Montgomery 1981). Because this research was conducted over 40 years ago, the lettuce varieties and 2,4-D formulations tested are less relevant today. The most recent 2,4-D response study using 2,4-D dimethylamine salt (label rate 670 g a.i. ha^{-1}) on the lettuce variety 'Stella' at 30 days after transplanting showed significant biomass reduction with observed death when treated with above 80.4 g a.i. ha^{-1} (Roesler et al. 2020). At a dose of 4.69 g a.i. ha^{-1}, lettuce were able to recover with the yield close to the control (Roesler et al. 2020).

MCPA (2-methyl-4-chlorophenoxyacetic acid) is another phenoxy herbicide similar to 2,4-D. A sublethal dose study of MCPA on summer cabbage lettuce 'Borough Wonder' showed significant yield reduction with abnormal leaves at the dose of 33.6 g ha^{-1}(Lettuce and Way 1962). A field study of 2,4-D on cabbage found the rate of 161.2 g ae ha^{-1} caused reduction in marketable cabbage (Nascimento et al. 2020). A 50% yield reduction was observed when broccoli at eight leaf stage received 16.8 g ae ha^{-1} (1.6% of the label rate) of 2,4-D (Mohseni-Moghadam and Doohan 2015). A sublethal dose study of tomatoes showed 2% (13.44 g ae ha^{-1}) of 2,4-D caused 92% reduction in number of fruits per plant and 93% crop yield reduction (Fagliari et al. 2005). A greenhouse study averaged over grape cultivars 'Riesling', 'Chardonnay', 'Chardonel', 'Vidal blanc', and 'Traminette' reported that 2,4-D at an application rate of 28 g ha^{-1} caused 66% injury and 42 days after treatment shoot length was reduced by 84% (Mohseni-Moghadam et al. 2015).

It is often hard to quantify the effect from herbicide drift on specialty crops because herbicide injury, yield loss, and economic damage varies by crop type and growth stage, herbicide type, and effective rate (Australia and Primary Industries Standing Committee 2002). There has also been a lack of knowledge of growth regulators on crops especially when drift occurs at early growth stages (Lettuce and Way 1962). Therefore, the aim of this study was to assess the effects of a novel 2,4-D choline formulation on injury and yield loss of three modern lettuce varieties, including 'Allstar', 'Green Forest' and 'Vulcan' at seedling and mature growth stages.

1.2 MATERIALS AND METHODS

1.2.1 GREENHOUSE SET UP

Studies were conducted in 2019 and 2020 between February and April at the University of Nebraska-Lincoln Plant Growth Facilities in Lincoln, NE (40° 50' 4.050" N, 96° 39' 54.612" W). Temperature was set as 21.1 to 26.7 C° during the day and at 15.6 to 21.1 C° during the night. Actual temperature around noon can be high as 28.3 C° but the coldest temperature at night was maintained at 15.6 C°. Supplemental lights in 2019 were 1000 watt metal halide fixtures. Supplemental lights in 2020 were replaced with 1000 watt high pressure sodium fixtures. Lights were on for 18 hours per day. Peters Professional 20-10-20 fertilizer (Scotts Co., Marysville, Ohio, USA) was applied at the concentration of 250 ppm by fertilizer injector three times per week after lettuce reached the seedling stage with cotyledon fully expanded until harvest. Lettuce was water once per day until harvest besides the day with fertigation.

1.2.2 PLANT MATERIAL

‘Allstar Gourmet’, ‘Green Forest’, and ‘Vulcan’ (Johnny’s Selected Seeds Company; Winslow, ME) were the lettuce varieties used for the experiment. ‘Allstar Gourmet’ is the popular spring mix lettuce sold in the grocery store with the color of dark reds and greens. The ruffed edges and unique shapes provide soft, interesting texture and fancy appearance. ‘Green Forest’ is the most attractive green romaine head lettuce that is tall with smooth ribs that are early to packs and handles. This lettuce is very common in Caesar salad. ‘Vulcan’ was the common red color head lettuce usually sold as organic at the grocery store. It has slightly-frilled leaves with candy apple red color over a light green background. Lettuce was directly seeded into the 15.3 centimeter diameter pots at a planting depth of 0.64 centimeter and filled with soilless potting mix, Berger mix BM6 (Saint-Modeste, QC, Canada). ‘Allstar Gourmet’ was direct-seeded into each pot with 30 seeds per pot. ‘Green Forest’ and ‘Vulcan’ were direct-seeded into each pot with two seeds per pot. ‘Allstar Gourmet’ was not thinned after emergence due to the nature of selling as a lettuce mix. ‘Green Forest’ and ‘Vulcan’ were thinned to one plant per pot after emergence to grow as individual lettuce head.

1.2.3 GREENHOUSE EXPERIMENTAL DESIGN AND TREATMENT APPLICATIONS

In 2019, lettuce pots were arranged in a completely randomized design with six replicates in the greenhouse. The treatment factors included sublethal rates of 2,4-D choline salt (Enlist One; Dow AgroSciences, Indianapolis, IN) with 0, 1/4, 1/10, 1/100, 1/1000, and 1/10000 of the label rate [1066 g ae ha^{-1} of Enlist One], and two application timings (seedling and mature stage). Seedling stage was defined as when lettuce plants had two fully expanded true leaves. Mature stage was defined as one week prior to harvest. There were a total of 216 experimental units (3 varieties × 6 sublethal rates × 2 application timings × 6 replications). In 2019, planting date of all lettuce was 12 Feb. The seedling stage application of all lettuce varieties was on 5 Mar. The mature stage application of 'Allstar Gourmet' was on 14 Mar, and 'Vulcan' and 'Green Forest' were on 20 Mar. 'Allstar Gourmet' was harvested on 19 Mar (25 days after planting), and 'Green Forest' and 'Vulcan' were harvested on 27 Mar (33 days after planting). At harvest, each lettuce in the pot was cut at soil level, placed in individual paper bags and placed in a drying ovens at 31 C° for 7 days until totally dried. Lettuce weight of each treated rate of each lettuce varieties then divided by the replication to obtain the average yield as gram per pot.

In 2020, the pot-grown lettuce followed a random complete block design with 6 replicates in the greenhouse. Each replications of the treatment combinations is a block and blocked by benches. We modified the rates based on the symptomology and dropped the lowest rate, 1/10000. 6 sublethal rates (0 was the control; 1/4; 1/10; 1/50; 1/100; 1/500; 1/1000) of the label rate [1066 g ae ha^{-1} of 2,4-D choline salt. Planting date of all lettuce were on 10 Feb. There were total of 36 plants for each treatment combination; 6 plants × 6 replicates. Total experimental unites are 252 (1 herbicide × 3 varieties × 7 sublethal rates × 2 application timings × 6 replications). The seedling stage application of all lettuce varieties was on 25 Feb.

The mature stage application of ‘Allstar Gourmet’ was on 5 Mar. ‘Vulcan’ and ‘Green Forest’ were on 16 Mar. ‘Allstar Gourmet’ with both seedling and mature stage treatment were harvested on 12 Mar (31 days after planting seeds). While the rest were all harvested on 23 Mar (42 days after planting seeds). Harvest procedures were the same as 2019 lettuce oven dried at 31C° for 7 days. Lettuce weight of each treated rate of each lettuce verities then divided by the replication to obtain the average yield as gram per pot. In both year, herbicide was applied using a single-tip chamber sprayer (DeVries Manufacturing Corp, Hollandale, MN) fitted with an 8001 E nozzle (TeeJet Technologies, Spraying systems Co., Wheaton, IL) calibrated to deliver 140 L ha^{-1} at 276 kPa at a speed of 4.8 km h^{-1}. Visual injury ratings were based on the percentage scale of 0 (no injury) to 100 (death of the plant) relative to the nontreated control (Appendix 1). This protocols was adapted from Frans et al. (1986). The injury rating data were collected at 3, 7, 12, 16, 22 days after treatment (DAT). Depending on the sublethal rates and the stage of the plant, injury symptoms from 2,4-D included leaf chlorosis, curling, cupping, stunting, and necrosis.

1.2.4 FIELD STUDY EXPERIMENTAL DESIGN AND TREATMENT APPLICATIONS

In 2019 summer, only ‘Vulcan’ and ‘Green Forest’ lettuce were tested in the field. Both lettuce varieties were seeded into the flats in the greenhouse on 10 Apr for seedling plugs preparation. On 24 May, lettuce seedlings were transplanted to the field located at Havelock (40°51’ 7.008” N, 96°36’ 52.980” W). Before transplanting, the field was prepared with rotary tillage. In a single field pass of the bed-shaper/mulch-layer (RB448; Nolt’s Produce Supplies), raised beds were shaped, and drip irrigation line was laid beneath a white on black plastic film. Each plot was 3.7 meters long by 1.2 meters wide which fit 12 lettuce plants within each pot.

The gap between plot was 2.4 meters long. There were total of 12 plots within each row serve as 1 replication. Of all 12 plots, each variety (‘Green Forest’ and ‘Vulcan) with 6 sublethal rates of herbicide were randomized within each row. There were total of 4 rows which serve as 4 replications. Due to no preplant fertilizer broadcast before shaping bed. A fertilizer injector was connected to the irrigation line later in the season for fertigation. Fertigation was done twice in the season which was one week before the herbicide treatment and one week after the herbicide treatment. Calcium nitrate (15N–0P–0K, YaraLiva Tropicote 15–0–0; Yara North America, Tampa, FL) was mixed in the fertilizer injector to deliver 44.8 kg ha^{-1} of the Nitrogen. Treatments in 2019 included six sublethal rates (0 was the control and 1/4; 1/10; 1/50, 1/100; 1/500 of the label rate (1066 g ae ha^{-1} 2,4-D choline salt).

In 2020 summer, seedling plugs of ‘Vulcan’ and ‘Green Forest’ were also prepared in the greenhouse on 8 Apr. On 18 May, lettuce seedlings were transplanted to the field located on east campus (40° 50’ 10.890” N, 96° 39’ 45.162” W). Before transplanting, the field was prepared with rotary tillage. All plots received an application of 112 kg ha^{-1} N with granular urea (46N-0P-0K) from PRO-AP (Wawaka, IN) applied as preplant broadcast fertilizer and incorporated into the soil. In a single field pass of the bed-shaper/mulch-layer (RB448; Nolt’s Produce Supplies), raised beds were shaped, and drip irrigation line was laid beneath a white on black plastic film. The set up for row and plot were the same as in 2019. Each plot was 3.7 meters long by 1.2 meters wide and the gap between was 2.4 meters long. There were total of 12 plot within each row. Due to labor restrain from COVID 19, only one lettuce variety was transplanted in each rows. Thus, each row is considered as 2 replications. 6 sublethal rates of herbicide were randomized within each row. 6 sublethal rates were (0 was the control; 1/4; 1/10; 1/50, 1/100; 1/500) of the label rate [1066 g ae ha^{-1} of 2,4-D choline, and 1 application timing (vegetative stage). For both year, herbicide was applied using a CO_2-pressurized tank sprayer with a two-nozzle booms spaced 51 cm apart. Sprayer was calibrated to deliver 140 L ha^{-1} at 276 kPa through TeeJet 8001E nozzle (TeeJet Technologies, Spraying systems Co., Wheaton, IL). Travel speed of the nozzle was based on the walking speed of 4.8 km/hr. In 2019 treatment with sublethal doses was on 20 Jun while treatment in 2020 was done in 12 Jun. At that time, lettuce reached the midpoint of the growing cycle which we referred as vegetative stage. The application was done on mid-June to simulate when 2,4-D was typically apply to Enlist Corn and Enlist E3 soybean as POST emergence weed control. Application window for Enlist corn is before V8 stage or 30 inches tall. For Enlist E3 soybean, application can be done before R2 or full flowering stage (Enlist 2021 Product Use Guide). Visual injury rating was conducted every seven days until harvest with the same protocols as the greenhouse experiment. All lettuce were harvested and collected as fresh weight on 10 July in 2019 and 23 Jun in 2020 for the yield data. Lettuce within each plot for each responding rate was averaged to gram per plant then further divided to 4 (replications) for average yield.

1.2.5 STATISTICAL ANALYSIS

Yield data were based on dry biomass and percentage yield loss relative to the controls. Due to the non-linear nature of plant response to sublethal rates of herbicide, a four-parameter log-logistic regression model was used to analyze the relationship between either sublethal rates of 2,4-D or dicamba with visual injury, average yield and percentage yield loss, utilizing approach described in Knezevic et al. (2007).

The four parameter model was defined by the equation

$Y = c + \{ d - c / 1 + \exp [b(\log x - \log e)]\}$

where c is lower limit, d is upper limit, b is slope and e is the ED 50 (dose giving 50% response) (Seefeldt et al. 1995). The regression analyses helped estimate the actual rate of 2,4-D or dicamba. For example, in the aspect of percent injury, ED10 will give the prediction rate that will cause 10% injury (same with percent yield loss). Regression analyses were conducted using drc package in R version 3.4.1 (R Core Team, 2019). Recorded days after treatment (DAT) injury ratings were averaged across the six replications and were fit to the response across the six sublethal rates within the corresponding application timing.

Percentage yield loss was calculated using the equation (Wortman, 2014):

$Y= [(C – T) / C] 100$

where Y represents the percentage yield loss compared to the nontreated control plot in the corresponding replication block, C represents the biomass of the nontreated control plot, and T represents the biomass of the treated plot. Graphs generated were non-linear regression and were in log scale. By fitting all 6 sublethal rates, the model gave the full range prediction of the actual drift rate. This can be done using the effective dosage value (ED). ED can be set in the range of 0-100% which represent percent injury and percent yield loss as described in Knezevic et al. (2007).

1.3 RESULTS

1.3.1 2019 GREENHOUSE 'GREEN FOREST' LETTUCE VISUAL RATINGS

Visual symptoms of 2,4-D injury on seedling 'Green Forest' included leaf curling and twisting on the newest leaves (Figure 1.1a). Newly growing leaves were stunted and the petiole of the leaves was thickened. The symptoms of 2,4-D on 'Green Forest' were detected as early as one day after the herbicide treatment, particularly following exposure to the two highest sublethal rates of 1/4 and 1/10 of the labeled rate. The lowest rate that caused visible injury was 1/100. This showed in Figure 1.2a of 'Green Forest' treated at seedling stage 7 and 22 DAT with injury ranged 10 to 15% compared with control. Mature lettuce 'Green Forest' on the other hand did not exhibit severe injury except at the two highest rates 1/4 and 1/10 at 7 DAT (Figure 1.1b, Figure 1.3a). Overall, mature stage 'Green Forest' showed higher tolerance to 2,4-D compared to seedling stage because 1/100 did not shown any symptoms. Interestingly, the laminae of both old and newly growing leaves of mature staged 'Green Forest' were strongly recurved, rugose or papillose. The newly growing leaves of the two highest rates (1/4 and 1/10 rate) of seedling plants appeared stunted and slowly growing. Until harvest, those seedling plants were not able to recover and the percentage yield loss was nearly to 100% (Figure 1.5a). Newly growing leaves of mature plants at the two highest rates (1/4 and 1/10 rate) showed abnormal growth with the center of the leaves wide open. This is not normal for head forming lettuce as 'Green Forest'. The texture of the injured leaves became quite rigid and less flexible. Leaves and petioles were thicker than the nontreated plants. For seedling stage 'Green Forest', as time proceed, percentage injury got worse. At 22 DAT, the two highest rates showed 90 to 100% injury (Figure 1.2a). By comparing the estimation rate to cause 5% and 50% injury (ED 5 and ED 50) from the dose response model for seedling stage and mature stage lettuce. It is clear the rate caused 5% and

50% injury for seedling stage 7 and 22 DAT ranged from 5.38 to 8.03 and 34.50 to 46.09 g ae ha^{-1} respectively (Table 1.1). Mature stage showed ED 5 value of 41.89 g ae ha^{-1} and ED 50 value of 101.66 g ae ha^{-1} (Table 1.2) which is higher than the seedling stage estimation. This confirmed the observation that mature 'Green Forest' lettuce had higher tolerance than seedling lettuce to 2,4-D off-target injury.

1.3.2 2019 GREENHOUSE 'GREEN FOREST' LETTUCE YIELD

Seedling stage 'Green Forest' showed good fit for dose-response model (Figure 1.4a). Average dry weight is at the approximate 15 g plant^{-1}. The two highest rate of 1/4 and 1/10 have the highest yield reduction of nearly 0 g plant^{-1} (Figure 1.4a). Dose response estimated 4.00 ± 2.09 g ae ha^{-1} reduced yield by 5% while rate of 20.14 ± 5.77 reduced yield by 50% (Table 1.1). Mature stage 'Green Forest' had high variation with poor model fitting (Figure 1.4a). Dose response estimated 1.41 ± 2.53 g ae ha^{-1} reduced yield by 5% while rate of 1.91 ± 8.17 g ae ha^{-1} reduced yield by 50% (Table 1.2).

1.3.3 2019 GREENHOUSE 'GREEN FOREST' LETTUCE YIELD LOSS

Regardless of growth stage, effective dose (ED) estimated for 5%, 10%, 20%, and 50% yield loss was lower than the corresponding rate for percentage injury. This showed lettuce are very susceptible to yield loss at lower rate. For example, the estimated rate that caused 10% injury 7 DAT was equivalent to 20% yield loss at harvest. On seedling 'Green Forest' lettuce, a dose range of 4.00 ± 1.72 to 20.14 ± 4.73 g ae ha^{-1} caused 5% and 50% yield loss respectively (Table 1.1). On mature 'Green Forest', a dose of 19.78 ± 22.47 g ae ha^{-1} caused an estimated 5% yield loss. Whereas a dose of 95.50 ± 60.13 g ae ha^{-1} caused 50% yield loss (Table 1.2). When

‘Green Forest’ was treated at seedling stage, 1/4, 1/10, and 1/100 sublethal doses showed yield loss and the top two highest rates showed 100% yield loss (Figure 1.5a). In mature ‘Green Forest’, the two highest sublethal doses 1/4, and 1/10 caused 25% yield loss (Figure 1.5a). Dose response curves measure total biomass yield loss (not marketable yield loss), but injury symptoms observed (Figure 1.1b) clearly suggest that mature lettuce impacted by rates of 1/4 and 1/10 would not be marketable.

1.3.4 2020 GREENHOUSE ‘GREEN FOREST’ LETTUCE VISUAL RATINGS

Visual injury symptoms observed on seedling ‘Green Forest’ showed exact same symptoms as in 2019. For seedling ‘Green Forest’, the most visible injury symptoms were observed on 1/4 and 1/10 sublethal rates. Sublethal rates of 1/50, 1/100, 1/500, and 1/1000 all showed minor injuries in the form of slight leaf curling compared with the control (0) (Figure 1.6a). The percentage injury estimation generated from the dose response model in 2020 matched with our estimation in 2019. Estimated sublethal doses for seedling ‘Green Forest’ 7 DAT in 2019 matched with 7 DAT in 2020 across ED 5, 10, 20, and 50. Estimated value for seedling ‘Green Forest’ 22 DAT in 2019 also matched with 27 DAT in 2020 despite the observation date in 2020 was 5 days late (Table 1.1 and Table 1.3). Initial percentage injury observed on seedling ‘Green Forest’ 7 DAT in 2020 (Figure 1.7a) were less severe compare with 2019 7 DAT (Figure 1.2a). However, as time proceed, injuries got worse and the top three rates, 1/4, 1/10, and 1/50 showed 100%, 80%, and 25% injury respectively (Figure 1.7a). The rate caused 5% and 50% injury for seedling stage 7 and 22 DAT ranged from 3.39 ± 2.83 to 9.16 ± 1.51 and 47.48 ± 3.96 to 80.66 ± 19.16 g ae ha^{-1} respectively (Table 1.3). Mature stage 3 DAT showed ED 5 value of 47.52 ± 11.00 g ae ha^{-1} and ED 50 value of 111.27 ± 4.62 g ae ha^{-1}. When observed 7 DAT for the mature ‘Green Forest’, injury got worse as all 4 highest rate 1/4, 1/10, 1/50, and 1/100 all showed injury as 90%, 50%, 30%, and 15% respectively (Figure 1.8a). This trend also showed in the estimated dose value to cause injury as the number was much lower which proved lettuce were more susceptible to 2,4-D injury even plants were bigger.

1.3.5 2020 GREENHOUSE ‘GREEN FOREST’ LETTUCE YIELD

Same trend was observed on seedling stage ‘Green Forest’ with good model fitting (Figure 1.9a). The average lettuce yield was at approximate 20 g plant^{-1}. The two highest rate showed highest yield reduction. Dose response model estimated rate of 20.96 ± 11.45 g ae ha^{-1} resulted 5% yield reduction while rate of 64.57 ± 12.89 g ae ha^{-1} resulted 50% yield reduction (Table 1.3). Mature stage ‘Green Forest’ had high variation with poor model fitting. Due to this, the dose response model failed to converge (Table 1.4).

1.3.6 2020 GREENHOUSE ‘GREEN FOREST’ LETTUCE YIELD LOSS

Yield loss results in 2020 were different than 2019. On seedling ‘Green Forest’, a dose range of 23.82 ± 13.93 and 67.44 ± 13.66 g ae ha^{-1} caused 5% and 50% yield loss respectively (Table 1.3). On mature ‘Green Forest’, a dose range of 1.48 ± 4.13 and 38.42 ± 32.96 g ae ha^{-1} caused 5% and 50% yield loss respectively (Table 1.4). Estimated value for percentage yield loss across ED 5, 10, 20, and 50 for seedling stage ‘Green Forest’ were higher than the value in 2019. Mature stage estimate values were smaller than 2019. Due to plants were larger at mature stage, the biomass differences were harder to differentiate. On mature ‘Green Forest’, the dose response model did not converge because there was no consistent yield loss across rates (Figure 1.10a). This also showed high standard error (Table 1.4). As in 2019 and 2020, the visible injury symptoms on mature ‘Green Forest’ at the three highest rates would eliminate any opportunity to sell the lettuce; thus, the economic loss is likely much greater than the biomass yield loss.

1.3.7 2020 FIELD ‘GREEN FOREST’ LETTUCE

In 2020, the only sublethal rate that caused visible injury on field-grown ‘Green Forest’ was 1/4 of the labeled rate (Figure 1.9a). No consistent injury was observed on any other sublethal rates. However, despite a lack of visible injury in the 1/10 and 1/50 rates, there was measurable yield loss (Figure 1.9b). A dose of 147.96 ± 1.02 g ae ha^{-1} caused 5% injury whereas a dose of 168.04 ± 1.12 g ae ha^{-1} caused 50% injury (Table 1.5). In contrast, a dose of 55.92 ± 24.28 and 136.61 ± 21.63 g ae ha^{-1} caused 5% and 50% percentage yield loss respectively (Table 1.5). Dose response model generated from yield data also showed similar estimation (Table 1.5). A dose of 57.08 ± 24.93 g ae ha^{-1} resulted 5% yield reduction while dose of 134.26 ± 21.39 g ae ha^{-1} resulted 50% yield reduction (Table 1.5). In other words, 5% injury at 7 DAT resulted in 50% yield loss. Overall, field results for ‘Green Forest’ lettuce were similar to the greenhouse, but field-grown lettuce appears to be somewhat more tolerant of 2,4-D.

1.3.8 2019 GREENHOUSE ‘VULCAN’ LETTUCE VISUAL RATINGS

Visual injury of 2,4-D on ‘Vulcan’ lettuce shared similar symptomology as on ‘Green Forest’ lettuce. Symptoms included stunted growth on the youngest tissues, leaf curling and twisting, and thickened petioles (Figure 1.12). One unique symptoms on ‘Vulcan’ lettuce were bluish-green color and of a leathery texture. In the greenhouse, we observed injury as early as 1 day after treatment in the two highest rates. Symptoms of 2,4-D injury were prominent by 7 DAT and the lowest sublethal rate that caused visible injury at the seedling growth stage was 1/10 for

‘Vulcan’ (Figure 1.12a). Results were similar for mature Vulcan at 7 DAT (Figure 1.12b). Vulcan lettuce did show laminae strongly recurved and leaves were soft with leathery texture and bluish-green in color. We also observed loss of red pigmentation on the injured Vulcan lettuce treated with 1/4, 1/10, and 1/100 sublethal rate, and the color remained bluish-green through harvest. As time proceed, injuries got worse especially for the two highest rates in seedling stage ‘Vulcan’. The two highest rate showed approximate 85% injuries 7 DAT, however, at 22 DAT lettuce were died (100% injury) (Figure 1.2b). On seedling ‘Vulcan’, a dose of 4.64 to 26.46 and 21.62 to 33.33 g ae ha^{-1} caused 5% injury 7 and 22 DAT and 50% injury 7 and 22 DAT respectively (Table 1.1). On mature ‘Vulcan’, 73.02 ± 185.46 g ae ha^{-1} caused 5% injury while 104.68 ± 12.88 g ae ha^{-1} caused 50% injury (Table 1.2). The sublethal dose response for ‘Vulcan’ in 2019 was similar to ‘Green Forest’ in 2019.

1.3.9 2019 GREENHOUSE ‘VULCAN’ LETTUCE YIELD

‘Vulcan’ lettuce at seedling stage showed good model fitting and the two highest rates resulted dry yield at approximate 0 g plant^{-1} (Figure 1.4b). Dose response model estimated 11.46 ± 8.88 g ae ha^{-1} resulted 5% yield reduction whereas rate of 28.00 ± 32.33 g ae ha^{-1} resulted 50% yield reduction (Table 1.1). Mature stage ‘Vulcan’ showed high variation in yield. Dose response model estimated rate of 2.44 ± 10.25 g ae ha^{-1} caused 5% yield reduction whereas rate of 10.99 ± 7.75 g ae ha^{-1} caused 50% yield reduction (Table 1.2).

1.3.10 2019 GREENHOUSE ‘VULCAN’ LETTUCE YIELD LOSS

The top two highest rates of 2,4-D showed 100% yield loss on seedling ‘Vulcan’. For mature ‘Vulcan’, the top three highest rates showed yield loss ranged from 35%, 23%, and 10% for the rate of 1/4, 1/10, and 1/50 (Figure 1.5b). For seedling ‘Vulcan’, a dose of 11.16 ± 5.96 g ae ha^{-1} caused 5% yield loss whereas dose of 34.27 ± 12.02 g ae ha^{-1} caused 50% yield loss (Table 1.1). Mature stage ‘Vulcan’ was much more sensitive to 2,4-D compared with seedling stage. Much lower estimated rated was observed at mature stage ‘Vulcan’. A dose of 1.18 ± 1.85 g ae ha^{-1} caused 5% yield loss and dose of 15.24 ± 12.30 g ae ha^{-1} caused 50% yield loss (Table 1.2). The two lowest sublethal doses of 1/1000 and 1/10000 resulted in negative yield loss (i.e., growth increase or hormesis) when compared with the nontreated control (Figure 1.5b).

1.3.11 2020 GREENHOUSE ‘VULCAN’ LETTUCE VISUAL RATINGS

We observed similar results in 2020, but the 1/100 rate showed slight injury on seedling ‘Vulcan’ (Figure 1.13a). Injury symptoms on mature ‘Vulcan’ were again only visible on the two highest sublethal rates of 1/4 and 1/10 (Figure 1.13b). On seedling ‘Vulcan’, two highest rates 1/4 and 1/10 showed 38%, and 18% injury 7 DAT but the injury got worse at 27 DAT as 100% and 85% injury (Figure 1.7b). Estimated rate to cause 5% injury for seedling stage ‘Vulcan’ showed range of 11.46 to 46.41 g ae ha^{-1} on 7 and 27 DAT. For 50% injury, estimated rate was range from 48.09 to 122.42 g ae ha^{-1} on 7 and 27 DAT (Table 1.3). Mature ‘Vulcan’ 3 DAT showed 38% and 22% injury on the rate of 1/4 and 1/10. At 7 DAT, top four rates all showed varies percentage injury from highest as 90% and lowest of 22% (Figure 1.8b). For the estimation from the dose response model, mature ‘Vulcan’ for 3 and 7DAT showed the range of

1.67 to 18.80 g ae ha^{-1} for the 5% injury estimates. A range of 26.58 to 99.27 g ae ha^{-1} was estimated for 3 and 7 DAT for the 50% injury (Table 1.4).

1.3.12 2020 GREENHOUSE 'VULCAN' LETTUCE YIELD

Seedling stage 'Vulcan' had good model fitting with the two highest rates caused the highest yield reduction (Figure 1.9b). Dose response model estimated a rate of 11.26 ± 5.55 g ae ha^{-1} resulted 5% yield reduction whereas rate of 30.16 ± 8.80 resulted 50% yield reduction (Figure 1.4). Mature stage 'Vulcan' showed high variability in yield (Figure 1.9b) which caused dose response model failed to converge (Table 1.4).

1.3.13 2020 GREENHOUSE 'VULCAN' LETTUCE YIELD LOSS

Yield loss estimates for 2020 were similar to 2019 for Vulcan at the seedling stage. A dose of 9.25 ± 3.94 g ae ha^{-1} caused 5% yield loss while 33.23 ± 7.57 g ae ha^{-1} caused 50% yield loss (Table 1.3). On mature 'Green Forest' lettuce, the dose response model did not converge because there was no consistent yield loss across rates (Figure 1.10b). Mature stage 'Vulcan', just as 2020 greenhouse 'Green Forest', all sublethal rates showed no yield loss (Figure 1.10a). Yield was "increased" by the approximate range of 10-40% (Figure 1.10b). Even though there were "increase" in biomass, all top four rates showed injuries from 20 to 90%. Those visual injury would still make lettuce not marketable.

1.3.14 2020 FIELD 'VULCAN' LETTUCE

We observed injuries on field-grown 'Vulcan' lettuce at the two highest rates of 1/4 and 1/10 (Figure 1.14a). A dose of 62.49 ± 3.77 g ae ha^{-1} caused 5% injury while a dose of 135.61 ±

3.78 caused 50% injury. ‘Vulcan’ lettuce yield was especially sensitive to 2,4-D. All sublethal rates observed yield reduction (Figure 1.14b). A dose of 0.85 ± 1.07 g ae ha^{-1} resulted 5% yield reduction whereas dose of 43.30 ± 16.10 resulted 50% yield reduction (Table 1.5). Percentage yield loss have the similar trend (Figure 1.14c) and estimation (Table 1.5). A dose of 0.8 ± 1.02 caused 5% yield loss whereas 37.31 ± 14.27 caused 50% yield loss. Similar to results for ‘Green Forest’ lettuce, these models suggest that a dose causing just 5% visible injury would likely results in 50% yield reduction.

1.3.15 2019 ‘ALLSTAR’ LETTUCE VISUAL RATINGS

‘Allstar’ is a mixture of different lettuces including green oakleaf, red oakleaf, red romaine, green leaf, and red leaf lettuces. Because it is a mixture, plant stands are much denser compared with head lettuce. Injuries of 2,4-D on ‘Allstar’ also showed leaf curling and crinkling (Figure 1.15a, 1.15b). On seedling ‘Allstar’, the two highest rates 1/4 and 1/10 showed injuries (Figure 1.15a, Figure 1.2c). On 3 DAT, a dose of 6.36 ± 1.71 g ae ha^{-1} caused 5% injury whereas a dose of 46.44 ± 7.69 g ae ha^{-1} caused 50% injury. On 12 DAT, a dose of 21.19 ± 10.79 g ae ha^{-1} caused 5% injury whereas a dose of 66.77 ± 10.73 g ae ha^{-1} caused 50% injury (1.2c). Mature stage ‘Allstar’ also showed injuries at the top two rates (Figure 1.3c). A dose of 43.67 ± 9.37 g ae ha^{-1} caused 5% injury and dose of 108.29 ± 4.39 g ae ha^{-1} caused 50% injury (Table 1.2).

1.3.16 2019 ‘ALLSTAR’ LETTUCE YIELD

The two highest rates cause the highest yield reduction on seedling stage ‘Allstar’. Dose response model had good fit. Estimated rate of 11.70 ± 13.28 g ae ha^{-1} resulted 5% yield reduction and the rate of 83.28 ± 40.67 g ae ha^{-1} resulted 50% yield reduction (Table 1.1).

Mature stage ‘Allstar’ had poor model fitting. Estimated 33.19 ± 171.55 g ae ha^{-1} resulted 5% yield reduction and rate of 68.11 ± 143.99 g ae ha^{-1} resulted 50% yield reduction (Table 1.2).

1.3.17 2019 ‘ALLSTAR’ LETTUCE YIELD LOSS

In 2019, the top two rates caused significant yield loss in both seedling and mature stage ‘Allstar’ (Figure 1.5c). For seedling stage ‘Allstar’ top two rates caused 50 to 75% yield loss but the economic yield loss is 100% due to the present physical injury. The same rates caused 20 to 30% yield loss at mature stage also with 100% economic yield loss. On seedling ‘Allstar’, a dose of 11.71 ± 12.46 g ae ha^{-1} caused 5% yield loss and dose of 83.27 ± 38.06 g ae ha^{-1} caused 50% yield loss (Table 1.1). These doses closely mirrored the injury dose response for seedling stage ‘Allstar’. The dose response model for mature stage ‘Allstar’ estimated rate of 15.38 ± 26.64 g ae ha^{-1} caused 5% yield loss and rate of 86.03 ± 50.56 g ae ha^{-1} caused 50% yield loss (Table 1.2).

1.3.18 2020 ‘ALLSTAR’ LETTUCE VISUAL RATINGS

For both seedling and mature stage ‘Allstar’, the three highest rates 1/4, 1/10, and 1/50 showed injury (Figure 1.16a, 1.16b). Seedling stage percentage injury ranged from 20-80% (Figure 1.7c) whereas mature stage percentage injury ranged from 20-40% (Figure 1.7c). On seedling ‘Allstar’ at 7 DAT, a dose of 49.60 ± 9.95 g ae ha^{-1} caused 5% injury and a dose of 126.43 ± 7.95 g ae ha^{-1} caused 50% injury (Table 1.3). On 16 DAT, a dose of 52.90 ± 13.71 g ae ha^{-1} caused 5% injury and a dose of 126.92 ± 9.05 g ae ha^{-1}caused 50% injury (Table 1.3). For mature ‘Allstar’ at 3 DAT, a dose of 2.89 ± 1.20 g ae ha^{-1} caused 5% injury and a dose of 57.46

± 9.24 g ae ha^{-1} caused 50% injury (Table 1.4). At 7DAT, a dose of 0.98 ± 0.46 g ae ha^{-1} caused 5% injury and a dose of 67.07 ± 10.06 g ae ha^{-1} caused 50% injury (Table 1.4).

1.3.19 2020 'ALLSTAR' LETTUCE YIELD

Seedling stage 'Allstar' showed good model fitting. Estimated rate of 3.65 ± 2.19 g ae ha^{-1} resulted 5% yield reduction and rate of 54.20 ± 12.64 g ae ha^{-1} resulted 50% yield reduction (Table 1.3). Mature stage 'Allstar' estimated rate of 8.34 ± 5.95 g ae ha^{-1} resulted 5% yield reduction and rate of 16.96 ± 4.47 g ae ha^{-1} resulted 50% yield reduction (Table 1.4).

1.3.20 2020 'ALLSTAR' LETTUCE YIELD LOSS

The yield loss dose response curve in 2020 (Figure 1.10c) was similar to 2019 (Figure 1.5c). The three highest rates 1/4, 1/10, and 1/50 showed yield loss compared with the control ranged from 70 to 22%. Hormesis (growth promotion) was observed on both seedling and mature 'Allstar' at 1/500 and 1/100 sublethal rates. For seedling 'Allstar', a dose of 10.53 ± 5.57 g ae ha^{-1} caused 5% yield loss and a dose of 75.18 ± 16.28 g ae ha^{-1} caused 50% yield loss (Table 1.3), which was consistent with 2019 estimates. At the mature stage, a dose of 1.78 ± 1.92 g ae ha^{-1} caused 5% yield loss and a dose of 32.46 ± 16.34 g ae ha^{-1} caused 50% yield loss (Table 1.4).

1.4 DISCUSSION

Results suggest that three modern lettuce varieties ‘Green Forest’, ‘Vulcan’, and ‘Allstar’ were all highly susceptible to 2,4-D regardless of growth stage. Across all varieties, dose response models for injury and yield loss were better fit at the seedling stage compared to the mature stage, which suggests the impact of 2,4-D is more variable at the mature stage (Table 1.1 and Table 1.3). At the mature stage, the top three rates of 1/4, 1/10, 1/50 all showed severe injuries symptoms which would make those plants non-marketable and essentially a 100% economic loss. It might be possible for an impacted growers to cut off the injured portion of the lettuce, but the expense of labor and residual herbicide residues are practical limitations of this approach. Injury symptoms at high rates of 2,4-D became more pronounced over time, and injury symptoms were most evident on and around the growing point. Lettuce with injury symptoms showed curling and crinkly with thickened petioles on the newest growth.

The amount of herbicide interception determines the quantity available for entry which determines the efficacy of the herbicide (Hammerton 1967). Skoss (1955) demonstrated that young plants have more exposed growing points and the wax components are loosely connected with high wettability; these factors make 2,4-D acid much more easily diffused through the cuticles into the plant to cause injury at the seedling stage. Penetration study using 1-Naphthyl-[1-^{14}C] acetic acid (NAA) also showed highest rate of uptake at the period where plant leaf expansion was most rapid and reduced penetration rate as tissued aged (Baker and Hunt 1981). The rapid rate of leaf expansion is accompanied by a low rate of wax production, which creates a relatively thin surface of wax coverage. The result is increased likelihood of herbicide penetration into the plant (Baker and Hunt 1981). It is also known that plants are more sensitive to 2,4-D injury when they are applied during rapid cell division and during rapid growing stages (Song, 2014). This is consistent with observations in our seedling stage lettuce and has also been observed for many weed species. For example, Palmer amaranth (*Amaranthus palmeri*) control decreased from 93% to 74% 21 days after treatment with Enlist Duo^{TM} (2,4-choline + glyphosate) when applied to plants that were 3 to 5 cm tall versus 10 to 30 cm tall, respectively (Manuchehri et al. 2019).

A recent study of 2,4-D dimethylamine salt formula on 55-day-old, mature 'Stella' lettuce, showed lettuce were killed when treated with 80.4 g a.i. ha^{-1} (Roesler et al. 2020). Mature plants in our study were harvested 7 DAT so no mortality was observed, but injury was significant. Roesler et al. (2020) demonstrated 1/10 rate of 2,4-D dimethylamine salt (670 g a.i. ha^{-1}) on 'Stella' also resulted in significant biomass reduction which plant was killed. Similar results were observed when seedling 'Green Forest' and 'Vulcan' were treated with 1/10 rate (106.6 g ae ha^{-1}) of 2,4-D choline salt (1066 g ae ha^{-1}). Another study sprayed triethanolamine salts of 2,4-D on lettuce variety 'Borough Wonder' at 12 days and 25 days after emergence and reported 99% marketability on the older plants at rate of 33.6 g ae ha^{-1}. However, spraying plants at 25 days after emergence with 112.1 g ae ha^{-1} resulted in 0% marketability of lettuce. The injury was more severe on the younger plants. Application of 33.6 g ae ha^{-1} resulted in only 32% marketability of lettuce and 112.1 g ae ha^{-1} led to 0% marketability (Way 1964). 112.1 g ae ha^{-1} of 2,4-D triethanolamine salts is similar to the 1/10 labeled rate (106.6 g ae ha^{-1}) of the 2,4-D choline in our study that caused 0% marketability on both seedling and mature stage lettuce due to severe injury symptoms. In our study, 33.6 g ae ha^{-1} 2,4-D choline caused 50% yield loss on seedling stage ‘Green Forest’ and ‘Vulcan’ in both years. However, no yield loss or visual injury was observed at 1/50 (21.3 g ae ha^{-1}) on all three lettuce varieties at the mature stage.

We did not observe any reduction in lettuce marketability when plants were sprayed with 21.3 g ae ha^{-1} (1/50 rate) 2,4-D choline at the mature stage. Way (1964), however, showed ‘Borough Wonder’ sprayed at medium size of twenty-eight leaves were almost completely unmarketable after application rates ranging from 11.2 g ae ha^{-1} to 112 g ae ha^{-1}. This matched with our 2020 field study when lettuce was at vegetative stage, though ‘Vulcan’ showed higher sensitivity to 2,4-D choline than the ‘Green Forest’ variety (Table 1.5). When comparing with Way (1964), we conclude that the severity of off-target injury to lettuce is similar for 2,4-D choline salt and 2,4-D amine salt.

Visual symptoms for ‘Green Forest’, ‘Vulcan’, and ‘Allstar’ shared similar injury symptoms including leaf curling, twisting, and thickened petioles. Mature stage ‘Green Forest’ and ‘Vulcan’ showed leaves strongly recurved with the center loosely open which prevents lettuce head formation. These symptoms closely match those from the MCPA lettuce study where growth was reduced and leaves were flattened with the center exposed (Lettuce and Way 1962). Only ‘Green Forest’ appeared to be rigid, but ‘Vulcan’ showed bluish-green color with leathery texture. This was one unique symptom and could be a potential identifier for 2,4-D or phenoxy acid related drift. Similar symptoms as ‘Vulcan’ were found on the summer cabbage lettuce variety ‘Borough Wonder’ after treatment with MCPA, another form of the phenoxy acid (Lettuce and Way 1962). In our field study in 2020, the 1/4 rate was required to induce injury in ‘Green Forest’ (Figure 1.9a). ‘Vulcan’ lettuce showed injury at both 1/4 and 1/10 rates (Figure

1.12a). While injury was not visible at lower rates, yield loss was still observed on all sublethals rates (Figure 1.12b). This is very important for lettuce growers and herbicide applicators, because it demonstrates the importance of minimizing off-target herbicide drift.

An increase in plant biomass in response to sublethal doses of growth regulator herbicide is a phenomenon known as hormesis. We did observe hormesis in our greenhouse study in both 2019 and 2020 when lettuce was treated at the mature stage. Stimulatory effects on total plant length in *Ranunculus aquatilis* (an aquatic plant species) was observed when treated with 2,4-D at a concentration of 30 µg L^{-1} (Belgers et al. 2007). Plant growth stage significantly influences the hormesis potential of any species and herbicide combination (Belz and Duke 2014). Results of our experiment suggest that lettuce at the seedling stage is unlikely to exhibit hormesis in response to sublethal rates of 2,4-D, whereas off-target drift during the mature stage may lead to increases in biomass. However, that increase in biomass was inconsistent across varieties, years, and environments; and any gains in biomass were more than offset by the potentially negative economic consequences of visible herbicide injury on marketable portions of the plant. This unpredictable effect of hormesis is supported by several studies in field and controlled environments (Appleby, 1998; Belz & Cedergreen, 2010; Belz & Leberle, 2012). One practical application of hormesis is the use of glyphosate in sugar cane. For the ripening sugar cane, the application of sublethal doses of 160 to 460 g ae ha^{-1} can reduce the vegetative growth of sugar cane and increase the sucrose accumulation (Dalley and Richard 2010). However, in most cases the risk of losing the crop or reducing marketability far outweighs any potential benefits (Belz et al. 2011).

1.5 CONCLUSION

Based on our greenhouse and field studies, it is clear that 2,4-D negatively impacts the growth and yield of lettuce verities ‘Green Forest’, ‘Vulcan’, and ‘Allstar’. It is important to note that lettuce yield loss was sometimes recorded even when no plant injury was visible (e.g., 2020 field study). Lettuce growers should be vigilant and document suspected drift events even if visual injury is not detected on the plants. When comparing with older formulations of 2,4-D used in previous studies, it seems that lettuce are equally susceptible to injury and yield reduction. Even at relatively low sublethal rates, yield loss could be as great as 20%. Although 2,4-D choline salt is less likely to vaporize after the application and cause particle drift (Sosnoskie et al. 2015), our results suggest that injury and yield loss is still potent once the chemical reaches the susceptible plant. Given these results, herbicide applicator should take extra precautions when applying 2,4-D choline near sensitive specialty crops. Precautions include: follow the label, use proper nozzle size that create larger droplet, use adjuvants that increase the surface tension, lower boom height closer to the ground, and travel at moderate speeds. Applications should be avoided during temperature inversions and periods of high wind speed.

It is interesting to note that we observed a stimulus effect (hormesis) in our mature stage lettuce in the greenhouse study. However, any benefits of the small increase in biomass was offset by visual injury and reduced marketability from the 2,4-D. In addition, the mechanism of hormesis is still unknown and is difficult to replicate as it is related with many environmental factors.

Although we did not measure herbicide residue persistence, it could be possible that 2,4-D residues remaining on asymptomatic lettuce plants could exceed EPA thresholds of 0.1 ppm for vegetables (EPA § 180.142) that would further limit marketability after a drift event. These off-target drift events are particularly problematic for certified organic lettuce growers because they risk losing their certification status. Given the value at stake during any drift event, it is important to explore strategies for minimizing future events. DriftWatch^{TM} (driftwatch.org) is a helpful tool that helps farmers and pesticide applicators easily find neighboring specialty crop farms to facilitate communication and preventative measures before herbicide applications.

In conclusion, the new formulation of 2,4-D choline has great value for weed suppression with lower drift rates, but specialty crops like lettuce remain highly susceptible to injury and yield loss from this chemical. Thus, herbicide applicators should continue to practice good stewardship when using the herbicide.

FIGURES AND TABLES

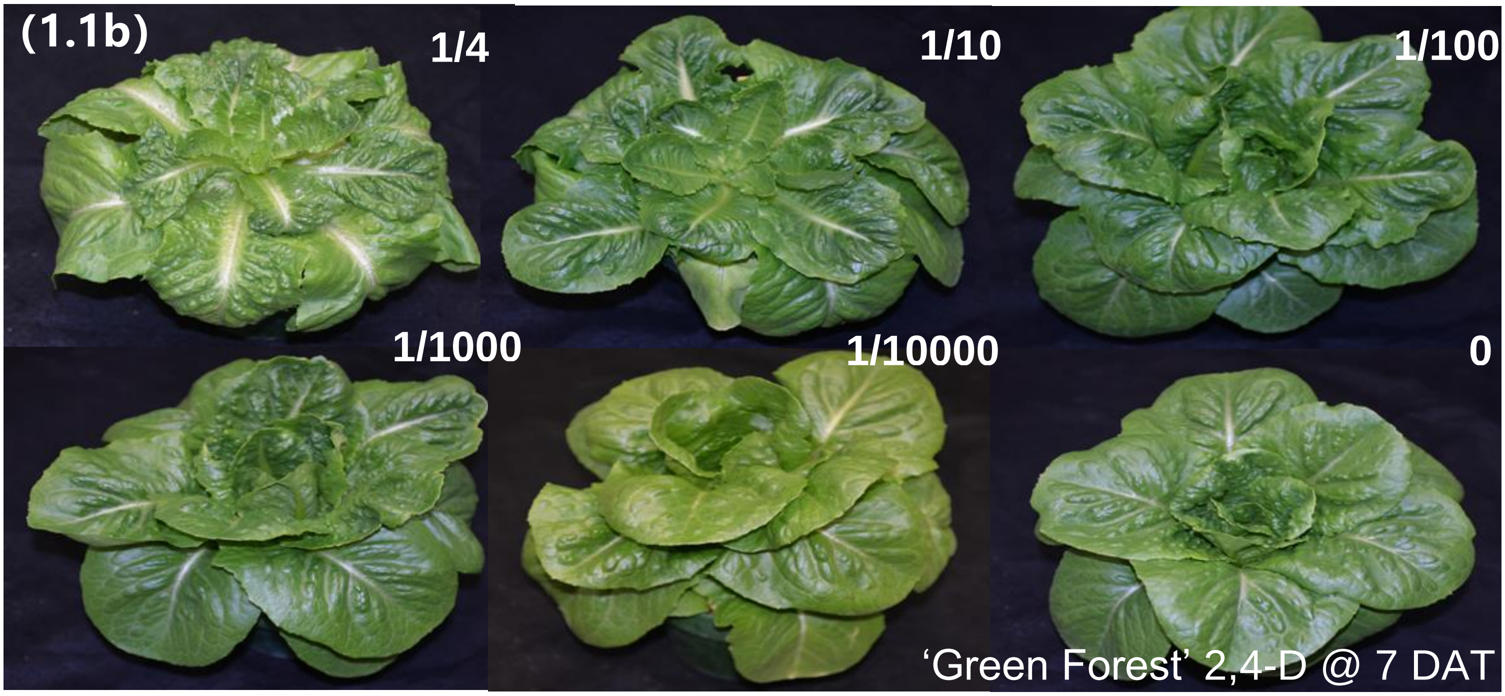
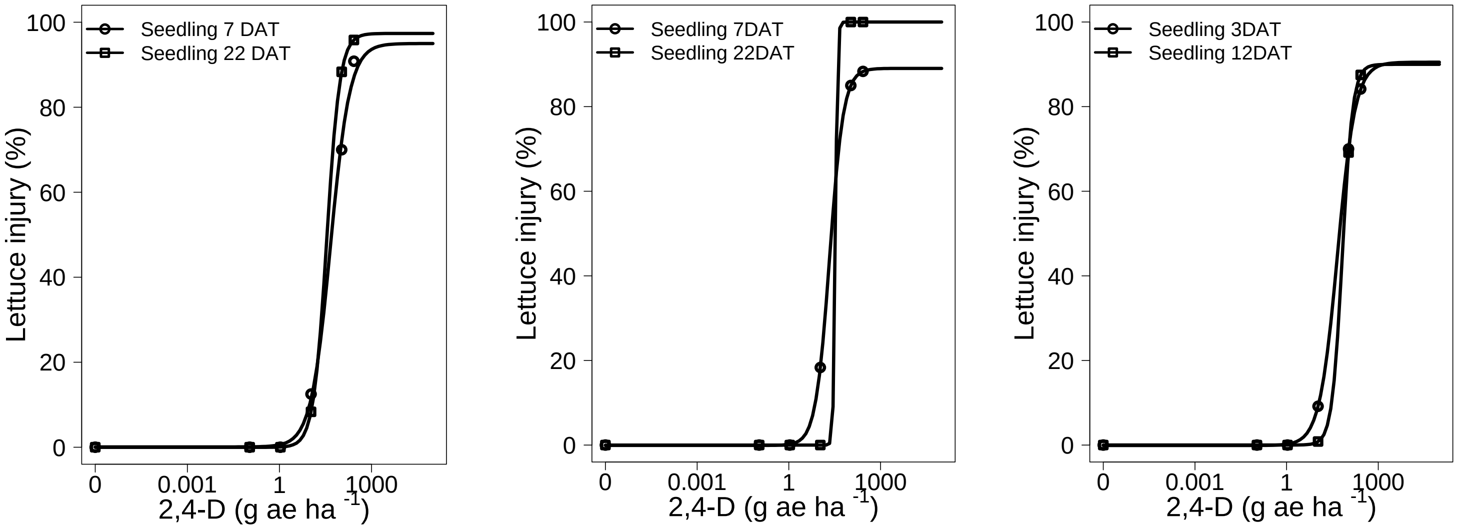


Figure 1.1: 2019 Greenhouse seedling ‘Green Forest’ (1.1a) and Mature ‘Green Forest’ (1.1b) injury from different sublethal rates of Enlist one® (2,4-D) at 7 DAT. On seedling ‘Green Forest’ dose of 1/4, 1/10, and 1/100 showed signs of chlorosis, curling, and twisting of young leaves as well as stunting, and necrosis. On mature ‘Green Forest’, dose of 1/4 and 1/10 showed backside curling of both old and newly growing leaves.

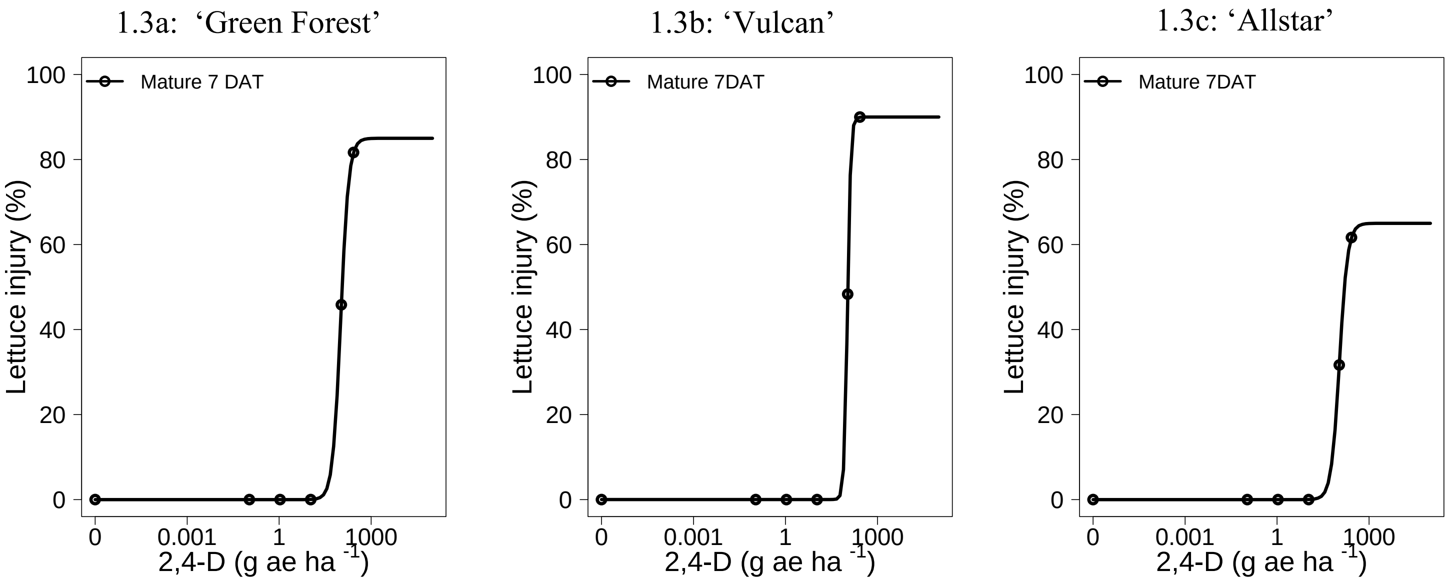


1.2a: ‘Green Forest’

1.2b: ‘Vulcan’

1.2c: ‘Allstar’

Figure 1.2: 2019 Greenhouse seedling stage comparison of 7 DAT and 22 DAT for ‘Green Forest’ (1.2a) and ‘Vulcan’ (1.2b) and 3 DAT and 12DAT for ‘Allstar’ (1.2c) percentage injury non-linear regression curve of Enlist one® (2,4-D). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/100; 1/1000; 1/10000.



1.3a: ‘Green Forest’

1.3b: ‘Vulcan’

1.3c: ‘Allstar’

Figure 1.3: 2019 Greenhouse mature stage of 7 DAT for ‘Green Forest’ (1.3a), ‘Vulcan’ (1.3b), and ‘Allstar’ (1.3c) percentage injury non-linear regression curve of Enlist one® (2,4-D). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/100; 1/1000; 1/10000.

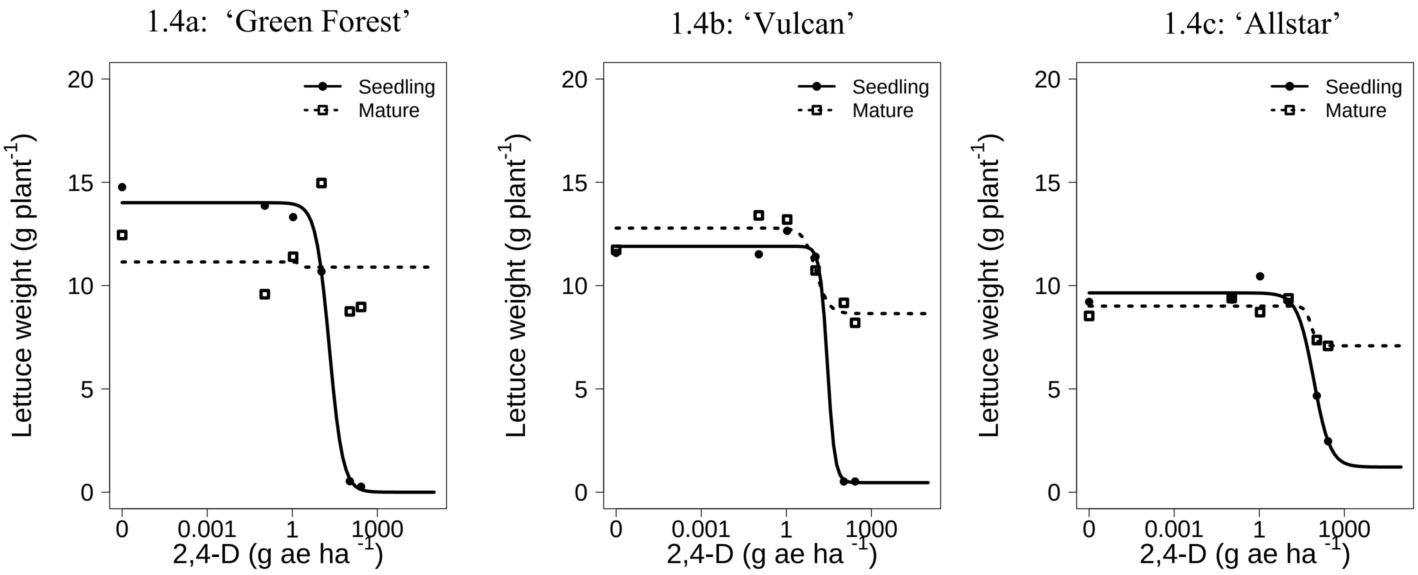
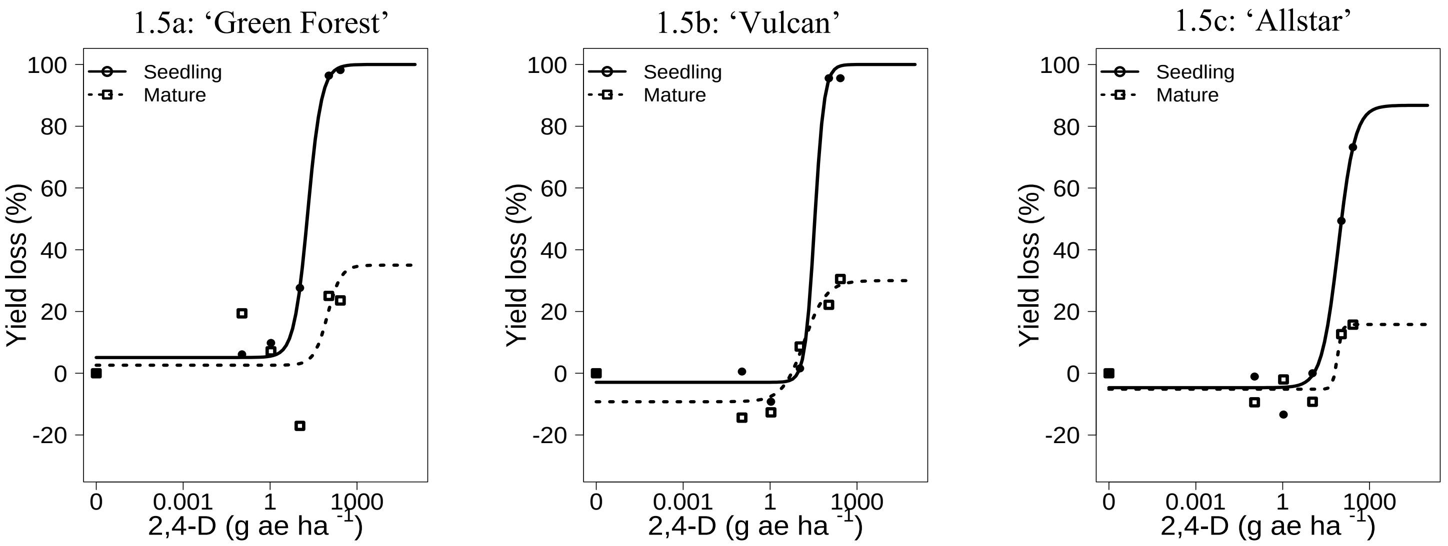


Figure 1.4: 2019 Greenhouse percentage yield upon harvest non-linear regression of Enlist one® (2,4-D) on 'Green Forest' (1.4a), 'Vulcan' (1.4b), and 'Allstar' (1.4c) lettuce at both seedling and mature stage. Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/100; 1/1000; 1/10000.

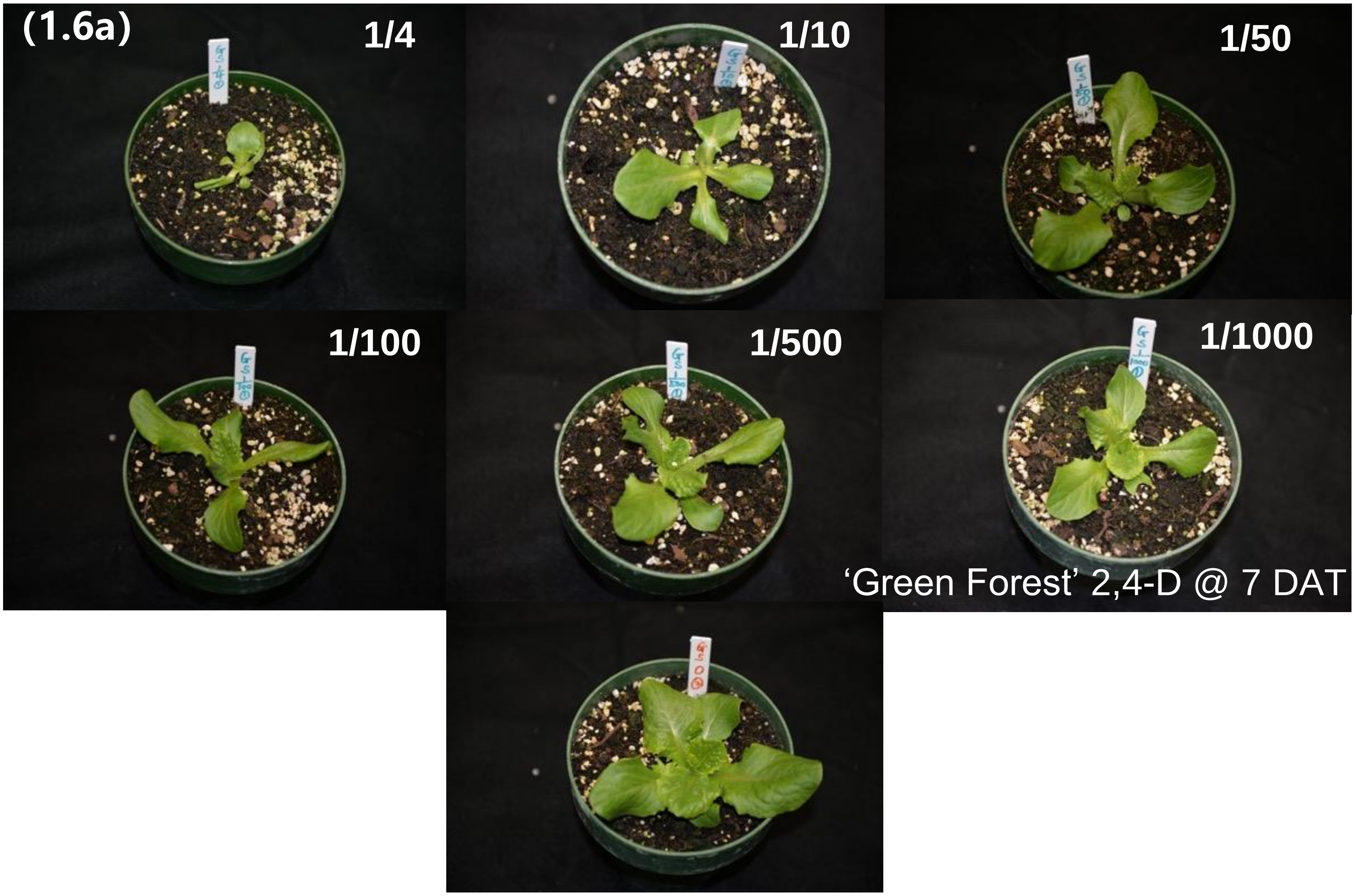


1.5a: ‘Green Forest’

1.5b: ‘Vulcan’

1.5c: ‘Allstar’

Figure 1.5: 2019 Greenhouse percentage yield loss upon harvest non-linear regression of Enlist one® (2,4-D) on ‘Green Forest’ (1.5a), ‘Vulcan’ (1.5b), and ‘Allstar’ (1.5c) lettuce at both seedling and mature stage. Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/100; 1/1000; 1/10000.


(1.6a)
1/4
1/10
1/50
1/100
1/500
1/1000
'Green Forest' 2,4-D @ 7 DAT

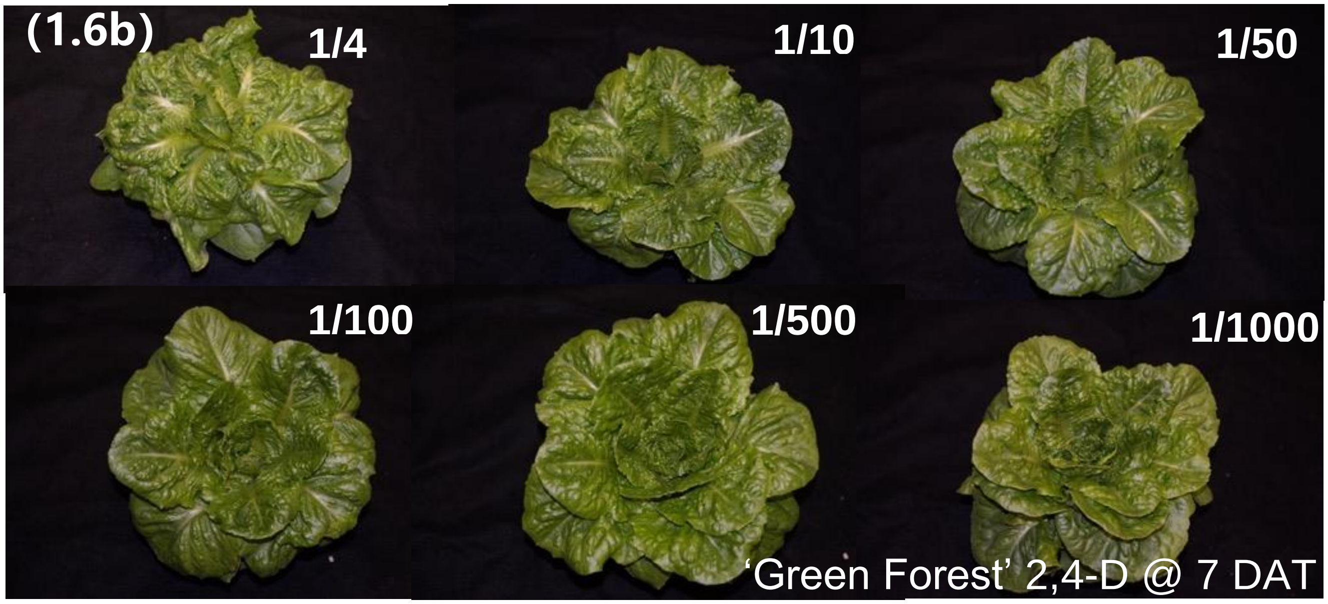
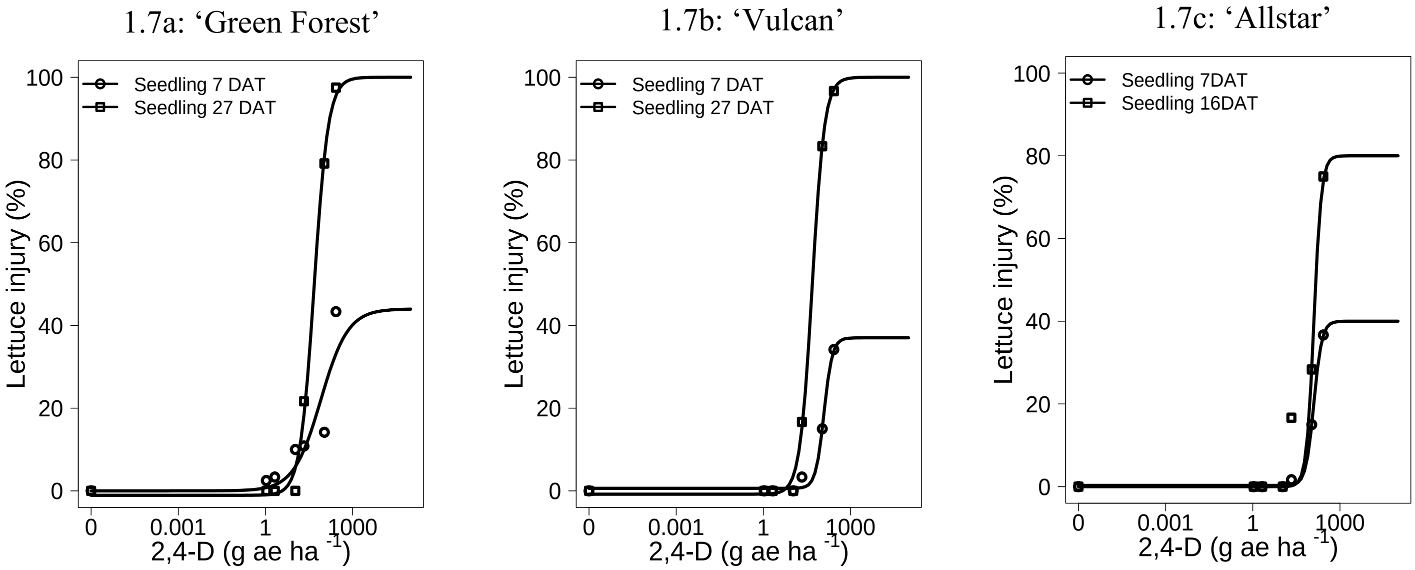

(1.6b)
1/4
1/10
1/50
1/100
1/500
1/1000
'Green Forest' 2,4-D @ 7 DAT

Figure 1.6: 2020 Greenhouse seedling ‘Green Forest’ (1.6a) and Mature ‘Green Forest’ (1.6b) injury from different sublethal rates of Enlist one® (2,4-D) at 7DAT. On seedling ‘Green Forest’, dose of 1/4, 1/10, 1/50, 1/100, 1/500, and 1/1000 showed signs of chlorosis, curling, and twisting of young leaves as well as stunting, and necrosis. On mature ‘Green Forest’, dose of 1/4 and 1/10 showed backside curling of both old and newly growing leaves.



1.7a: ‘Green Forest’

1.7b: ‘Vulcan’

1.7c: ‘Allstar’

Figure 1.7: 2020 Greenhouse seedling stage comparison of 7 DAT and 27 DAT for ‘Green Forest’ (1.7a) and ‘Vulcan’ (1.7b) and 7 DAT and 16 DAT for ‘Allstar’ (1.7c) percentage injury non-linear regression curve of Enlist one® (2,4-D). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500; 1/1000.

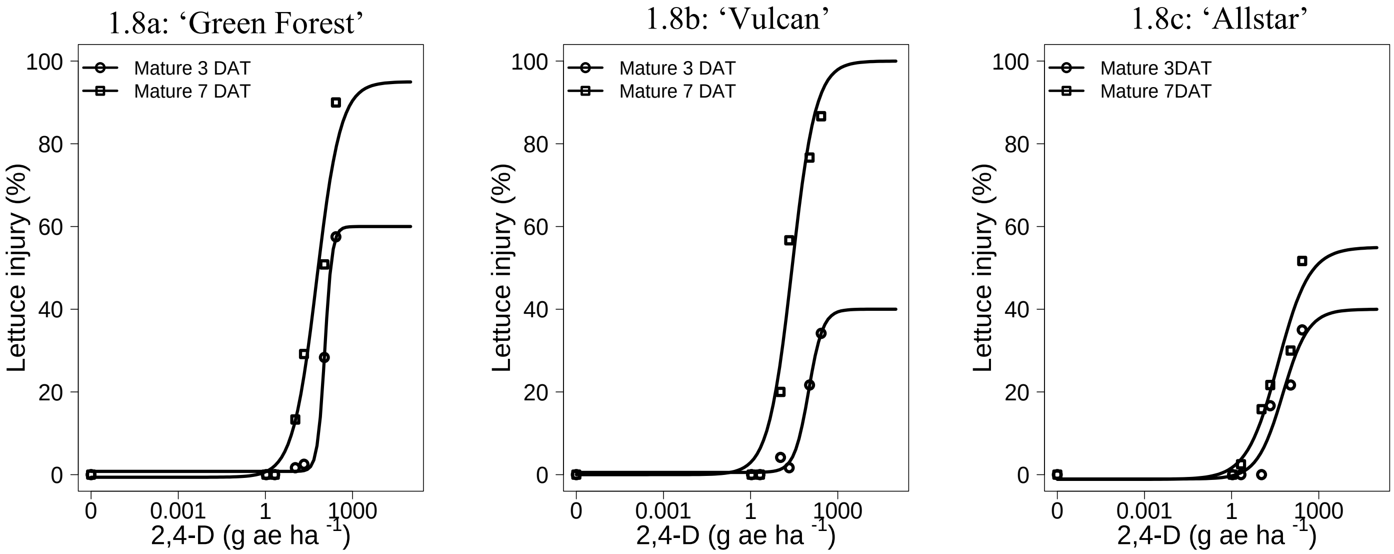
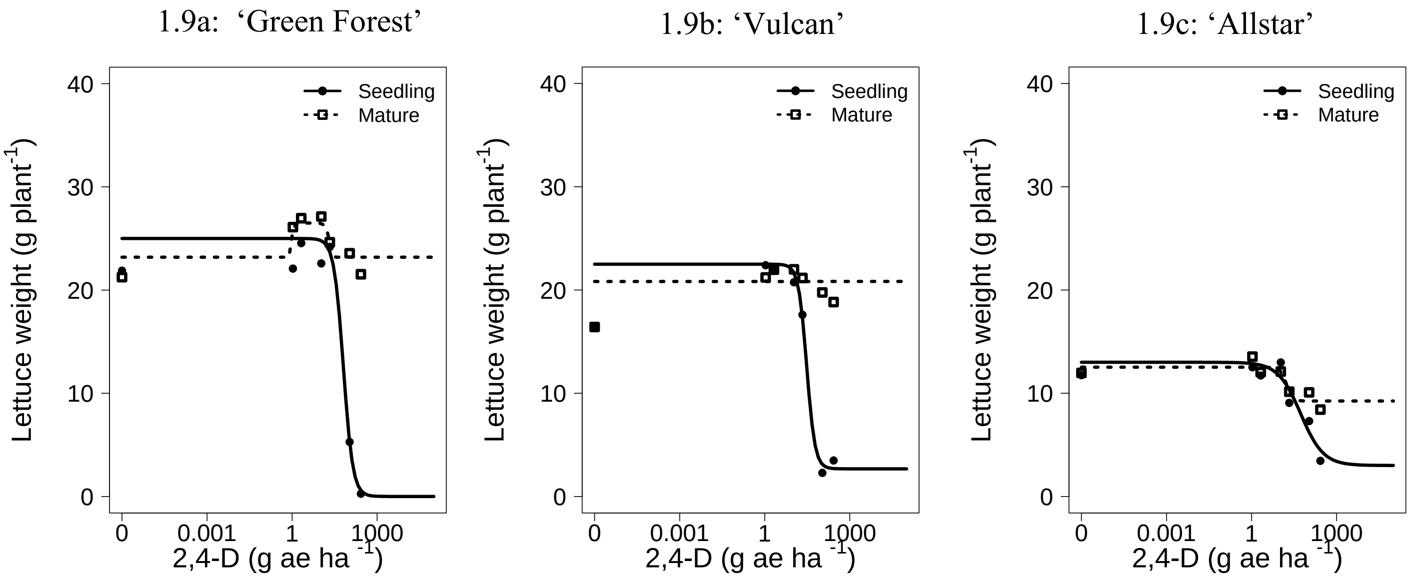


Figure 1.8: 2020 Greenhouse mature stage of 3 DAT and 7 DAT for ‘Green Forest’ (1.8a), ‘Vulcan’ (1.8b), and ‘Allstar’ (1.8c) percentage injury non-linear regression curve of Enlist one® (2,4-D). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500; 1/1000.

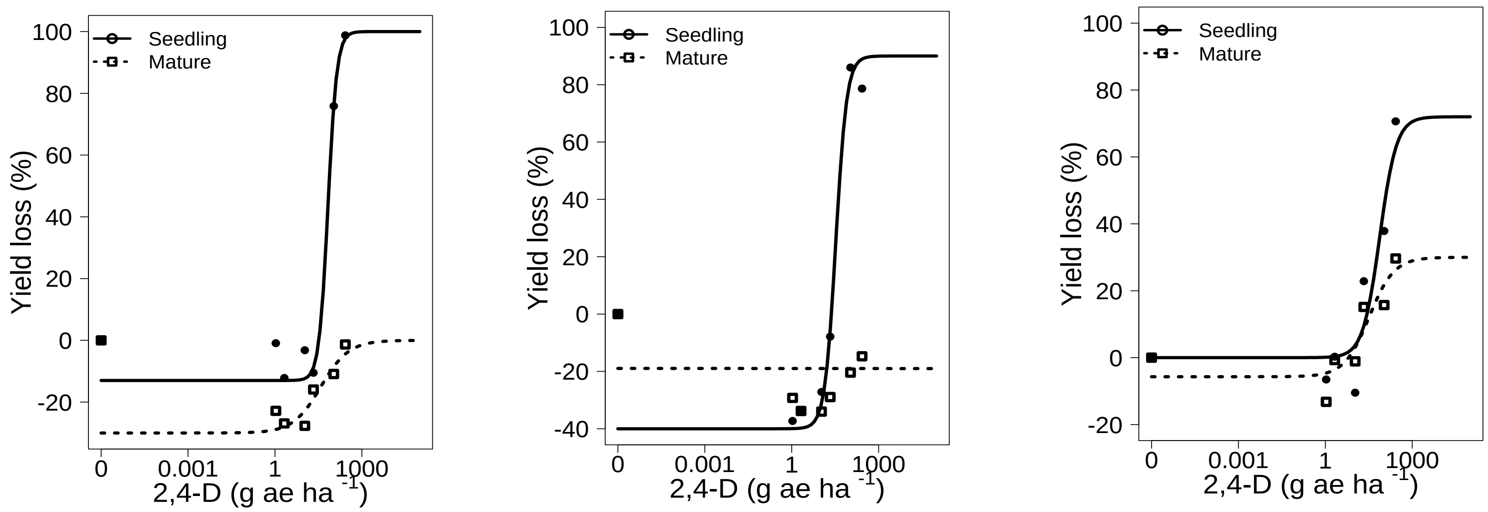


1.9a: 'Green Forest'

1.9b: 'Vulcan'

1.9c: 'Allstar'

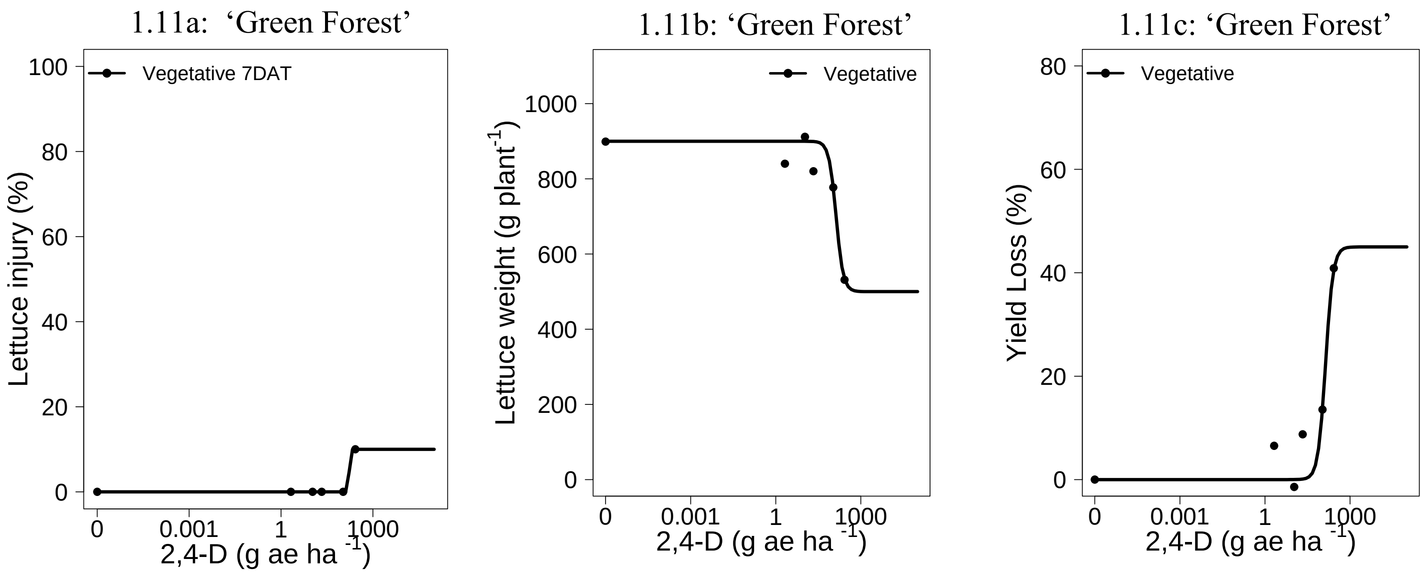
Figure 1.9: 2020 Greenhouse percentage yield upon harvest non-linear regression of Enlist one® (2,4-D) on 'Green Forest' (1.9a), 'Vulcan' (1.9b), and 'Allstar' (1.9c) lettuce at both seedling and mature stage. Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500; 1/1000.


1.10a: ‘Green Forest’

1.10b: ‘Vulcan’

1.10c: ‘Allstar’

Figure 1.10: 2020 Greenhouse percentage yield loss upon harvest non-linear regression of Enlist one® (2,4-D) on ‘Green Forest’ (1.10a), ‘Vulcan’ (1.10b), and ‘Allstar’ (1.10c) lettuce at both seedling and mature stage. Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500; 1/1000.

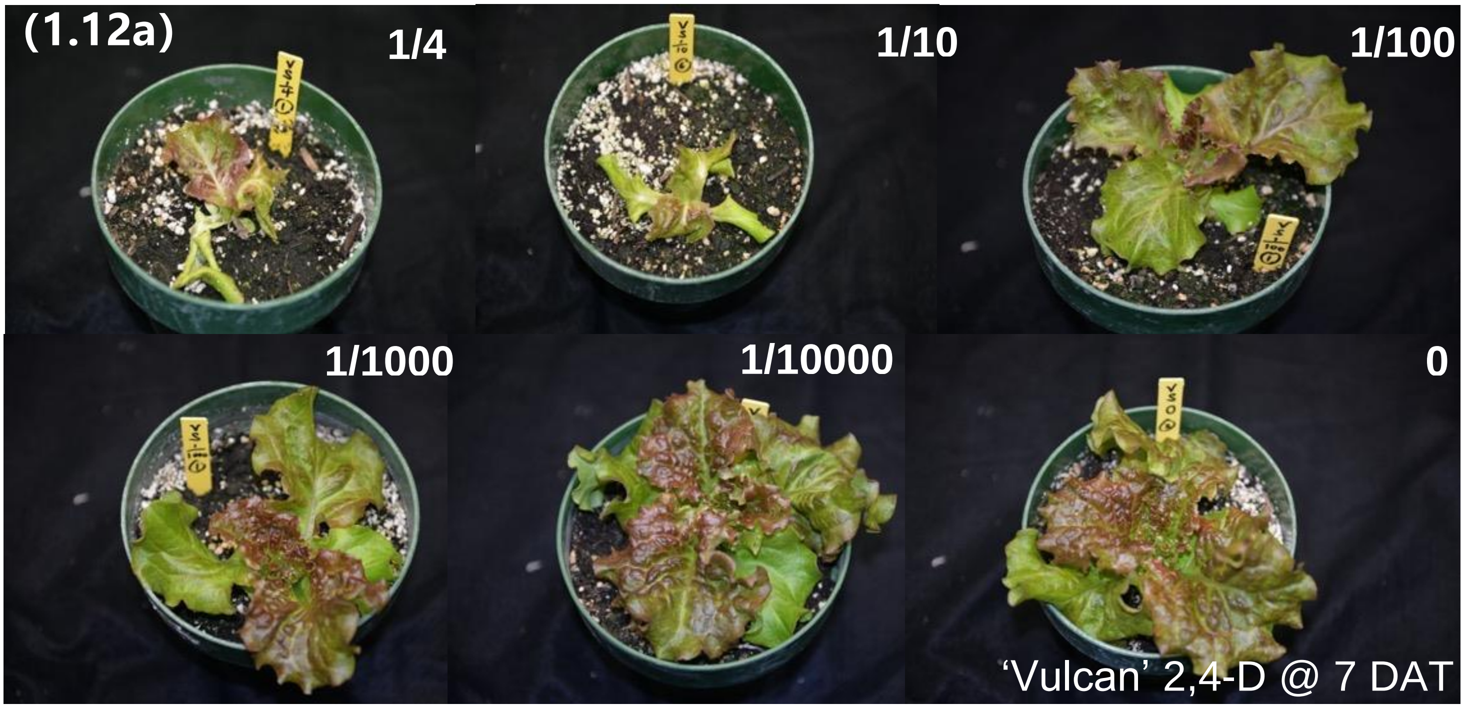


1.11a: ‘Green Forest’

1.11b: ‘Green Forest’

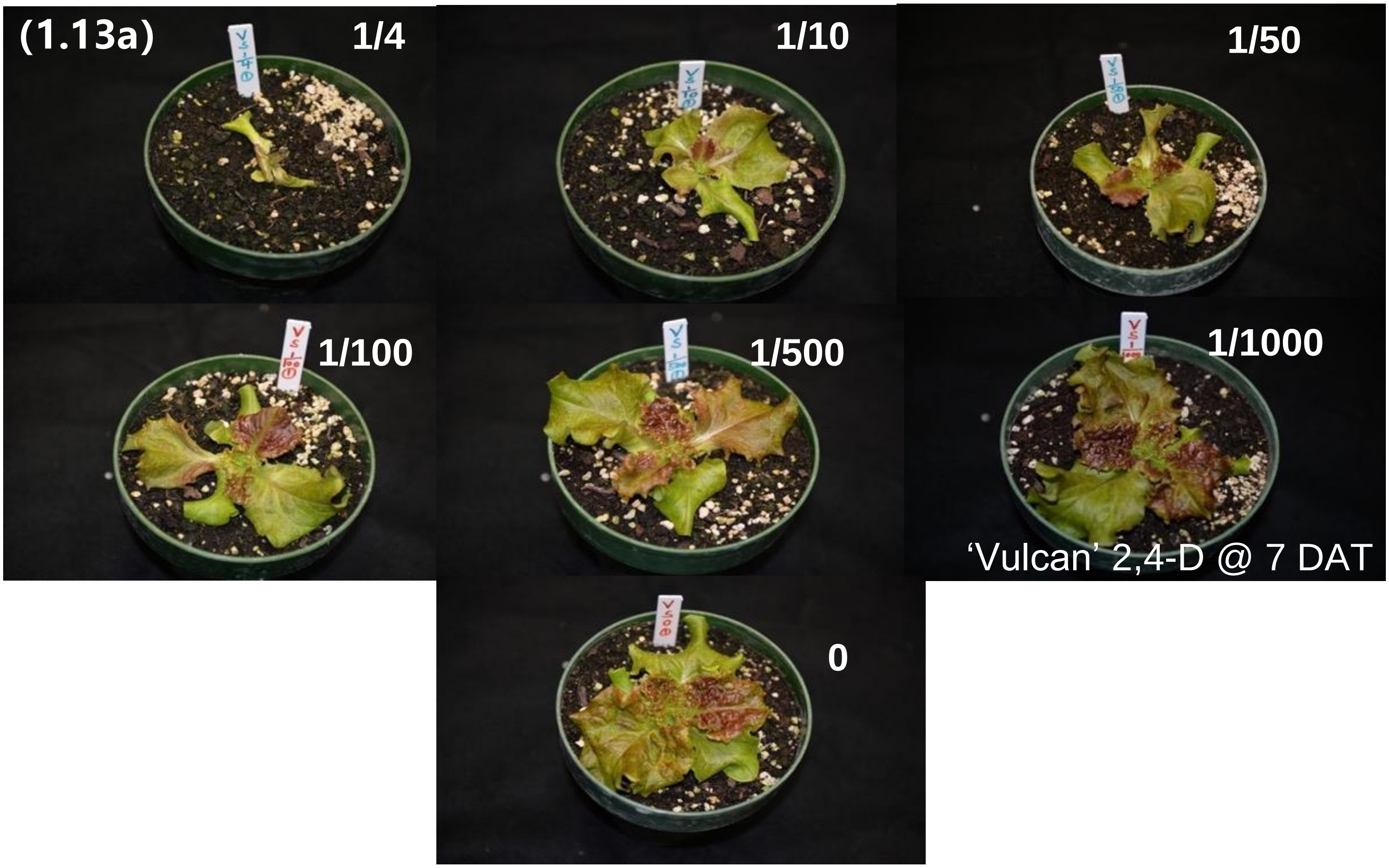
1.11c: ‘Green Forest’

Figure 1.11: 2020 Field 7 DAT at vegetative stage ‘Green Forest’ percentage injury (1.11a), yield (1.11b), and percentage yield loss upon harvest (1.11c) non-linear regression curve of Enlist one® (2,4-D). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500. Only the highest rate of 1/4 showed injury.


(1.12a)
1/4
1/10
1/100
1/1000
1/10000
0
‘Vulcan’ 2,4-D @ 7 DAT


(1.12b)
1/4
1/10
1/100
1/1000
1/10000
0
‘Vulcan’ 2,4-D @ 7 DAT

Figure 1.12: 2019 Greenhouse Seedling ‘Vulcan’ (1.12a) and Mature ‘Vulcan’ (1.12b) injury from different sublethal rates of Enlist one® (2,4-D) at 7DAT. On seedling ‘Vulcan’, dose of 1/4, and1/10 showed signs of chlorosis, curling, and twisting of young leaves as well as stunting, and necrosis. On mature ‘Vulcan’, dose of 1/4 and 1/10 showed backside curling of both old and newly growing leaves.


(1.13a)
1/4
1/10
1/50
1/100
1/500
1/1000
'Vulcan' 2,4-D @ 7 DAT
0

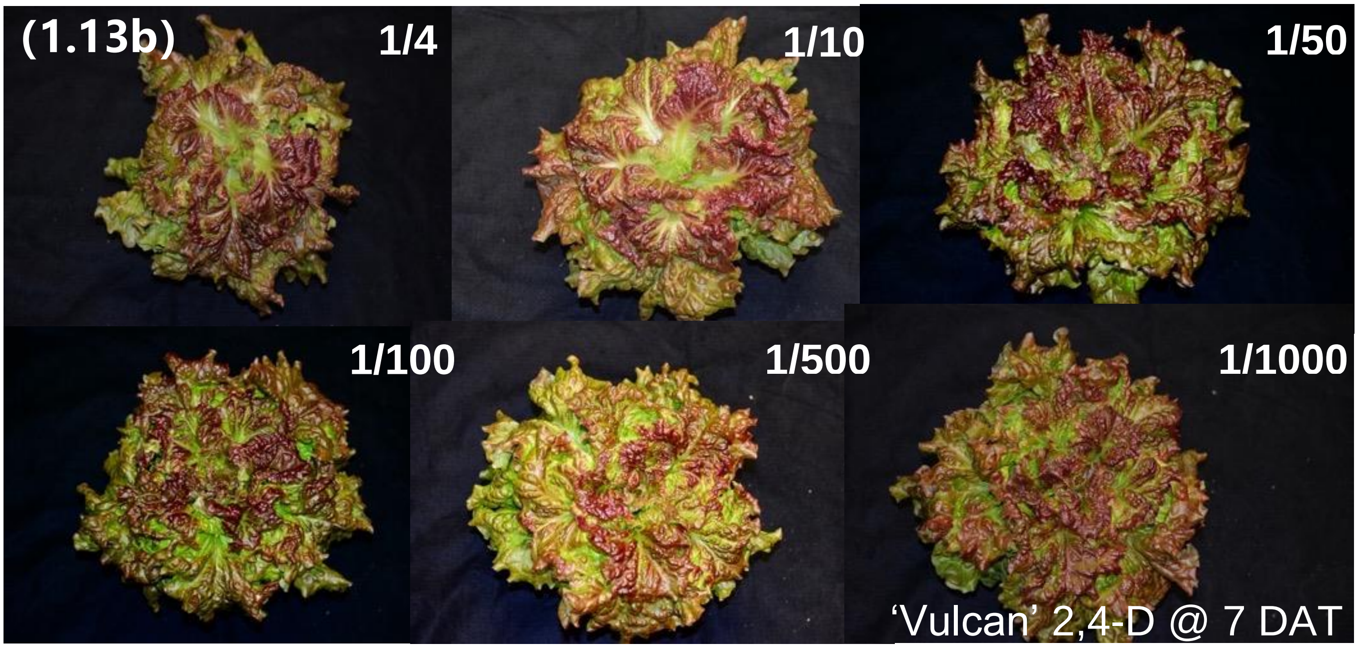
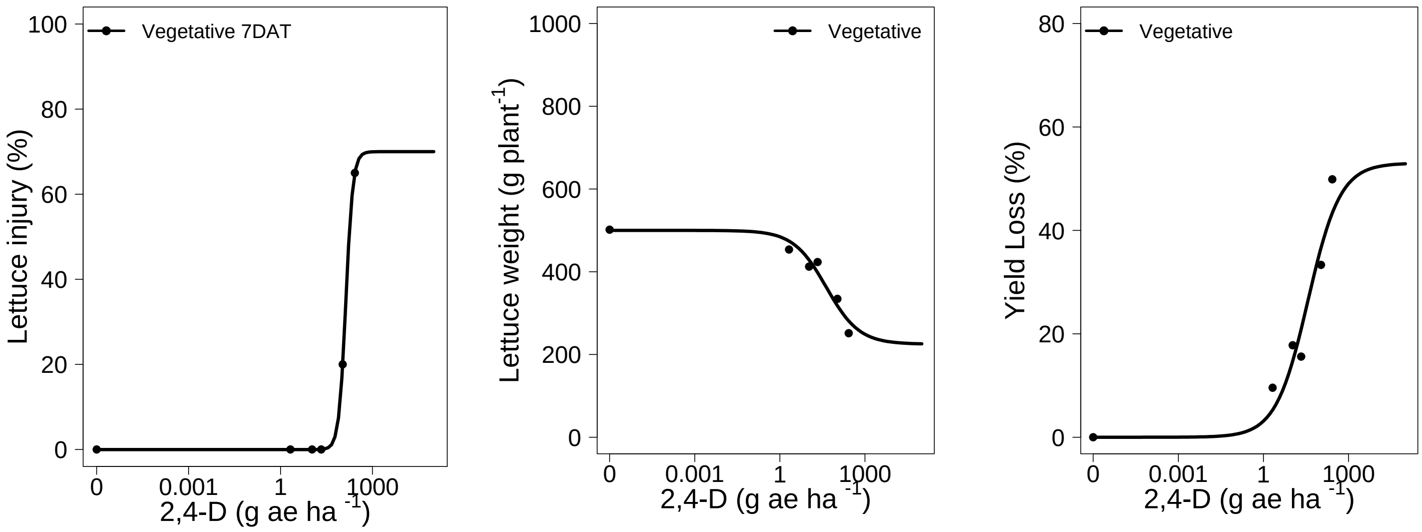

(1.13b)
1/4
1/10
1/50
1/100
1/500
1/1000
'Vulcan' 2,4-D @ 7 DAT

Figure 1.13: 2020 Greenhouse Seedling ‘Vulcan’ (1.13a) and Mature ‘Vulcan’ (1.13b) injury from different sublethal rates of Enlist one® (2,4-D) at 7 DAT. On seedling ‘Vulcan’, dose of 1/4, 1/10, and 1/100 showed signs of chlorosis, curling, and twisting of young leaves as well as stunting, and necrosis. On mature ‘Vulcan’, dose of 1/4 and 1/10 showed backside curling of both old and newly growing leaves.

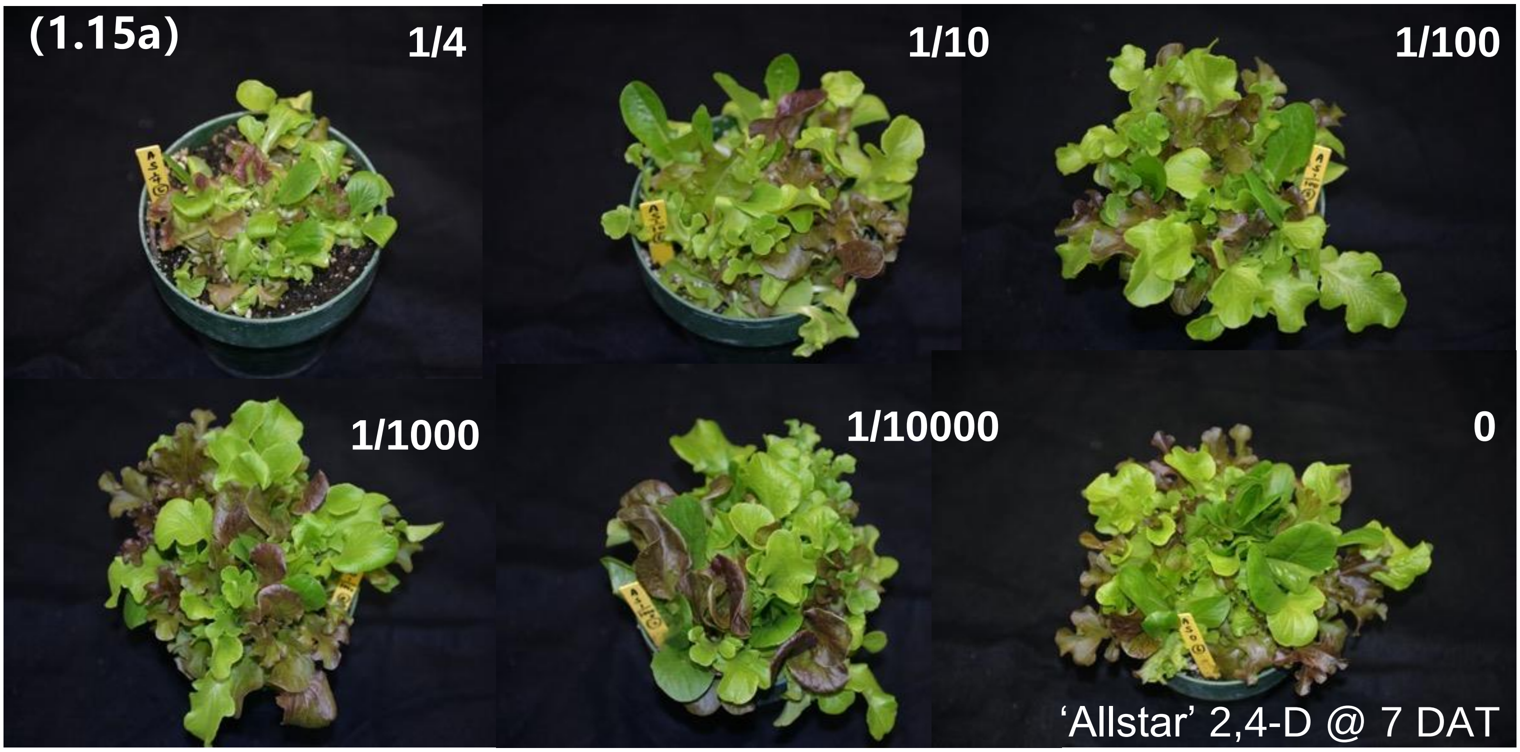


1.14a: ‘Vulcan’

1.14b: ‘Vulcan’

1.14c: ‘Vulcan’

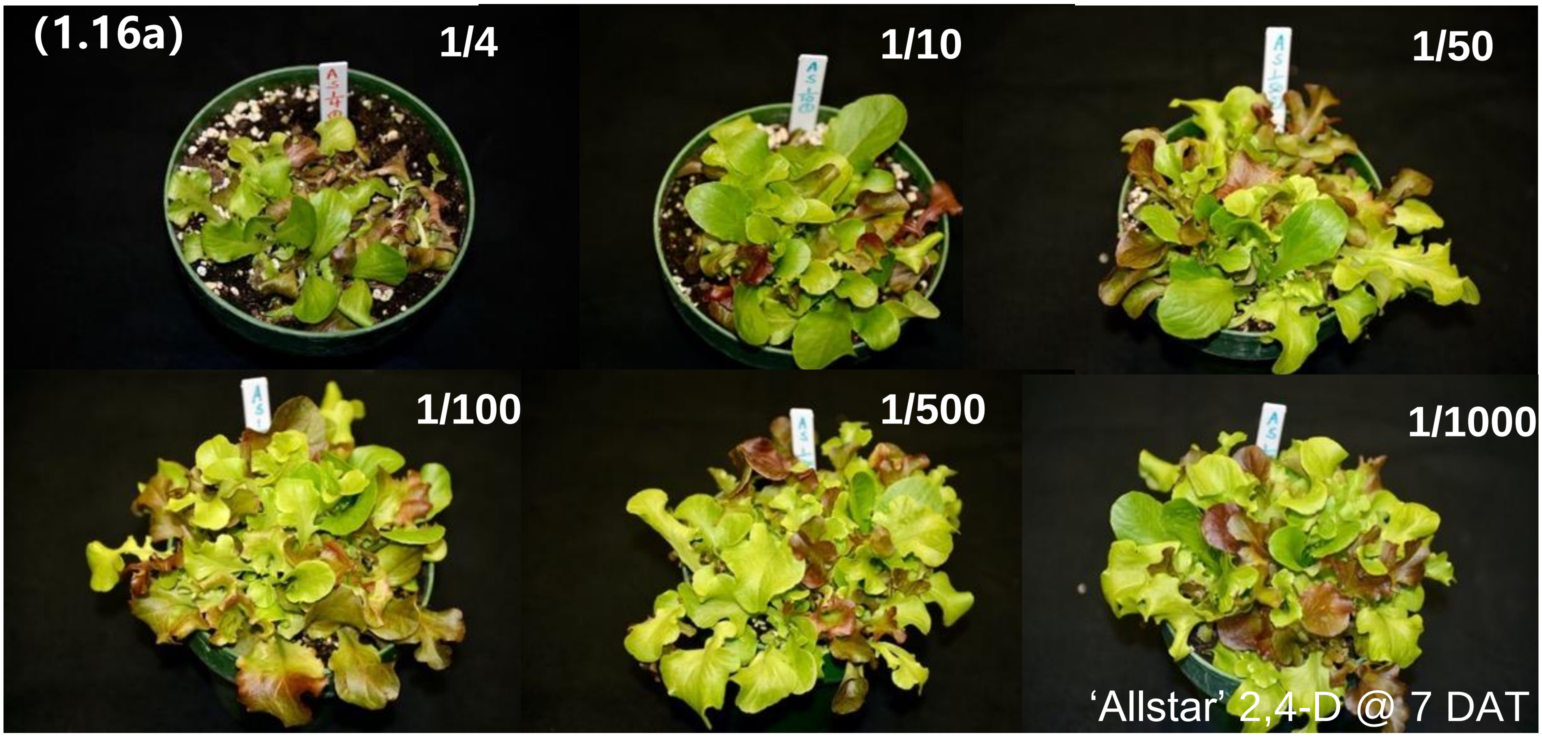
Figure 1.14: 2020 Field 7 DAT at vegetative stage ‘Vulcan’ percentage injury (1.14a), yield (1.14b), and percentage yield loss upon harvest (1.14c) non-linear regression curve of Enlist one® (2,4-D). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500. The two highest rate of 1/4 and 1/10 observed injury.



‘Allstar’ 2,4-D @ 7 DAT

‘Allstar’ 2,4-D @ 3 DAT

Figure 1.15: 2019 Greenhouse Seedling ‘Allstar’ (1.15a) and Mature ‘Allstar’ (1.15b) injury from different sublethal rates of Enlist one® (2,4-D) at 7 DAT. On seedling ‘Allstar’, dose of 1/4, 1/10, and 1/100 showed signs of chlorosis, curling, and twisting of young leaves as well as stunting, and necrosis. On mature ‘Allstar’, dose of 1/4 and 1/10 showed backside curling of both old and newly growing leaves.


(1.16a)
1/4
1/10
1/50
1/100
1/500
1/1000
'Allstar' 2,4-D @ 7 DAT

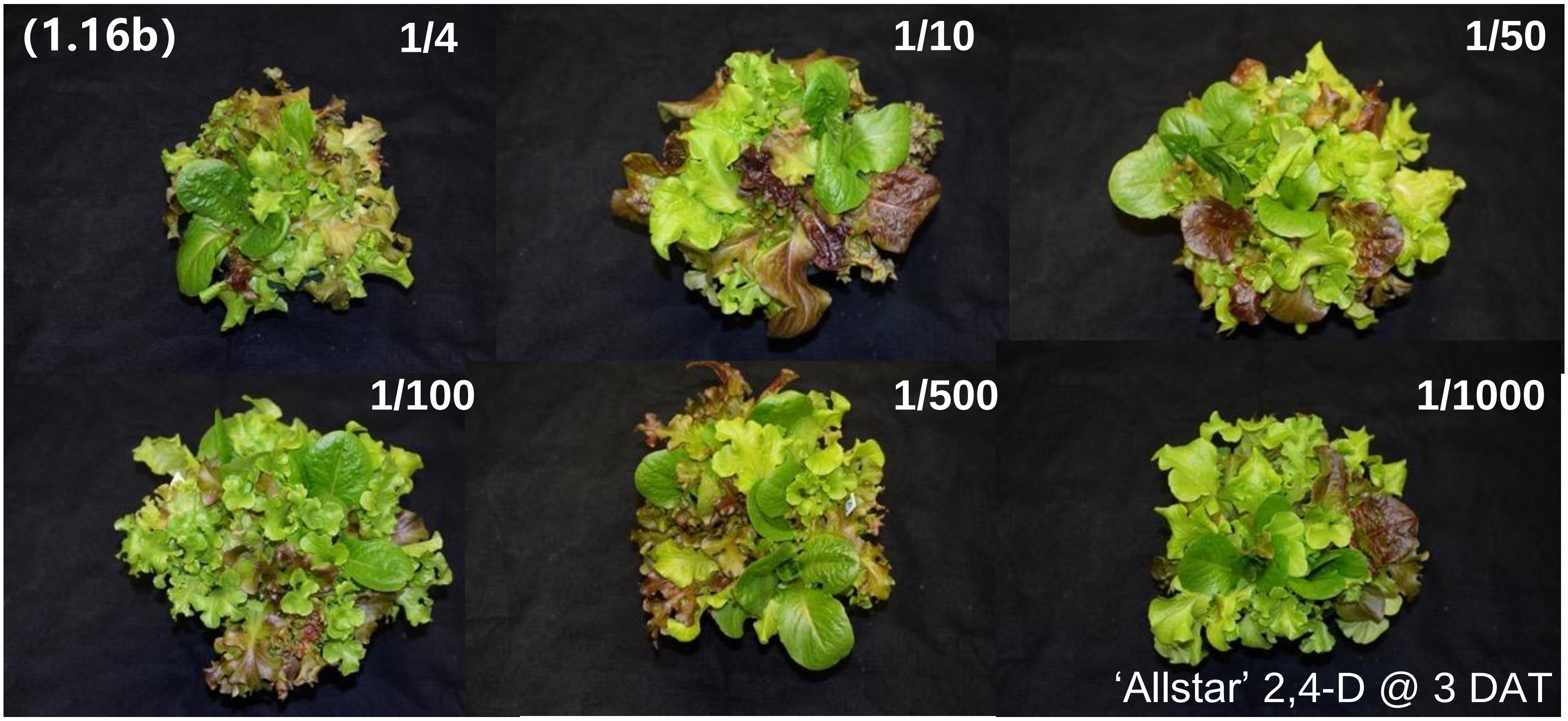

(1.16b)
1/4
1/10
1/50
1/100
1/500
1/1000
'Allstar' 2,4-D @ 3 DAT

Figure 1.16: 2020 Greenhouse Seedling ‘Allstar’ (1.16a) and Mature ‘Allstar’ (1.16b) injury from different sublethal rates Enlist one® (2,4-D) at 7 DAT. On seedling ‘Allstar’, dose of 1/4, 1/10, 1/50 and 1/100 showed signs of chlorosis, curling, and twisting of young leaves as well as stunting, and necrosis. On mature ‘Allstar’, dose of 1/4, 1/10, and 1/50 showed backside curling of both old and newly growing leaves.

Table 1.1: 2019 greenhouse dose of Enlist one® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on ‘Green Forest’, ‘Vulcan’, and ‘Allstar’ at SEEDLING stage

Lettuce Variety	**Measurement**	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
‘Green Forest’	**Percentage Injury**	**7 DAT**	5.38 (1.71)	0.5%	9.28 (2.46)	0.9%	16.77 (3.51)	1.6%	46.09 (6.01)	4.3%
		22 DAT	8.03 (1.06)	0.8%	11.63 (1.32)	1.1%	17.37 (1.74)	1.6%	34.50 (3.66)	3.2%
	Yield	**Seedling**	4.00 (2.09)	0.4%	6.02 (2.31)	0.6%	9.40 (2.46)	0.9%	20.14 (5.77)	1.9%
	Percentage Yield loss	**Seedling**	4.01 (1.72)	0.4%	6.04 (1.89)	0.6%	9.42 (2.01)	0.9%	20.14 (4.73)	1.9%
‘Vulcan’	**Percentage Injury**	**7 DAT**	4.64 (1.25)	0.4%	6.85 (1.17)	0.6%	10.47 (1.18)	1.0%	21.62 (5.15)	2.0%
		22 DAT	26.46 (0.25)	2.5%	28.06 (0.27)	2.6%	29.90 (0.29)	2.8%	33.33 (0.33)	3.1%
	Yield	**Seedling**	11.46 (8.88)	1.1%	14.38 (11.22)	1.3%	18.39 (15.97)	1.7%	28.00 (32.33)	2.6%
	Percentage Yield loss	**Seedling**	11.16 (5.96)	1.0%	14.83 (6.96)	1.4%	20.20 (8.29)	1.9%	34.27 (12.02)	3.2%
‘Allstar’	**Percentage Injury**	**3 DAT**	6.36 (1.71)	0.6%	10.53 (2.29)	1.0%	18.21 (3.17)	1.7%	46.44 (7.69)	4.4%
		12 DAT	21.19 (10.79)	2.0%	28.36 (11.90)	2.7%	38.90 (12.57)	3.6%	66.77 (10.73)	6.3%
	Yield	**Seedling**	11.70 (13.28)	1.1%	19.25 (16.18)	1.8%	33.05 (18.61)	3.1%	83.28 (40.67)	7.8%
	Percentage Yield loss	**Seedling**	11.71 (12.46)	1.1%	19.26 (15.17)	1.8%	33.06 (17.43)	3.1%	83.27 (38.06)	7.8%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 1.2: 2019 greenhouse dose of Enlist one® that resulted in 5%, 10%, 20%, and 50% injury at 7 DAT and yield loss on ‘Green Forest’, ‘Vulcan’, and ‘Allstar’ at MATURE stage.

Lettuce Variety	**Measurement**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
‘Green Forest’	**Percentage Injury**	41.89 (8.46)	4.0%	52.46 (8.16)	5.0%	66.97 (7.09)	6.3%	101.66 (3.31)	9.5%
	Yield	1.41 (2.53)	0.1%	1.47 (2.65)	0.1%	1.57 (2.83)	0.1%	1.91 (8.17)	0.2%
	Percentage Yield loss	19.78 (22.47)	1.9%	29.49 (26.00)	2.8%	45.50 (30.18)	4.3%	95.50 (60.13)	9.0%
‘Vulcan’	**Percentage Injury**	73.02 (185.46)	6.8%	80.01 (154.12)	7.5%	88.35 (111.36)	8.3%	104.68 (12.88)	9.8%
	Yield	2.44 (10.25)	0.2%	3.58 (10.90)	0.3%	5.41 (9.84)	0.5%	10.99 (7.75)	1.0%
	Percentage Yield loss	1.18 (1.85)	0.1%	2.25 (2.76)	0.2%	4.56 (4.18)	0.4%	15.24 (12.30)	1.4%
‘Allstar’	**Percentage Injury**	43.67 (9.37)	4.1%	54.99 (8.98)	5.2%	70.61 (7.71)	6.6%	108.29 (4.39)	10.2%
	Yield	33.19 (171.55)	3.1%	39.83 (174.12)	3.7%	48.55 (170.71)	4.6%	68.11 (143.99)	6.4%
	Percent Yield loss	15.38 (26.64)	1.4%	23.81 (32.67)	2.2%	38.25 (38.47)	3.6%	86.03 (50.56)	8.1%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 1.3: 2020 greenhouse dose of Enlist one® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on ‘Green Forest’, ‘Vulcan’, and ‘Allstar’ at SEEDLING stage

Lettuce Variety	**Measurement**	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
‘Green Forest’	**Percentage Injury**	**7 DAT**	3.39 (2.83)	0.3%	7.58 (4.96)	0.7%	18.15 (8.51)	1.7%	80.66 (19.16)	7.6%
		27 DAT	9.16 (1.51)	0.9%	13.91 (1.90)	1.3%	21.88 (2.39)	2.1%	47.48 (3.96)	4.5%
	Yield	**Seedling**	20.96 (11.45)	2.0%	27.89 (12.61)	2.6%	38.02 (13.40)	3.6%	64.57 (12.89)	6.1%
	Percentage Yield loss	**Seedling**	23.82 (13.93)	2.2%	31.02 (15.00)	2.9%	41.32 (15.51)	3.9%	67.44 (13.66)	6.3%
‘Vulcan’	**Percentage Injury**	**7 DAT**	46.41 (17.02)	4.4%	59.36 (16.57)	5.6%	77.54 (14.64)	7.3%	122.42 (11.95)	11.5%
		27 DAT	11.46 (1.70)	1.1%	16.49 (2.03)	1.5%	24.48 (2.44)	2.3%	48.09 (3.70)	4.5%
	Yield	**Seedling**	11.26 (5.55)	1.1%	14.46 (4.86)	1.4%	18.97 (3.83)	1.8%	30.16 (8.80)	2.8%
	Percentage Yield loss	**Seedling**	9.25 (3.94)	0.9%	12.79 (4.02)	1.2%	18.20 (3.95)	1.7%	33.23 (7.57)	3.1%
‘Allstar’	**Percentage Injury**	**7 DAT**	49.60 (9.95)	4.7%	62.89 (9.57)	5.9%	81.38 (8.40)	7.6%	126.43 (7.95)	11.9%
		16 DAT	52.90 (13.71)	5.0%	66.05 (12.78)	6.2%	84.06 (10.60)	7.9%	126.92 (9.05)	11.9%
	Yield	**Seedling**	3.65 (2.19)	0.3%	7.24 (3.44)	0.7%	15.22 (5.36)	1.4%	54.20 (12.64)	5.1%
	Percent Yield loss	**Seedling**	10.53 (5.57)	1.0%	17.34 (7.51)	1.6%	29.80 (10.02)	2.8%	75.18 (16.28)	7.1%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 1.4: 2020 greenhouse dose of Enlist one® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on 'Green Forest', 'Vulcan', and 'Allstar' at MATURE stage

Lettuce Variety	**Measurement**	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
'Green Forest'	**Percentage Injury**	**3 DAT**	47.52 (11.00)	4.5%	58.97 (10.30)	5.5%	74.54 (8.53)	7.0%	111.27 (4.62)	10.4%
		7 DAT	3.58 (1.36)	0.3%	7.27 (2.25)	0.7%	15.69 (3.71)	1.5%	58.48 (8.25)	5.5%
	Yield	**Mature**	NA	NA	NA	NA	NA	NA	NA	NA
	Percentage Yield loss	**Mature**	1.48 (4.13)	0.1%	3.38 (7.33)	0.3%	8.28 (12.70)	0.8%	38.42 (32.96)	3.6%
'Vulcan'	**Percentage Injury**	**3 DAT**	18.80 (6.80)	1.8%	28.67 (8.24)	2.7%	45.34 (9.48)	4.3%	99.27 (9.77)	9.3%
		7 DAT	1.67 (0.43)	0.2%	3.38 (0.66)	0.3%	7.23 (0.99)	0.7%	26.58 (2.81)	2.5%
	Yield	**Mature**	NA	NA	NA	NA	NA	NA	NA	NA
	Percentage Yield loss	**Mature**	NA	NA	NA	NA	NA	NA	NA	NA
'Allstar'	**Percentage Injury**	**3 DAT**	2.89 (1.20)	0.3%	6.17 (2.08)	0.6%	14.06 (3.59)	1.3%	57.46 (9.24)	5.4%
		7 DAT	0.98 (0.46)	0.1%	2.86 (1.06)	0.3%	9.16 (2.51)	0.9%	67.07 (10.06)	6.3%
	Yield	**Mature**	8.34 (5.95)	0.8%	9.99 (5.72)	0.9%	12.15 (5.23)	1.1%	16.96 (4.47)	1.6%
	Percent Yield loss	**Mature**	1.78 (1.92)	0.2%	3.72 (3.25)	0.3%	8.27 (5.59)	0.8%	32.46 (16.34)	3.0%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 1.5: 2020 field study dose of Enlist one® that resulted in 5%, 10%, 20%, and 50% injury at 7 DAT and yield loss on 'Green Forest' and 'Vulcan' at VEGETATIVE stage.

Lettuce Variety	**Measurement**	**Observation time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
'Green Forest'	**Percentage Injury**	**7 DAT**	147.96 (1.02)	13.9%	152.82 (1.04)	14.3%	158.27 (1.06)	14.8%	168.04 (1.12)	15.8%
	Yield	**Vegetative**	57.08 (24.93)	5.4%	70.92 (23.25)	6.7%	89.76 (19.84)	8.4%	134.26 (21.39)	12.6%
	Percentage Yield loss	**Vegetative**	55.92 (24.28)	5.2%	70.15 (23.09)	6.6%	89.71 (20.29)	8.4%	136.61 (21.63)	12.8%
'Vulcan'	**Percentage Injury**	**7 DAT**	62.49 (3.77)	5.9%	76.06 (3.44)	7.1%	94.16 (2.95)	8.8%	135.61 (3.78)	12.7%
	Yield	**Vegetative**	0.85 (1.07)	0.1%	2.30 (2.26)	0.2%	6.80 (4.69)	0.6%	43.30 (16.10)	4.1%
	Percentage Yield loss	**Vegetative**	0.80 (1.02)	0.1%	2.12 (2.12)	0.2%	6.10 (4.34)	0.6%	37.31 (14.27)	3.5%

References

Australia, Primary Industries Standing Committee (2002) Spray drift management: principles, strategies and supporting information. Collingwood, Vic., Australia: CSIRO Publishing

Baker EA, Hunt GM (1981) Developmental Changes in Leaf Epicuticular Waxes in Relation to Foliar Penetration. New Phytologist 88:731–747

Belgers JDM, Van Lieverloo RJ, Van der Pas LJT, Van den Brink PJ (2007) Effects of the herbicide 2,4-D on the growth of nine aquatic macrophytes. Aquatic Botany 86:260–268

Belz RG, Cedergreen N, Duke SO (2011) Herbicide hormesis - can it be useful in crop production?: Hormesis and herbicide use. Weed Research 51:321–332

Belz RG, Duke SO (2014) Herbicides and plant hormesis. Pest Management Science 70:698–707

Dalley CD, Richard EP (2010) Herbicides as Ripeners for Sugarcane. Weed sci 58:329–333

Delbert D. Hemphill Jr, Montgomery ML (1981) Response of Vegetable Crops to Sublethal Application of 2,4-D. Weed Science 29:632–635

Fagliari JR, Jr.* RS de O, Constantin J (2005) Impact of Sublethal Doses of 2,4-D, Simulating Drift, on Tomato Yield. Journal of Environmental Science and Health, Part B 40:201–206

Hammerton JL (1967) Environmental Factors and Susceptibility to Herbicides. Weeds 15:330–336

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R Software Package for Dose-Response Studies: The Concept and Data Analysis. wete 21:840–848

Lettuce I, Way JM (1962) The Effects of Sub-Lethal Doses of Mcpa on the Morphology and Yield of Vegetable Crops. Weed Research 2:233–246

Manuchehri MR, Dotray PA, Keeling JW (2019) Efficacy of 2,4-D Choline as Influenced by Weed Size in the Texas High Plains. Journal of Experimental Agriculture International:1–8

Mohseni-Moghadam M, Doohan D (2015) Response of Bell Pepper and Broccoli to Simulated Drift Rates of 2,4-D and Dicamba. Weed Technology 29:226–232

Mohseni-Moghadam M, Wolfe S, Dami I, Doohan D (2015) Response of Wine Grape Cultivars to Simulated Drift Rates of 2,4-D, Dicamba, and Glyphosate, and 2,4-D or Dicamba Plus Glyphosate. wete 30:807–815

Nascimento ALV, Pereira G a. M, Pucci LF, Alves DP, Gomes CA, Reis MR, Nascimento ALV, Pereira G a. M, Pucci LF, Alves DP, Gomes CA, Reis MR (2020) Tolerance of Cabbage Crop to Auxin Herbicides. Planta Daninha 38

Norris LA, Freed VH (1966) The Absorption and Translocation Characteristics of Several Phenoxyalkyl Acid Herbicides in Bigleaf Maple*. Weed Research 6:203–211

Peterson GE (1967) The Discovery and Development of 2,4-D. Agricultural History 41:243–254

Peterson MA, McMaster SA, Riechers DE, Skelton J, Stahlman PW (2016) 2,4-D Past, Present, and Future: A Review. Weed Technology 30:303–345

Roesler GD, Jonck LCG, Silva RP, Jeronimo AV, Hirata ACS, Monquero PA (2020) Decontamination methods of tanks to spray 2,4-D and dicamba and the effects of these herbicides on citrus and vegetable species. Aust J Crop Sci:1302–1309

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-Logistic Analysis of Herbicide Dose-Response Relationships. Weed Technology 9:218–227

Skoss JD (1955) Structure and Composition of Plant Cuticle in Relation to Environmental Factors and Permeability. Botanical Gazette 117:55–72

Song Y (2014) Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide. Journal of Integrative Plant Biology 56:106–113

Sosnoskie LM, Culpepper AS, Braxton LB, Richburg JS (2015) Evaluating the Volatility of Three Formulations of 2,4-D When Applied in the Field. wete 29:177–184

Way JM (1964) The Effects of Sub-Lethal Doses of Mcpa on the Morphology and Yield of Vegetable Crops. Weed Research 4:319–337

Eytcheson AN, Reynolds D, Irby J, Steckel L, Walton L, Haygood R, Ellis D, Richburg J (2012) Volatility of GF-2726 as Compared with Other Auxin Herbicides. Orlando, FL: Beltwide Cotton Conference

Hanner, D. 1984. Herbicide drift prompts state inquiry. Dallas Morning News, July 25.

CHAPTER 2, IMPACT OF MICRO-RATES OF DICAMBA ON LETTUCE INJURY, RESIDUE PERSISTENCE, AND YIELD LOSS

Dicamba is a synthetic growth regulator herbicide that selectively controls broadleaves plants. Due to the increase of herbicide resistant weeds, the number of farmers using dicamba-tolerant crops has also increased. This could increase the risk of potential drift of dicamba to specialty crops, especially in the Midwest where specialty crops are grown in close proximity to conventional row-crops. Lettuce (*Lactuca sativa L.*) is a broadleaf plant with limited knowledge on the susceptibility to dicamba. The goal of this study was to quantify crop injury and yield loss of greenhouse and field-grown lettuce exposed to simulated sub-lethal doses of dicamba. Sublethal rates were determined based on a percentage of commercial labeled rate (560 g ae ha^{-1}) ranging from 1/10000 to 1/4. Lettuce varieties ‘Green Forest’, ‘Vulcan’, and ‘Allstar’ were sprayed at the seedling and mature growth stage. Extra two replications of mature stage ‘Green Forest’ were used for residue persistence test and results were in parts per billion (ppb). Plant injury ratings were recorded every 4 days after herbicide application until harvest maturity. Lettuce was harvested and dry (greenhouse) or fresh (field) weights were recorded. Dose-response curves were generated to determine effective dose (ED) values and to relate drift rates with crop injury and yield loss. Regardless of growth stage, years, locations, all lettuce varieties were highly susceptible to dicamba with nearly 100% marketable yield loss at rates between 56 and 140 g ae ha^{-1}. In 2019 greenhouse, seedling ‘Vulcan’ lettuce showed recovery in 22-27 days after treatment (DAT) at the rate of 23.03 ± 9.65 g ae ha^{-1} with 5% yield loss reduction (22-27 DAT). However the same rate caused more than 50% yield loss in seedling ‘Green Forest’ which showed ‘Green Forest’ were more susceptible to dicamba. Mature stage lettuce showed higher variability on yield loss but injuries showed consistently at the rates between 56 to 140 g ae ha^{-1}

which made lettuce unmarketable. Field of both lettuce varieties observed yield loss of 20-40% at the rate of 56 and 140 g ae ha^{-1} which matched the greenhouse study. Surprisingly, yield loss was often observed even in absence of visible dicamba injury. dicamba residue test showed the detection were persistent within the seven days after the application. Results confirm the susceptibility of lettuce to low rates of dicamba, typical of off-target injury, and highlight the importance of herbicide drift prevention for specialty crop production throughout the Midwest.

2.1. INTRODUCTION

Dicamba (3,6-dichloro-o-anisic acid) is a synthetic growth regulator herbicide that mimics the action of auxin. For the past 50 years, dicamba has been widely used and selectively controls broadleaf weeds, primarily in pasture and cereal crops (Jones et al. 2019a). Use can be traced back to the early 1960s and there have been only few reported cases of weed resistance (Cao et al. 2011). Up to data, there were only nine weed species confirmed resistance to group 4 (auxin mimics) in the United States including *Amaranthus palmeri*, *Amaranthus tuberculatus (=A. rudis)*, *Centaurea solstitialis*, *Commelina diffusa*, *Daucus carota*, *Digitaria ischaemum*, *Echinochloa crus-galli var. crus-galli*, *Kochia scoparia*, *Lactuca serriola*, and *Plantago lanceolata* (Heap, 2021). The few resistant made dicamba a good candidate in controlling other confirmed resistant weeds. dicamba showed effective control in glyphosate resistant *Conyza canadensis* (Byker et al. 2013), *Ambrosia trifida* (Vink et al. 2012), *Chenopodium album (Chahal and Johnson 2012)*.

The repeated use of herbicide with the same mode of action greatly increases the potential for herbicide resistant weeds (Behrens et al. 2007). In 2004, the adoption rate for glyphosate in the U.S. was almost 90% on planted soybean acreage and approximately 60% of cotton acreage (Duke 2005). As a result, some weeds developed resistance including *Ambrosia artemissifolia*, *Ambrosia trifida*, *Amaranthus palmeri*, *Amaranthus rudis*, *Amaranthus tuberculatus*, and various *Conyza* and *Lolium spp* (Powles 2008). To combat the resistant weeds, novel herbicide tolerant traits have been deployed in field crops. These technologies provided alternative tools and expanded windows to manage the existent glyphosate resistance weeds (Behrens et al. 2007). In addition, the use of dicamba-resistant traits in combination with other herbicide-resistant traits allows rotation or mixtures of herbicides with different modes of action – a potentially important strategy in resistance management. Bayer Crop Science (St. Louis, MO) developed transgenic herbicide-resistant crops with the Xtend® technology and VaporGrip®. This included Roundup Ready 2 Xtend® Soybeans with tolerance to dicamba and glyphosate and Xtendflex® Soybeans and Xtendflex® Cotton that include tolerance to dicamba, glyphosate, and glufosinate (Bayer Crop Science, St. Louis, MO). These crop traits could help manage current herbicide resistant weeds and slow the development of new herbicide resistant weeds (Behrens et al. 2007).

The parent acid of dicamba (methoxybenzoic acid) has a higher vapor pressure that tends to vaporize and cause drift issues (Hartzler 2017). Xtendimax® with Vapor Grip® technology and Engenia® were formulated to dissociate the parent acid of dicamba, which reduces the likelihood of vaporization (Hartzler 2017). Many independent researchers have verified these formulations do reduce the volatilization when compared with the old dicamba formulations but potential drift could still occur (Hartzler 2017). Roesler et al. (2020) showed dicamba can drift up to 152 m from the target application area and yield loss was noted in non-dicamba-resistant soybean at the R1 reproductive stage located 42.8 m from the application area. In the 2017 growing season, there were 2,708 dicamba-related injury cases on conventional soybeans affecting approximately 1.5 million hectares (Bradley, 2017). Dicamba is typically used as a post-emergence herbicide which means it is sprayed after crops have emerged, which increases the risk of off-target injury to surrounding susceptible plants. In dicamba-resistant soybean, dicamba applications can be made as preplant, at planting, and POST. The wider window of application during the growing season will increase the risk of off-target movement to adjacent fields. Dicamba has a relatively high vapor pressure of 4.5 x 10^{-3} Pa (25 °C) (Senseman, 2007) which increases its susceptibility to vaporization. Applying dicamba later in the growing season especially in Midwest are more likely encounter hot and humid weather conditions which can results in vaporization and off-target movement of the chemical.

Temperature inversion occurs when a layer of hot air smothers a cool air layer which could happen when elevated temperatures during the day turn into evening with inverted temperatures (Bentley 2019). An inversion is defined as an increase in air temperature with an increase in height above the surface which is the opposite of the normal day time temperature profile. During a temperature inversion, the vapor from recently applied dicamba can be held close to the ground for a longer period where it can injure susceptible plants (Bentley 2019). Temperature inversions are surprisingly common; a four-year study in Missouri showed temperature inversion formed more than 60% of the evenings across the growing season from April to July (Bish and Bradley, 2019).

Many specialty crops are broadleaf and susceptible to auxinic herbicides. The University of Georgia at Tifton collected visual injury data of various specialty crops and discovered grapes, lima bean, snap bean, southern pea, soybean, sweet potato, and tobacco are extremely sensitive to dicamba even under the fraction of the label rate of 1/800x (0.7 g ae ha^{-1}) (Culpepper, 2018). Leafy greens like cabbage and kale were found to be less sensitive to dicamba but visible injury was still detected at drift rates greater than 1/75x (7.5 g ae ha^{-1}) of the labeled dicamba rate (Culpepper, 2018). Although actual drift rate depends on factors like nozzle size, travel speed, and boom height, etc. Egan and Mortensen (2012) in a field study on soybean detected vapor drift of dicamba DMA salt at a mean concentration of 0.56 g ha^{-1} (1/1,000th of the applied rate of 560 g ha^{-1}). In this case, a drift rate of 7.5 g ae ha^{-1} could likely occur.

Lettuce (*Lactuca sativa* L.) is a major fresh vegetables and are commonly used in salad mixtures and sandwiches (Mou 2008). The United States has the largest production of lettuce as a salad crop, and produced 22% of the world's lettuce supply (Mou 2008). Lettuce is a popular leafy crop that plays an important role in American diet and nutrition (Mou 2009). In 2020, both head lettuce, Romaine lettuce, and tomatoes were the highest value vegetable crops in the US accounting for 30% of the total value when combined (USDA, National Agricultural Statistics Service, Vegetables 2020 Summary). However, there is limited information on the susceptibility to dicamba drift. A recent study by Roesler et al. (2020) on lettuce variety 'Stella' (*Lactuca sativa* var. Stella) fifty-five days after planting showed dicamba with the labeled rate of 560 g ae ha^{-1} and half labeled rate of 280 g ae ha^{-1} (50%) caused the death of plant twenty-eight days after treatment. Dicamba at the rate of 67.2 g ae ha^{-1} (12%) also caused plant death but took a longer time at forty-five days after treatment (Roesler et al. 2020). Interestingly, lettuce treated with the rate of or under 16.8 g ae ha^{-1} (3%) recovered at 28 days after treatment with no biomass reduction compared with the control (Roesler et al. 2020). However, there is still a lack of knowledge about the effect of growth regulators on lettuce at seedling and early vegetative stages (Lettuce and Way 1962). Therefore, the objective of the study was to assess the effect of the new dicamba formulation (Xtendimax) on injury and yield loss of three modern lettuce varieties including 'Allstar', 'Green Forest', and 'Vulcan' at seedling and mature growth stages.

2.2. MATERIALS AND METHODS

2.2.1 GREENHOUSE SETUP

Studies were conducted in 2019 and 2020 between February and April at the University of Nebraska-Lincoln Plant Growth Facilities in Lincoln, NE (40° 50’ 4.050” N, 96° 39’ 54.612” W). Temperature was set as 21.1 to 26.7 C° during the day and at 15.6 to 21.1 C° during the night. The highest recorded temperature in the greenhouse was 28.3 C° and the lowest temperature was15.6 C°. Supplemental lights in 2019 were 1000-W metal halide fixtures. Supplemental lights in 2020 were replaced with 1000-W high-pressure sodium fixtures. Lights were on for 18 hours per day. Lettuce was watered daily by hand sprinkler and a 20-10-20 N-P-K fertilizer (Peters Professional; Scotts Co., Marysville, Ohio, USA) was applied at the concentration of 250 ppm by fertilizer injector three times per week after lettuce reached the seedling stage (two fully expanded true leaves) through harvest.

2.2.2 PLANT MATERIAL

‘Allstar’, ‘Green Forest’, and ‘Vulcan’ (Johnny’s Selected Seeds Company; Winslow, ME) were the lettuce varieties used for the experiment. ‘Allstar’ is a popular loose-leaf spring mix that includes a mix of red and green leaf. The ruffled edges and unique shapes provide soft, interesting textures and a fancy appearance. ‘Green Forest’ is a green romaine head lettuce that is tall with smooth ribs. This lettuce is very common in Caesar salad. ‘Vulcan’ is a common red, head lettuce popular among organic growers and consumers. It has slightly frilled leaves with a candy apple red color over a light green background. Lettuce was directly seeded into 15.3-cm diameter pots at a planting depth of 0.64 centimeters and filled with soilless potting mix (Berger mix BM6; Saint-Modeste, QC, Canada). ‘Allstar’ was direct-seeded into each pot with 30 seeds per pot. ‘Green Forest’ and ‘Vulcan’ were direct-seeded into each pot with two seeds per pot. ‘Allstar’ was not thinned after emergence because loose-leaf lettuce is commonly seeded at high densities. ‘Green Forest’ and ‘Vulcan’ were thinned to one plant per pot after emergence to grow as an individual lettuce head.

2.2.3 GREENHOUSE EXPERIMENTAL DESIGN AND TREATMENT APPLICATIONS

In 2019, lettuce pots were arranged in a completely randomized design with six replicates in the greenhouse. The treatment factors included sublethal rates of dicamba (Xtendimax®; Bayer CropScience, St. Louis, MO) with 0, 1/4, 1/10, 1/100, 1/1000, and 1/10000 of the label rate (560 g ae ha^{-1}) and two application timings (seedling and mature stage). Seedling stage was defined as when lettuce plants had two fully expanded true leaves. Mature stage was defined as one week prior to harvest. There were a total of 216 experimental units (3 varieties × 6 sublethal rates × 2 application timings × 6 replications). In 2019, the planting date of all lettuce was 12 Feb. The seedling stage application of all lettuce varieties was on 5 Mar. The mature stage application of ‘Allstar’ was on 14 Mar, and ‘Vulcan’ and ‘Green Forest’ were on 20 Mar. ‘Allstar’ was harvested on 19 Mar (25 days after planting), and ‘Green Forest’ and ‘Vulcan’ were harvested on 27 Mar (33 days after planting). At harvest, each lettuce in the pot was cut at soil level, placed in individual paper bags, and placed in a drying oven at 31 C° for 7 days until totally dried. Lettuce weight of each treated rate of each lettuce varieties then divided by the replication to obtain the average yield as gram per pot.

In 2020, the pot-grown lettuce was arranged in a randomized complete block design with 6 replicates in the greenhouse. Each replication of the treatment combinations is a block and blocked by benches according to distance from the south-facing greenhouse wall.

In 2020, we modified application rates based on 2019 observations and replaced the lowest rate of 1/10000 with 1/500 and added the rate of 1/50. 7 sublethal rates were now (0 was the control; 1/4; 1/10; 1/50; 1/100; 1/500; 1/1000) of the label rate [560 g ae ha^{-1} of Xtendimax®. Planting date for all lettuce was 10 Feb. There was a total of 252 experimental units (1 herbicide × 3 varieties × 7 sublethal rates × 2 application timings × 6 replications). The seedling stage application of all lettuce varieties was on 25 Feb. The mature stage application of 'Allstar' was on 5 Mar. 'Vulcan' and 'Green Forest' were on 16 Mar. 'Allstar' was harvested on 12 Mar (31 days after planting seeds) and 'Vulcan' and 'Green Forest' were harvested on 23 Mar (42 days after planting seeds). Harvest procedures and yield data collection were the same as in 2019.

In both years, herbicide was applied using a single-tip chamber sprayer (DeVries Manufacturing Corp, Hollandale, MN) fitted with an 8001 E nozzle (TeeJet Technologies, Spraying Systems Co., Wheaton, IL) calibrated to deliver 140 L ha^{-1} at 276 kPa at a speed of 4.8 km h^{-1}. Visual injury ratings were based on the percentage scale of 0 (no injury) to 100 (death of the plant) relative to the nontreated control (Appendix 1 injury rating guide). This protocol was adapted from Frans et al. (1986). The injury rating data were collected at 3, 7, 12, 16, and 22 days after treatment (DAT). Depending on the sublethal rates and the stage of the plant, injury symptoms from dicamba included leaf chlorosis, curling, twisting, cupping, and stunting.

2.2.4 2020 GREENHOUSE DICAMBA RESIDUE ANALYSIS

In 2020, dicamba residue analysis was conducted on the mature stage treated 'Green Forest' variety in the greenhouse. An extra 6 replications of 'Green Forest' lettuce were planted and treated at the mature stage with dicamba at the rate of 0, 1/4, 1/10, 1/50, 1/100, and 1/500 on 16 Mar. Dicamba residue present on or in the lettuce was analyzed from two replicate samples collected at 3, 7, and 16 DAT. Plants were cut at the soil surface, packed in individual sample bags and shipped overnight for next-day analysis (South Dakota Agricultural Laboratories; Brookings, SD, U.S.). Dicamba residues from samples were quantified using Gas Chromatography – Tandem Mass Spectrometry (GC/MS-MS).

2.2.5 FIELD STUDY EXPERIMENTAL DESIGN AND TREATMENT APPLICATIONS

In 2019, ‘Vulcan’ and ‘Green Forest’ lettuce were tested in the field. Both lettuce varieties were seeded into flats in the greenhouse on 10 Apr. for transplant plugs. On 24 May, lettuce seedlings were transplanted to the field located at the UNL Havelock Research Farm (40°51’ 7.008” N, 96°36’ 52.980” W). Before transplanting, the field was prepared with rotary tillage. In a single field pass of the bed-shaper/mulch-layer (RB448; Nolt’s Produce Supplies), raised beds were shaped, and a drip irrigation line was laid beneath a white on black plastic film. Each plot was 3.7 meters long by 1.2 meters wide with a single row of 12 lettuce plants in each plot. A within row gap of 2.4 m between plots was included to minimize herbicide drift among plots. Treatments in 2019 included six sublethal rates (0 was the control and 1/4; 1/10; 1/50, 1/100; 1/500 of the label rate (560 g ae ha^{-1} Xtendimax®) and two lettuce varieties, resulting in a total of 12 experimental units within each of four replicate blocks. Fertilizer was delivered to plants via drip irrigation two times; one week prior to and one week after herbicide application. Calcium nitrate (15N–0P–0K, YaraLiva Tropicote 15–0–0; Yara North America, Tampa, FL) was mixed in the fertilizer injector to deliver 44.8 kg ha^{-1} N at each fertigation. Irrigation was done by deliver through drip tape once per week with running time of 4 hours until harvest.

In 2020, seeds of ‘Vulcan’ and ‘Green Forest’ were planted to seedling flats in the greenhouse on 8 Apr. On 18 May, lettuce seedlings were transplanted to the field located at the

UNL East Campus Research Farm (40° 50’ 10.890” N, 96° 39’ 45.162” W). Before transplanting, the field was prepared with rotary tillage. All plots received an application of 112 kg ha^{-1} N with granular urea (46N-0P-0K; PRO-AP, Wawaka, IN) applied as preplant broadcast fertilizer and incorporated into the soil. In a single field pass of the bed-shaper/mulch-layer (RB448; Nolt’s Produce Supplies), raised beds were shaped, and a drip irrigation line was laid beneath a white on black plastic film. Irrigation was done by deliver through drip tape once per week with running time of 4 hours until harvest. Plot dimensions, treatment structure, and field layout was identical to 2019. For both years, herbicide was applied using a CO_2-pressurized tank sprayer with a two-nozzle boom spaced 51 cm apart. The sprayer was calibrated to deliver 140 L ha^{-1} at 276 kPa through TeeJet 8001E nozzle (TeeJet Technologies, Spraying Systems Co., Wheaton, IL). Travel speed of the nozzle was based on walking speed of approximate 4.8 km/hr. In 2019 treatment with sublethal doses was 20 Jun. while treatment in 2020 was 12 Jun. The application was done in mid-June to stimulate when dicamba was typically apply to dicamba ready soybeans.

Visual injury rating was conducted every seven days until harvest with the same protocols as the greenhouse experiment. All lettuce were harvested and fresh weights were recorded on 10 July in 2019 and 23 Jun in 2020 to determine yield. Lettuce yields in each plot were adjusted to a per plant basis to account for minor differences in final plant populations within plots (e.g., plants lost to transplant shock or herbivory).

2.2.6 STATISTICAL ANALYSIS

Yield data were based on dry biomass in the greenhouse trials, fresh weight in the field trials and percentage yield loss relative to the controls. Due to the non-linear nature of plant response to sublethal rates of herbicide, a four-parameter log-logistic regression model was used to analyze the relationship between sublethal rates of dicamba with visual injury, average yield and percentage yield loss utilizing approach described in Knezevic et al. (2007).

The four parameter model was defined by the equation

$Y = c + \{ d - c / 1 + \exp [b(\log x - \log e)]\}$

where c is lower limit, d is upper limit, b is slope and e is the ED 50 (effective dose giving 50% response) (Seefeldt et al. 1995). The regression analyses and ED values were used to estimate doses of dicamba corresponding to critical thresholds of injury or yield loss (ED values). Regression analyses were conducted using the ‘drc’ package in R version 3.4.1 (R Core Team, 2019) as described in Knezevic et al. (2007). Injury ratings were averaged across replications and fit to the dose response model across the six sublethal rates within each application timing (greenhouse trials) and DAT sampling interval.

Percentage yield loss was calculated using the equation:

$Y= [(C – T) / C] 100$

where Y represents the percentage yield loss compared to the nontreated control plot in the corresponding replication block, C represents the biomass of the nontreated control plot, and T represents the biomass of the treated plot.

2.3. RESULTS

2.3.1 2019 GREENHOUSE 'GREEN FOREST' LETTUCE VISUAL RATINGS

Injury symptoms of dicamba on 'Green Forest' were prominent on new leaf growth especially for the two highest sublethal rates of 1/4 and 1/10. The young leaves appeared to be curling and twisting (Figure 2.1a and 2.1b). Seedling lettuce treated with 1/100 sublethal rate showed approximate 35% injury at 7 DAT, but was able to recover at 22 DAT with no injury observed (Figure 2.2a). However, even with no visual injury observed by 22 DAT, 38% yield loss was observed at harvest (Figure 2.5a). Mature stage 'Green Forest' observed at 7 DAT showed injury on both rates of 1/4 and 1/10 (Figure 2.3a). On seedling stage 'Green Forest' at 7 DAT, dose response model ED values suggest a dose of 0.91 ± 0.40 g ae ha^{-1} would cause 5% injury and a dose of 7.87 ± 1.26 g ae ha^{-1} corresponds to 50% injury measured at 7 DAT (Table 2.1). At 22 DAT, a dose of 8.84 ± 3.15 g ae ha^{-1} would be required to cause 5% injury, and dose of 30.63 ± 3.41 g ae ha^{-1} to cause 50% injury (Table 2.1). Mature stage 'Green Forest' exhibited higher tolerance to dicamba compared with the seedling stage. At 7 DAT, a dose of 23.00 ± 2.48 g ae ha^{-1} would be required for 5% injury and a dose of 65.50 ± 2.14 g ae ha^{-1} for 50% injury (Table 2.2).

2.3.2 2019 GREENHOUSE 'GREEN FOREST' LETTUCE YIELD

'Green Forest' at seedling stage showed good model fitting (Figure 2.4a). Dose response model estimated rate of 0.44 ± 0.48 g ae ha^{-1} resulted 5% yield reduction while rate of 16.31 ± 6.28 g ae ha^{-1} resulted 50% yield reduction (Table 2.1). High variation of yield was observed when 'Green Forest' was treated at the mature stage which resulted dose response model converge with high standard error (Figure 2.4a, Table 2.2).

2.3.3 2019 GREENHOUSE ‘GREEN FOREST’ LETTUCE YIELD LOSS

‘Green Forest’ lettuce treated with dicamba at the seedling stage was highly susceptibility to yield loss. A rate of 0.44 ± 0.47 g ae ha^{-1} caused a 5% yield loss and a rate of 16.31 ± 6.11 g ae ha^{-1} caused 50% yield loss (Table 2.1). The dose-response model failed to converge when ‘Green Forest’ was treated at the mature stage due to high variability in plant response within and among treatments (Table 2.2). However, yield was reduced by approximately 45% in lettuce treated with the highest rate (140 g ae ha^{-1}) and by 10% when treated with 1/10 of the label rate (56 g ae ha^{-1}) (Figure 2.5a); however, any plant showing dicamba-related symptoms (e.g., visual injury) would be unmarketable and represents 100% economic loss (Figure 2.1b). Yield loss ranged from approximately 30% to 40% for the three lowest rates of 1/100, 1/1000, and 1/10000 of the label rate (Figure 2.5a).

2.3.4 2020 GREENHOUSE ‘GREEN FOREST’ LETTUCE VISUAL RATINGS

Injury symptoms of dicamba in 2020 matched with the symptoms observed in 2019. On ‘Green Forest’ treated at the seedling stage, leaves twisting and curling were the dominant symptoms observed at the rate of 1/4 and 1/10 (Figure 2.6a). The rate of 1/50 and 1/100 also showed slight injury of curling on the newly emerged leaves (Figure 2.6a). Also consistent with 2019, injury symptoms worsened from 7 to 27 DAT, especially in the 1/4 and 1/10 treatments. At 1/4 rate, visible injury was 45% at 7 DAT and increased to nearly 90% by 27 DAT (Figure 2.7a). At 1/10 rate, the injury was 35% at 7 DAT and 70% at 27 DAT (Figure 2.7a). Visible injury was observed in the 1/50 and 1/100 rates (20-25% injury at 7 DAT), but no injury was observed by 27 DAT (Figure 2.7a).

The dose response model suggests that at 7 DAT a rate of 0.49 ± 0.16 g ae ha^{-1} in seedling 'Green Forest' caused 5% injury whereas a rate of 11.30 ± 1.55 g ae ha^{-1} showed 50% injury (Table 2.3). At 27 DAT, a rate of 44.19 ± 37.91 g ae ha^{-1} caused 50% injury (Table 2.3). In 2020, the visual injury ratings on mature stage 'Green Forest' were conducted at 3 and 7 DAT. The two highest rates of 1/4 and 1/10 showed injury symptoms worsening from 3 to 7 DAT (55% to 84% at 1/4 and 30% to 60% at 1/10, respectively; Figure 2.8a). The ED 50 for mature 'Green Forest' was 37.04 ± 4.38 g ae ha-1 at 3 DAT and 27.89 ± 2.14 g ae ha-1 at 7 DAT (Table 2.4).

2.3.5 2020 GREENHOUSE 'GREEN FOREST' LETTUCE YIELD

Seedling 'Green Forest' showed good model fitting (Figure 2.9a). Dose response model estimated a rate of 9.09 ± 5.51 g ae ha^{-1} resulted 5% yield reduction and a rate of 39.97 ± 8.02 resulted 50% yield reduction (Table 2.3). Mature stage 'Green Forest' showed high variation in yield (Figure 2.9a) which resulted dose response model failed to converge (Table 2.4).

2.3.6 2020 GREENHOUSE 'GREEN FOREST' LETTUCE YIELD LOSS

In 2020, the two highest rates (1/4 and 1/10) in seedling stage 'Green Forest' caused yield loss of 100% and 58%, respectively (Figure 2.10a). The dose response model suggests a rate of 10.46 ± 5.68 g ae ha^{-1} can cause a 5% yield loss and a rate of 41.71 ± 6.98 g ae ha^{-1} a 50% yield loss (Table 2.3). In contrast to 2019 results, mature stage 'Green Forest' did not suffer yield loss in any of the treatments and the dose response model did not converge (Figure 2.10a). However, the three highest rates of 1/4, 1/10, and 1/50 all showed injury symptoms which would render them almost entirely non-marketable and cause severe economic injury (Figure 2.6b).

2.3.7 2020 GREENHOUSE ‘GREEN FOREST’ DICAMBA ANALYSIS

The amount of dicamba residue detected increased with application rate. At 3 DAT, 27 ppb dicamba was detected in lettuce treated with the 1/500 rate and 1162 ppb dicamba was detected in the 1/4 rate (Figure 2.11). It was noted the amount of dicamba detected decreased significantly especially at 16 DAT on the rate of 1/4 dicamba. By 16 DAT, dicamba residue in the 1/4 rate had decreased to 296.3 ppb (Figure 2.11). Overall, the dicamba residue in the lettuce was most stable within 7 days of treatment and became more variable by 16 DAT (Figure 2.11).

2.3.8 2020 FIELD ‘GREEN FOREST’ LETTUCE VISUAL RATINGS

Field-grown ‘Green Forest’ at the vegetative stage showed injury by 7 DAT ranging from 9% in the 1/50 rate to 45% in the 1/4 rate (Figure 2.12a). The dose response model for 7 DAT suggests a rate of 8.61 ± 4.01 g ae ha^{-1} results in 5% visible injury and a rate of 40.45 ± 6.21 g ae ha^{-1} causes 50% injury (Table 2.5).

2.3.9 2020 FIELD ‘GREEN FOREST’ LETTUCE YIELD

Only the two highest rate of 1/4 and 1/10 showed significant yield reduction (Figure 2.12b). Dose response model suggested rate of 15.80 ± 11.23 g ae ha^{-1} resulted 5% yield reduction and rate of 59.01 ± 10.92 g ae ha^{-1} resulted 50% yield reduction (Table 2.5).

2.3.10 2020 FIELD ‘GREEN FOREST’ LETTUCE YIELD LOSS

The 1/4 rate resulted in 40% yield loss and the 1/10 rate resulted in 23% yield loss in field-grown ‘Green Forest’ (Figure 2.12c). Interestingly, we measured a yield loss of 5-10% in the lowest rates of 1/100 and 1/500 even though no visible injury was observed. The dose response model for field-grown ‘Green Forest’ indicates an approximate rate of 7.35 ± 7.70 g ae ha^{-1} will cause 5% yield loss and 44.92 ± 12.55 g ae ha^{-1} will result in 50% yield loss (Table 2.5).

2.3.11 2019 GREENHOUSE ‘VULCAN’ LETTUCE VISUAL RATINGS

Injury symptoms of dicamba on ‘Vulcan’ lettuce also appeared as leaf curling and twisting and these symptoms were most prominent at the two highest rates of 1/4 and 1/10 applied at the seedling growth stage (Figure 2.13a). The leaves of ‘Vulcan’ lettuce are soft with leathery texture and stayed soft even after the dicamba injury. This contrasted with ‘Green Forest’ where the texture was rigid and easily snapped after dicamba injury. ‘Vulcan’ lettuce is known as red-leaf lettuce because of the development of red pigmentation as plants mature. However, it was noted at the two highest rates of 1/4 and 1/10, the plants treated at the seedling stage stayed green and failed to develop the normal red pigmentation (Figure 2.13a).

At seedling stage 7 DAT, the 1/10 rate was observed as the threshold of visual injury for ‘Vulcan’. Dose response models suggest that at 7 DAT a rate of 0.65 ± 0.32 g ae ha^{-1} results in 5% injury and a rate of 14.70 ± 2.98 g ae ha^{-1} results in 50% injury (Table 2.1). Effective dose (ED) values for visible injury increased substantially by 22 DAT suggesting some recovery from initial symptoms (Table 2.1). At 7 DAT, the 1/4 rate caused approximately 63% injury and the rate of 1/10 caused 25% injury (Figure 2.2b). By 22 DAT, both 1/4 and 1/10 caused severe injury that ranged from 82% to 90% (Figure 2.2b). ‘Vulcan’ lettuce treated at mature stage only showed injury at the rate of 1/4 by 7 DAT (Figure 2.13b, Figure 2.3b). The dose response model suggests a rate of 57.74 ± 7.27 g ae ha^{-1} is required for 5% injury, but a rate of only 64.76 ± 18.38 g ae ha^{-1} caused 50% injury (Table 2.2).

2.3.12 2019 GREENHOUSE ‘VULCAN’ LETTUCE YIELD

Seedling stage ‘Vulcan’ showed good model fitting (Figure 2.4b). Dose response model suggested rate of 23.05 ± 10.98 g ae ha^{-1} caused 5% yield reduction and rate of 55.41 ± 4.42 g ae ha^{-1} caused 50% yield reduction (Table 2.1). Mature stage ‘Vulcan’ showed significant yield reduction on only the two highest rates of 1/4 and 1/10 (Figure 2.4b). Dose response model converged but with high standard error. A rate of 1.14 ± 8.28 was suggested to cause 5% yield reduction and rate of 1.72 ± 7.80 g ae ha^{-1} was suggested to cause 50% yield reduction (Table 2.2).

2.3.13 2019 GREENHOUSE ‘VULCAN’ LETTUCE YIELD LOSS

‘Vulcan’ lettuce treated at the seedling stage with the 1/4 rate reduced yield 100% and the 1/10 rate reduced yield by 50% (Figure 2.5b). There was no yield loss at 1/100, but there was 10% yield loss in the 1/1000 rate. Dose response for seedling stage ‘Vulcan’ suggests 23.03 ± 9.65 g ae ha^{-1} will cause 5% yield loss and 55.41 ± 3.90 g ae ha^{-1} is required to reach50% yield loss (Table 2.1). Mature stage ‘Vulcan’ yield loss was less severe. The 1/4 rate reduced yield by 38% and 1/10 reduced yield by 15% (Figure 2.5b). Lower rates of 1/1000 and 1/10000 caused 10% to 13% yield loss (Figure 2.5b). Dose response suggests that for mature stage ‘Vulcan’ a rate of 26.30 ± 21.03 g ae ha^{-1} results in 5% yield loss and 73.05 ± 21.52 can cause 50% yield loss (Table 2.2).

2.3.14 2020 GREENHOUSE ‘VULCAN’ LETTUCE VISUAL RATINGS

Visible injury symptoms for the 2020 greenhouse ‘Vulcan’ were the same as in 2019, and the lack of red pigmentation was observed for the three highest rates (1/4, 1/10, and 1/50; Figure

2.14a). Injury worsen as time increased from 7 DAT to 27 DAT for both seedling (Figure 2.7b) and mature stage (Figure 2.8b) at the two highest rate of 1/4 and 1/10. Dose response estimates for ‘Vulcan’ at the seedling stage 7 DAT suggest 1.26 ± 0.71 g ae ha^{-1} results in 5% injury and 17.74 ± 4.08 g ae ha^{-1} results in 50% injury (Table 2.3). By 27 DAT, 33.08 ± 3.56 g ae ha^{-1} would cause 5% injury and 69.18 ± 3.22 g ae ha^{-1} for 50% injury (Table 2.3). Increased ED values over time are an indication of plant recovery, at least at the lower application rates in ‘Vulcan’ (Figure 2.7b). For mature stage ‘Vulcan’, the 1/10 rate caused injury which was different than in 2019 where only the 1/4 rate caused injury (Figure 2.3b, Figure 2.8b). The dose-response model for mature stage ‘Vulcan’ at 7 DAT indicates 26.01 ± 2.60 g ae ha^{-1} results in 5% injury and 63.06 ± 1.73 g ae ha^{-1} results in 50% injury (Table 2.4).

2.3.15 2020 GREENHOUSE ‘VULCAN’ LETTUCE YIELD

Seedling stage ‘Vulcan’ showed good model fitting (Figure 2.9b). Dose response model suggested rate of 29.82 ± 8.06 g ae ha^{-1} resulted 5% yield reduction and rate of 68.23 ± 6.35 g ae ha^{-1} resulted 50% yield reduction (Table 2.3). Mature stage ‘Vulcan’ showed high variation in yield which resulted dose response model converge with high standard error. The response model suggested rate of 1.58 ± 1.37 g ae ha^{-1} resulted 5% yield reduction and rate of 1.97 ± 6.05 g ae ha^{-1} resulted 50% yield reduction (Table 2.4).

2.3.16 2020 GREENHOUSE ‘VULCAN’ LETTUCE YIELD LOSS

In the 2020 greenhouse study, seedling ‘Vulcan’ showed yield loss only at the two highest rates of 1/4 (95% yield loss) and 1/10 (15% yield loss) (Figure 2.10b). However, other rates resulted in small increases in yield, which contrasts with 2019 results where even the lowest rates of dicamba caused yield loss. The dose response model for seedling stage ‘Vulcan’ suggests 29.23 ± 7.89 g ae ha^{-1} causes 5% yield loss and 67.63 ± 5.92 g ae ha^{-1} causes 50% yield loss (Table 2.3). Mature stage ‘Vulcan’ did not exhibit yield loss at any of the tested rates and the dose response model did not converge (Figure 2.10b, Table 2.4).

2.3.17 2020 FIELD ‘VULCAN’ LETTUCE VISUAL RATINGS

The three highest rates of 1/4, 1/10, and 1/50 all resulted in injuries 7 DAT. Visible injury ranged from 9% in 1/50 to 45% in the 1/4 rate (Figure 2.13a). The dose response model suggests 11.40 ± 6.48 g ae ha^{-1} results in 5% injury and 46.00 ± 6.87 g ae ha^{-1} results in 50% injury to ‘Vulcan’ at 7 DAT (Table 2.5).

2.3.18 2020 FIELD ‘VULCAN’ LETTUCE YIELD

Only the two highest rates showed significant yield reduction (Figure 2.15b). Dose response model suggested a rate of 2.96 ± 2.02 g ae ha^{-1} resulted 5% yield reduction and rate of 25.72 ± 7.59 g ae ha^{-1} g ae ha^{-1} resulted 50% yield reduction (Table 2.5).

2.3.19 2020 FIELD ‘VULCAN’ LETTUCE YIELD LOSS

The four highest rates of 1/4, 1/10, 1/50, and 1/100 all caused yield loss ranging from 5% to 40% (Figure 2.15c). The lowest rate of 1/1000 increased yield of ‘Vulcan’ by 12%. The dose response model suggests 1.06 ± 0.66 g ae ha^{-1} results in 5% yield loss and 17.65 ± 4.12 g ae ha^{-1} results in 50% yield loss (Table 2.5).

2.3.20 2019 GREENHOUSE 'ALLSTAR' LETTUCE VISUAL RATINGS

The injury symptoms on 'Allstar' included leaf curling and twisting especially on the newly growing leaves. Injury symptoms were more severe at the seedling stage and plants appeared to be stunted especially at the two highest rates of 1/4 and 1/10 (Figure 2.16a). The dose-response model indicated the two highest rates of 1/4 and 1/10 caused 50% and 43% injury at 3 DAT, respectively (Figure 2.2c). Injury symptoms worsened by 12 DAT (Figure 2.2c). At 3 DAT, a rate of 0.23 ± 0.31 g ae ha^{-1} caused 5% injury and a rate of 12.88 ± 6.02 g ae ha^{-1} caused 50% injury (Table 2.1). At 12 DAT, a rate of 1.83 ± 0.38 g ae ha^{-1} was required for 5% injury, and a rate of 12.92 ± 1.42 g ae ha^{-1} for 50% injury (Table 2.1). When sprayed at the mature stage, 'Allstar' visible injury reached 60% in the 1/4 rate and 20% in the 1/10 rate by 7 DAT (Figure 2.3c). The dose response model suggests a rate of 29.28 ± 3.09 g ae ha^{-1} results in 5% injury and 68.38 ± 2.73 g ae ha^{-1} results in 50% injury (Table 2.2).

2.3.21 2019 GREENHOUSE 'ALLSTAR' LETTUCE YIELD

Seedling stage 'Allstar' do not have good model fitting. Dose response model suggested rate of 0.07 ± 0.21 g ae ha^{-1} resulted 5% yield reduction and rate of 19.71 ± 110.40 g ae ha^{-1} resulted 50% yield reduction. Mature stage 'Allstar' also have poor fitting. Dose response model suggested rate of 7.85 ± 24.69 g ae ha^{-1} resulted 5% yield reduction and rate of 22.04 ± 49.47 g ae ha^{-1} resulted 50% yield reduction (Table 2.2).

2.3.22 2019 GREENHOUSE ‘ALLSTAR’ LETTUCE YIELD LOSS

‘Allstar’ lettuce treated at the seedling stage experienced yield loss when treated with the top four rates of 1/4, 1/10, 1/100, and 1/1000 (Figure 2.5c). The 1/4 rate caused 32% yield loss and 1/10 caused 20% yield loss (Figure 2.5c). The lowest rate of 1/10000, however, increased yield by 15% (Figure 2.5c). The dose response model indicates a rate of 0.01 ± 0.01 g ae ha^{-1} results in 5% yield loss and a rate of 3.27 ± 2.35 g ae ha^{-1} results in 50% yield loss when applied to seedling stage ‘Allstar’ (Table 2.1). At the mature stage, only the 1/4 and 1/10 rates reduced ‘Allstar’ yield (Figure 2.5c), which is consistent with visible injury symptoms observed for mature ‘Allstar’ 7 DAT (Figure 2.16b). The 1/100, 1/1000, and 1/10000 rates increased yield by up to 20% (Figure 2.5c). Dose response for mature stage ‘Allstar’ suggests 0.06 ± 0.22 g ae ha^{-1} results in 5% yield loss and 5.72 ± 6.82 results in 50% yield loss (Table 2.2).

2.3.23 2020 GREENHOUSE ‘ALLSTAR’ LETTUCE VISUAL RATINGS

Visual injury symptoms on ‘Allstar’ lettuce showed nearly identical symptoms as in 2019 (Figure 2.17a). At seedling stage, the 1/4 rate caused approximately 50% injury at 7 DAT and symptoms worsened by 16 DAT to 78% injury (Figure 2.7c). The 1/10 rate showed 30% injury consistently over time (Figure 2.7c). The 1/50 rate had 20% injury at 7 DAT, but symptoms improved to 8% injury by 16 DAT (Figure 2.7c). There was no visible injury at rates below 1/50. Dose response for seedling ‘Allstar’ at 7 DAT suggests 3.14 ± 1.73 g ae ha^{-1} results in 5% injury and 33.74 ± 7.41 g ae ha^{-1} results in 50% injury (Table 2.3). By 16 DAT, 28.57 ± 4.85 g ae ha^{-1} is required to observe 5% injury and 65.92 ± 3.52 g ae ha^{-1} for 50% injury (Table 2.3). ‘Allstar’ was more tolerant of dicamba when treated at the mature stage. Visual injury at 7 DAT was 38% in the 1/4 rate and 15% in 1/10 (Figure 2.8c). Dose response at 7 DAT suggests 27.72 ± 4.31 g ae ha^{-1} results in 5% injury and 66.68 ± 3.52 g ae ha^{-1} results in 50% injury when ‘Allstar’ is sprayed at the mature growth stage (Table 2.4).

2.3.24 2020 GREENHOUSE ‘ALLSTAR’ LETTUCE YIELD

Seedling stage ‘Allstar’ showed the trend of yield reduction as sublethal rate increase (Figure 2.9c). The rate of 1/4 reduced yield to only 4 g pot^{-1}. Dose response model estimated rate of 1.13 ± 0.92 g ae ha^{-1} caused 5% yield reduction and rate of 25.92 ± 7.51 g ae ha^{-1} caused 50% yield reduction (Table 2.3). Mature stage ‘Allstar’ had some variability in yield (Figure 2.9c). Dose response model suggested rate of 24.88 ± 27.45 g ae ha^{-1} resulted 5% yield reduction and rate of 64.73 ± 49.81 g ae ha^{-1} resulted 50% yield reduction (Table 2.4).

2.3.25 2020 GREENHOUSE ‘ALLSTAR’ LETTUCE YIELD LOSS

The trend for yield loss in 2020 was similar to 2019. However, in 2020, seedling ‘Allstar’ showed yield loss only at the two highest rates of 1/4 and 1/10 while rates lower than 1/10 showed an increase in yield (Figure 2.10c). Dose response suggested 1.68 ± 1.27 g ae ha^{-1} results in 5% yield loss and 32.28 ± 8.62 results in 50% yield loss (Table 2.3). ‘Allstar’ sprayed at the mature stage experienced yield loss of 18% in the 1/4 rate, but yield loss was not significant at rates below that (Figure 2.10c). Dose response indicates a rate of 43.09 ± 26.00 g ae ha^{-1} resulted in 50% yield loss (Table 2.4).

2.4 DISCUSSION

2.4.1 DICAMBA INJURY ON LETTUCE MAINLY AFFECT THE NEW GROWING REGION

All three lettuce varieties – ‘Green Forest’, ‘Vulcan’, and ‘Allstar’ – were highly susceptible to injury and yield reduction after exposure to sublethal rates of dicamba. When all three lettuce varieties were at either seedling or mature stage, the two highest rates of 1/4, and 1/10 consistently showed injury symptoms and those symptoms progressed over time. It is consistent the injury symptoms were prominent only at the newly growth part of the lettuce. Similar effect was observed in soybean new growth leaves (Jones et al. 2019b), and cotton (Marple et al. 2008). This is due to dicamba translocate to newly formed meristematic tissue (Senseman 2007). Texture of the ‘Green Forest’ leaves with dicamba injury showed as rigid and easy to break which is the opposite for ‘Vulcan’ as the leaves stayed soft and leathery.

2.4.2 AUXINIC HERBICIDE CAN INHIBIT THE ANTHOCYANIN BIOSYNTHESIS IN RED LEAF ‘VULCAN’ LETTUCE

Seedling ‘Vulcan’ lettuce treated with 1/4 and 1/10 rate and mature stage ‘Vulcan’ treated with 1/4 rate in both years greenhouse condition showed poor development of the red pigment which is common on red leaf lettuce. The red coloration in red leaf lettuce is anthocyanin which are natural water soluble red pigments belongs to the flavonoid polyphenol compounds (Harborne and Williams 2000). Anthocyanin also known for its antioxidant properties and free radical scavenging capacity with the benefit of anti-inflammatory, anti-carcinogenic activity, cardiovascular disease prevention, obesity control, and diabetes alleviation properties (He and Giusti 2010). It is known that auxins regulate secondary metabolic pathways including phenylpropanoid, flavonoid, and anthocyanin metabolism (Meyer and Van Staden 1995, Zhou et al. 2008). An early study on carrots using 2,4-D showed the strongest inhibition on the anthocyanin biosynthesis (Ozeki and Komamine 1986). The application of exogenous NAA and 2,4-D showed decrease in the anthocyanin content in tobacco callus cultures (Zhou et al. 2008). Study on red-flesh apple showed increase in auxin concentration can significantly inhibit the anthocyanin biosynthesis (Ji et al. 2015a, 2015b). This explained the lack of red pigmentation on our ‘Vulcan’ lettuce in the greenhouse condition. Dicamba as an synthetic auxinic herbicide could inhibit the biosynthesis of anthocyanin. As color is an important quality index and this could also influence the purchasing behavior of consumers to some degree (Cliff 2002). Although in the field study in 2020, ‘Vulcan’ did not showed lack of anthocyanin in the two highest rates as showed in the greenhouse. However, lack of anthocyanin was not observed in the field research which showed other environmental factors like light and temperatures could play a part of the anthocyanin development.

2.4.3 LETTUCE RESPONSE HIGHLY VARIABLE IN DIFFERENT YEARS TREAT WITH THE SAME RATES

In 2019, ‘Green Forest’ at seedling stage treated with 5.6 g ae ha^{-1} showed 35% injury 7 DAT but able to recover with no injury 22 DAT. However, 38% yield loss was still observed. Other rates of 0.56 and 0.056 g ae ha^{-1} on seedling ‘Green Forest’ also showed yield loss range approximate 10%. ‘Vulcan’ at the seedling stage responded the same in 2019 as ‘Green Forest’ with the exception of the 1/10000 rate showed yield increase. The same effect was observed in 2020 field ‘Green Forest’, a yield loss of 5-10% occurred in the lowest rates of 1/100 and 1/500 even though no visible injury was observed. This showed even lettuce were able to recover with no injury, yield loss could already occurred and proved lettuce production were highly susceptible to dicamba off-target injury. In 2020, seedling stage 'Green Forest' and 'Vulcan' treated with rates of 1/50 and lower responded with yield increases of up to 20%. This was an interesting found as synthetic auxin herbicides like dicamba are widely reported to cause stimulus effects at low doses also known as hormesis (Egan et al. 2014). Hormesis was observed in our 2020 'Green Forest' and 'Vulcan' lettuce which stimulating the growth and increase the biomass. Despite the observed hormesis, the response was not consistent across years and would not be a sustainable approach to increasing lettuce yield because of the risk of injury and yield loss. Any observed yield gain via hormesis at low dicamba rates is outweighed by the risk of severe injury and yield loss in lettuce and other crops. Similarly, all lettuce varieties treated at the mature stage in 2019 experienced yield loss at the 1/4 and 1/10 rates. However, in 2020 only 'Allstar' yield was reduced when treated with dicamba rates of 1/4 and 1/10 – 'Green Forest' and 'Vulcan' were not affected. Small evidence of hormesis in 'Green Forest' and 'Vulcan' lettuce treated at the mature stage is irrelevant to growers because most of the lettuce would be non-marketable due to visible injury symptoms.

2.4.4 'GREEN FOREST' WERE HIGHLY SUSCEPTIBLE TO DICAMBA COMPARED OTHER LETTUCE VARIETIES

There has been extensive research on the susceptibility of specialty crops to drift injury with phenoxy herbicides, particularly 2,4-D, but less research on benzoic herbicides like dicamba (Masiunas 1991). To our knowledge, there has been only one other study on the effects of dicamba on lettuce (Roesler et al. 2020). Roesler et al. (2020) found that the lettuce variety 'Stella' in the greenhouse condition experienced 100% mortality within 4 weeks when treated with full- (560 g ae ha^{-1}) and half-label (280 g ae ha^{-1}) rates of dicamba 55 days after planting.

The two highest rates in our study were 1/4 (140 g ae ha^{-1}) and 1/10 (56 g ae ha^{-1}) of the labeled rate and when sprayed on seedling stage lettuce we observed 80% to 90% injury after 22 to 27 DAT. Roesler et al. (2020) reported that 67.2 g ae ha^{-1} dicamba caused plant death at forty-five days after treatment which is close to our 1/10 rate (56 g ae ha^{-1}). Roesler et al. (2020) also found lettuce treated with 16.8 g ae ha^{-1} or less at the stage of 55 days after planting recovered by 28 days after treatment with no biomass reduction compared with the control. This result is consistent with dose response estimates for 2019 seedling ‘Vulcan’ in the greenhouse condition as a 5% yield loss was estimated at the rate of 23.03 ± 9.65 g ae ha^{-1}. Similarly, 5% yield loss was estimated for 2020 seedling ‘Vulcan’ in the greenhouse at a treatment rate of 29.23 ± 7.89 g ae ha^{-1}. However, ‘Green Forest’ was more sensitive to dicamba and the dose response estimate in 2019 was 50% yield loss after treatment with 16.8 g ae ha^{-1}. In 2020, 21.75 ± 7.19 g ae ha^{-1} was the effective dose for 20% yield loss in ‘Green Forest’ treated at the seedling stage. Results were similar in field trials where rates of 1/4, 1/10, 1/50, 1/100, and 1/500 reduced ‘Green Forest’ yield, but ‘Vulcan’ yield was not affected at the 1/500 rate.

2.4.5 DETECTION FOR DICAMBA RESIDUE ON ‘GREEN FOREST’ LETTUCE IS MORE STABLE IN THE WINDOW OF 7 DAT

Our study found out dicamba detection on ‘Green Forest’ lettuce is stable within a week of the herbicide application. However, in 16 DAT, we observed inconsistence of increased and decreased of detected dicamba residue. The highest rate of 1/4 at 16 DAT was detected 296.3 ppb which was significantly lower than 1161.5 ppb at 3 DAT and 1046.5 ppb at 7 DAT. This can be explained by the nature of plant metabolize herbicide. For example, 2,4-D metabolism in plants follow three pathways 1) direct conjugation, 2) hydroxylation mediated by cytochrome

P450 (Hock and Elstner 2004) and, 3) side-chain cleavage (Peterson et al. 2016). All of those innate process will make dicamba less toxic and decrease the amount detected. However, the second rate of 1/10 showed an increase in detection at 16 DAT at 712.5 ppb compared with 3 DAT at 297 ppb and 7 DAT at 327 ppb. This could due to plant were at the rapid rate of leaf expansion accompanied with low rate of wax production which increased herbicide penetration into the plant (Baker and Hunt 1981). In addition, we have limited replications of only two for the dicamba detection per rate per stage and could not be enough with high variation.

2.5 CONCLUSION

Based two years of greenhouse and field studies, we conclude that dicamba negatively impacts the growth and yield of modern lettuce varieties. Regardless of lettuce growth stage, simulated dicamba drift rates of 140 (25% of labeled rate) and 56 (10% of labeled rate) g ae ha^{-1} caused nearly 100% yield loss across all three lettuce varieties. Yield loss was often observed at rates lower than 56 g ae ha^{-1} even when no injury was visible on the plant. There were differences in the susceptibility of lettuce varieties to dicamba injury. Seedling stage ‘Green Forest’ was more susceptible to dicamba compared with seedling stage ‘Vulcan’. Dose response models suggest that the same rate of 23.03 ± 9.65 g ae ha^{-1} had little effect on ‘Vulcan’ yield but caused nearly 50% yield loss in ‘Green Forest’. Residue analysis on ‘Green Forest’ suggested that dicamba residue is stable when collected and analyzed with seven days of the drift event. Therefore, farmers should submit lettuce sample within 7 days to get an accurate approximation of the actual drift rate. An accurate estimate of the actual drift rate is necessary for using the dose response models from this study to explain possible yield loss. Given the nature of dicamba used as post-emergence herbicide with a high risk for off-target drift, it is important to explore strategies for minimizing drift events. DriftWatch^{TM} (driftwatch.org) is a useful tool that provides information not only for growers but the herbicide applicators about neighboring specialty crops and helps facilitate communication before herbicide applications and potential damage occur.

Herbicide applicators should take extra precautions when applying dicamba near sensitive specialty crops, including: follow the label, use proper nozzle size that creates larger droplets, uses adjuvants that increase the surface tension, lower boom height closer to the ground, and travel at moderate speeds. Applications should be avoided during temperature inversions and periods of high wind speed.

FIGURES AND TABLES

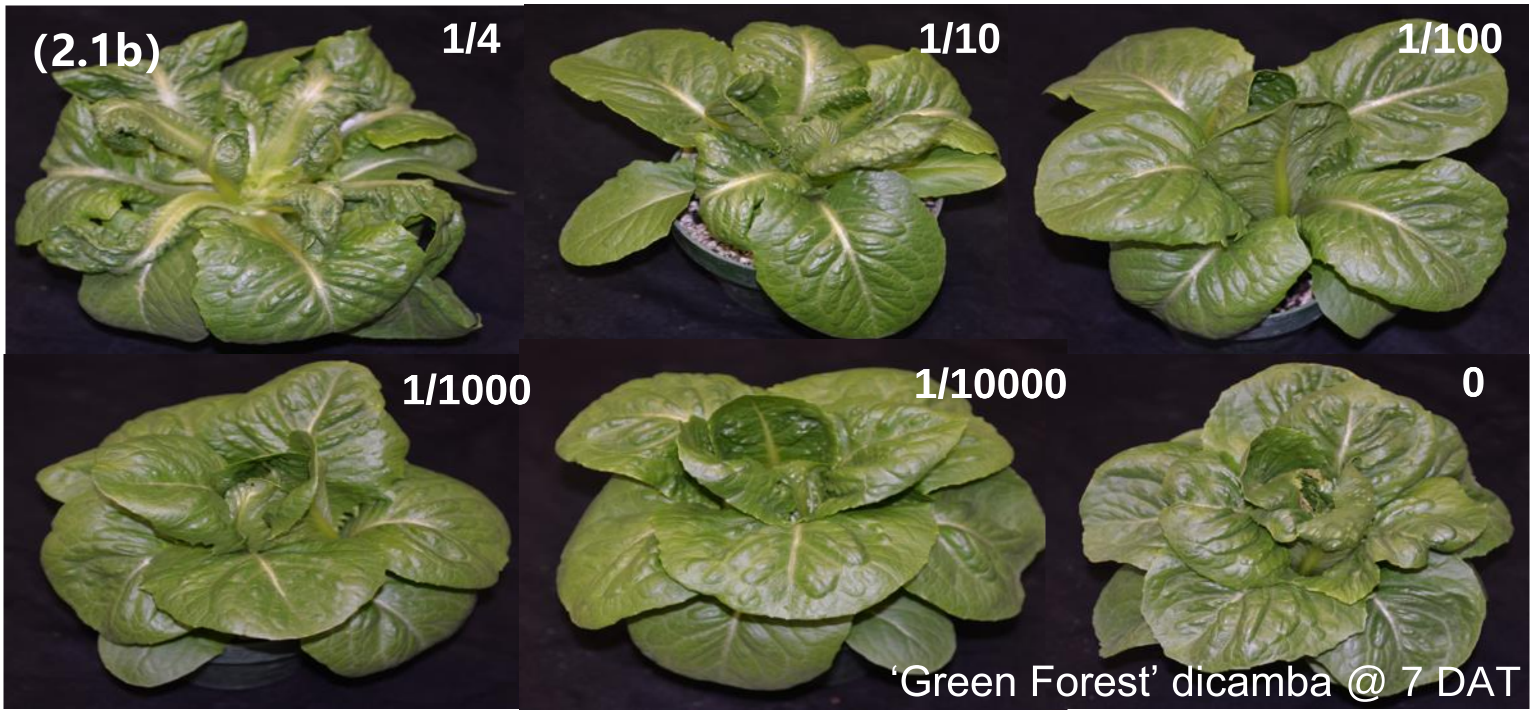
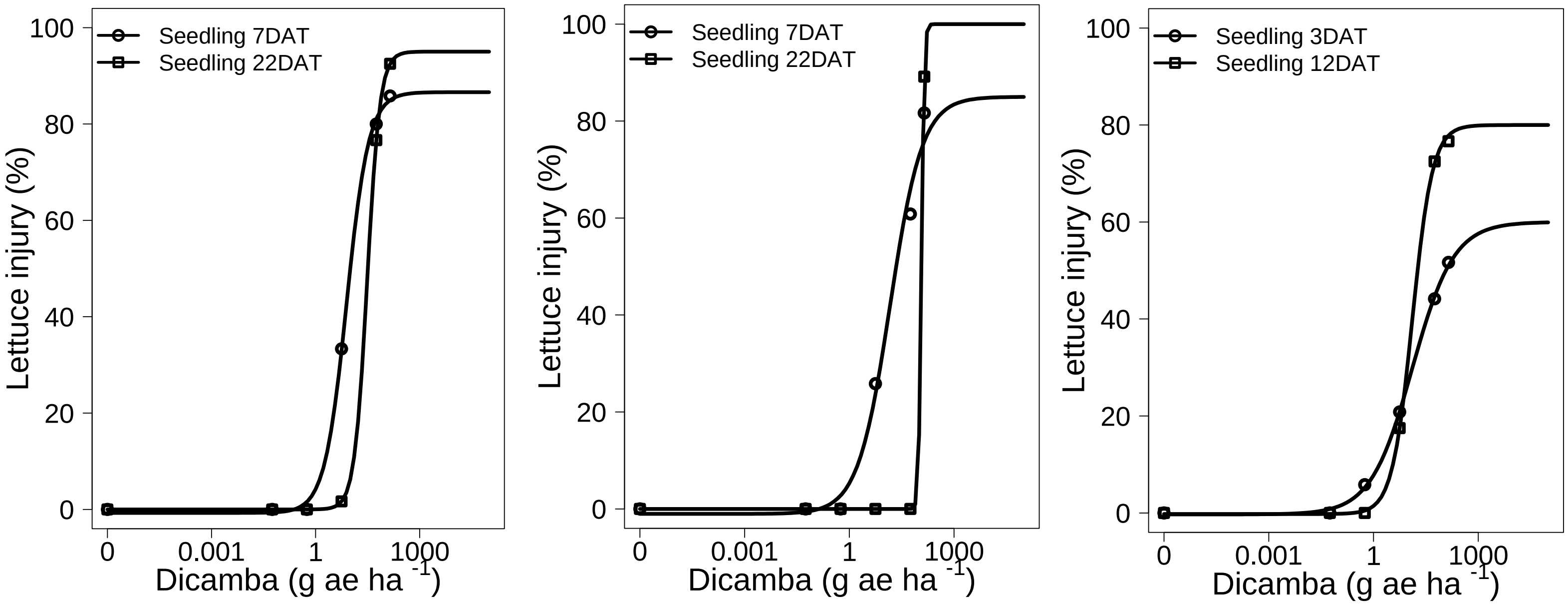


Figure 2.1: 2019 Greenhouse seedling ‘Green Forest’ (2.1a) and Mature ‘Green Forest’ (2.1b) injury from different sublethal rates of XtendiMax® (dicamba) at 7 DAT. On seedling ‘Green Forest’ dose of 1/4, 1/10, and 1/100 showed signs of chlorosis, curling, and twisting of young leaves as well as stunting, and necrosis. On mature ‘Green Forest’, dose of 1/4 and 1/10 showed backside curling mainly on newly growing leaves.

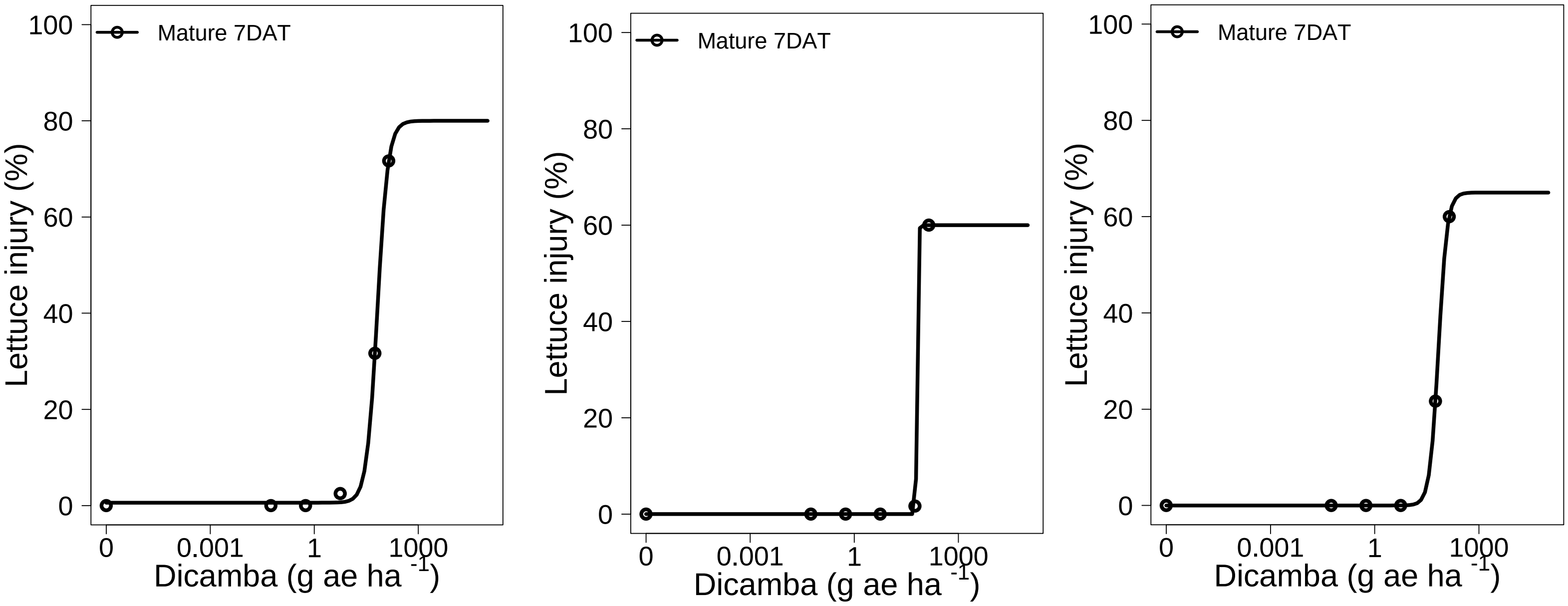


2.2a ‘Green Forest’

2.2b ‘Vulcan’

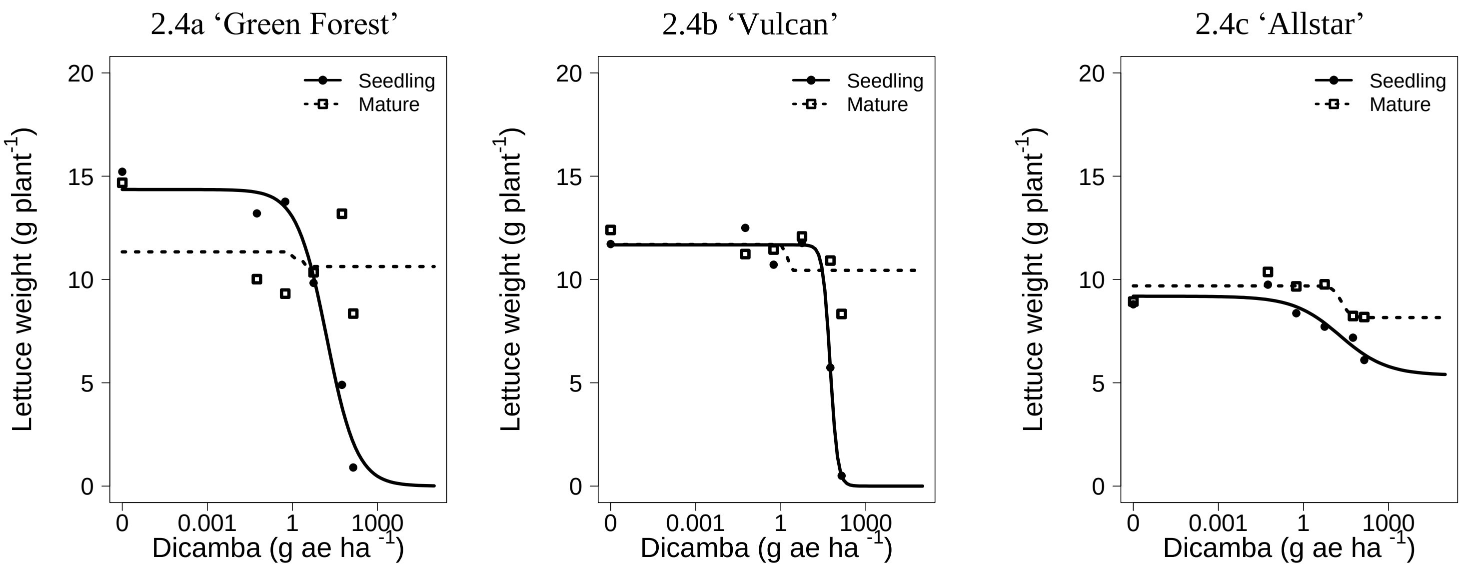
2.2c ‘Allstar’

Figure 2.2: 2019 Greenhouse seedling stage comparison of 7 DAT and 22 DAT for ‘Green Forest’ (2.2a) and ‘Vulcan’ (2.2b) and 3 DAT and 12DAT for ‘Allstar’ (2.2c) percentage injury non-linear regression curve of XtendiMax® (dicamba). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/100; 1/1000; 1/10000.



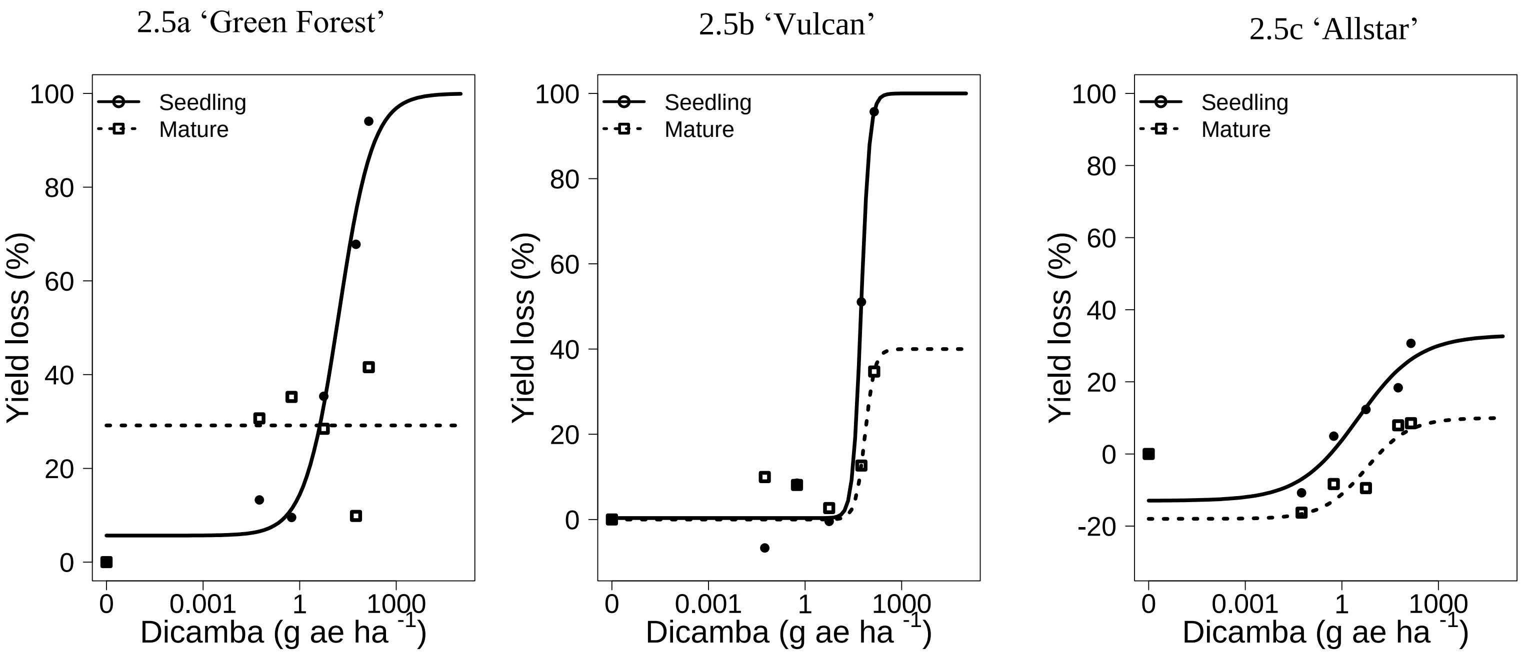
2.3a ‘Green Forest’ 2.3b ‘Vulcan’ 2.3c ‘Allstar’

Figure 2.3: 2019 Greenhouse mature stage of 7 DAT for ‘Green Forest’ (2.3a), ‘Vulcan’ (2.3b), and ‘Allstar’ (2.3c) percentage injury non-linear regression curve of XtendiMax® (dicamba). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/100; 1/1000; 1/10000.



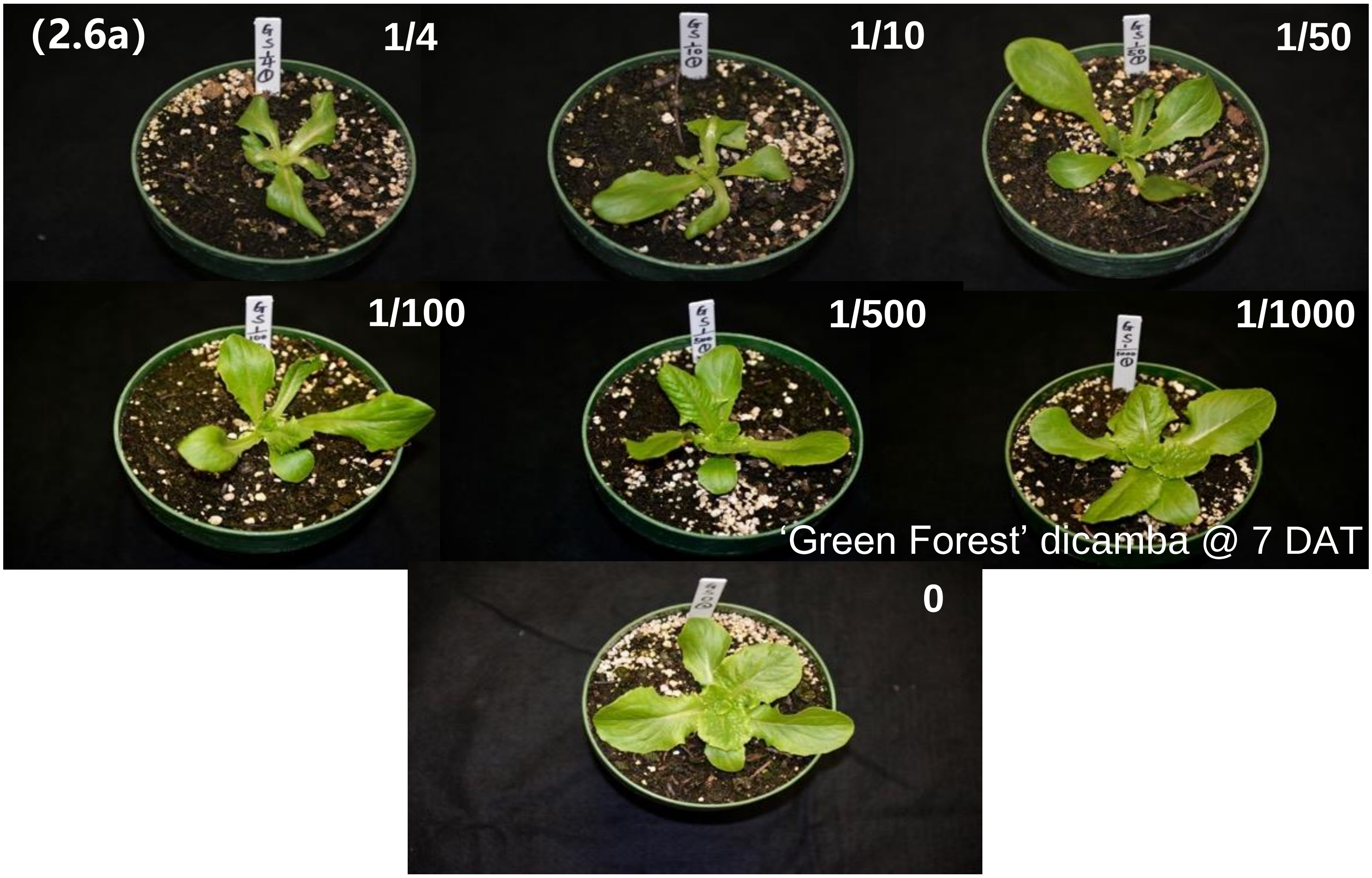
2.4a ‘Green Forest’ 2.4b ‘Vulcan’ 2.4c ‘Allstar’

Figure 2.4: 2019 Greenhouse percentage yield upon harvest non-linear regression of XtendiMax® (dicamba) on ‘Green Forest’ (2.4a), ‘Vulcan’ (2.4b), and ‘Allstar’ (2.4c) lettuce at both seedling and mature stage. Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/100; 1/1000; 1/10000.



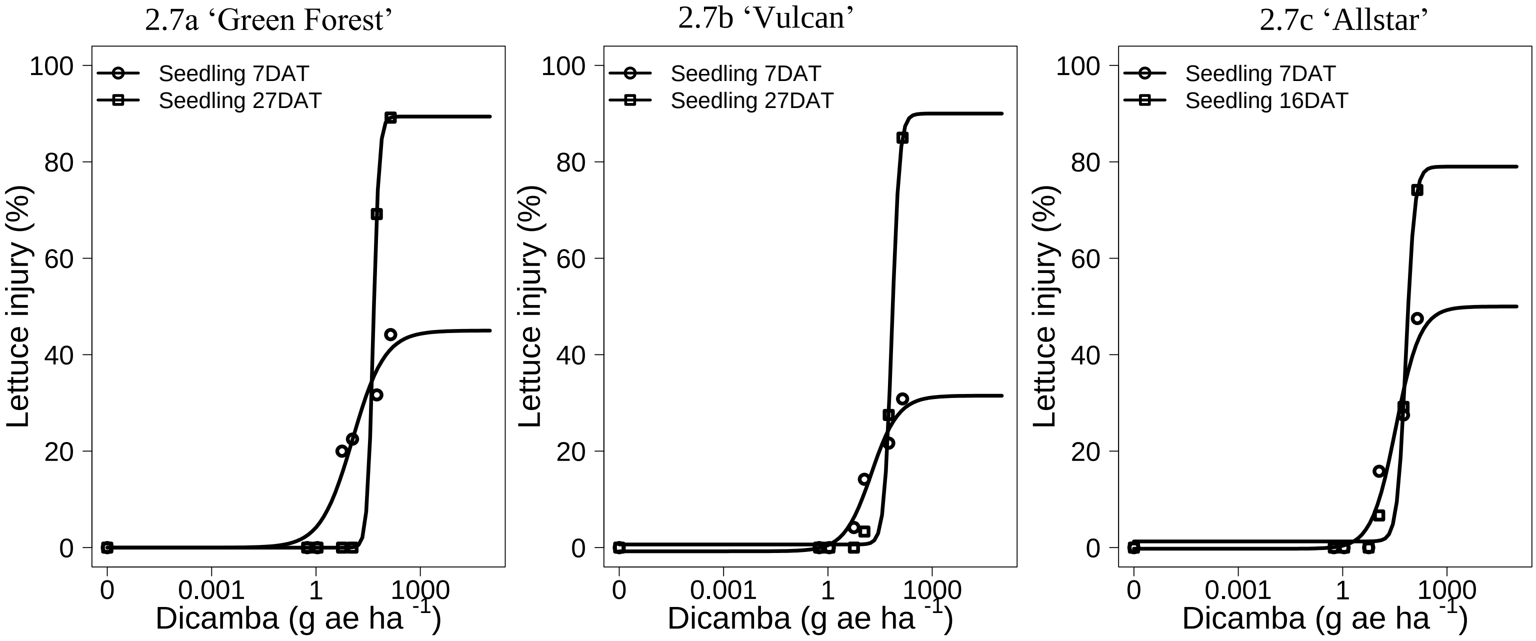
2.5a ‘Green Forest’ 2.5b ‘Vulcan’ 2.5c ‘Allstar’

Figure 2.5: 2019 Greenhouse percentage yield loss upon harvest non-linear regression of XtendiMax® (dicamba) on ‘Green Forest’ (2.5a), ‘Vulcan’ (2.5b), and ‘Allstar’ (2.5c) lettuce at both seedling and mature stage. Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/100; 1/1000; 1/10000.


(2.6a)
1/4
1/10
1/50
1/100
1/500
1/1000
'Green Forest' dicamba @ 7 DAT
0


(2.6b)
1/4
1/10
1/50
1/100
1/500
1/100
'Green Forest' dicamba @ 7 DAT

Figure 2.6: 2020 Greenhouse seedling ‘Green Forest’ (2.6a) and Mature ‘Green Forest’ (2.6b) injury from different sublethal rates of XtendiMax® (dicamba) at 7 DAT. On seedling ‘Green Forest’, dose of 1/4, 1/10, 1/50, 1/100, and 1/500 showed signs of curling, twisting of young leaves as well as stunting. On mature ‘Green Forest’, dose of 1/4 and 1/10 showed backside curling of both old and newly growing leaves.

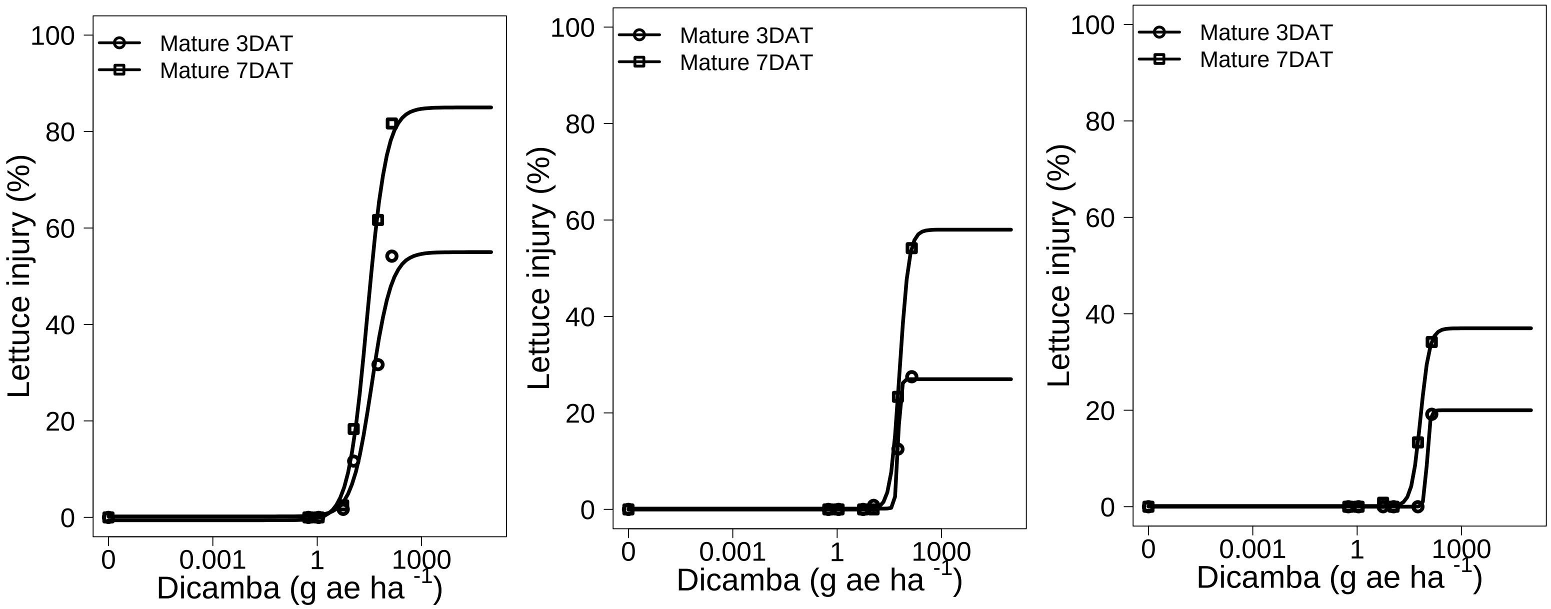


2.7a ‘Green Forest’

2.7b ‘Vulcan’

2.7c ‘Allstar’

Figure 2.7: 2020 Greenhouse seedling stage comparison of 7 DAT and 27 DAT for ‘Green Forest’ (2.7a), ‘Vulcan’ (2.7b) and 7 DAT and 16 DAT for ‘Allstar’ (2.7c) percentage injury non-linear regression curve of XtendiMax® (dicamba). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500; 1/1000.

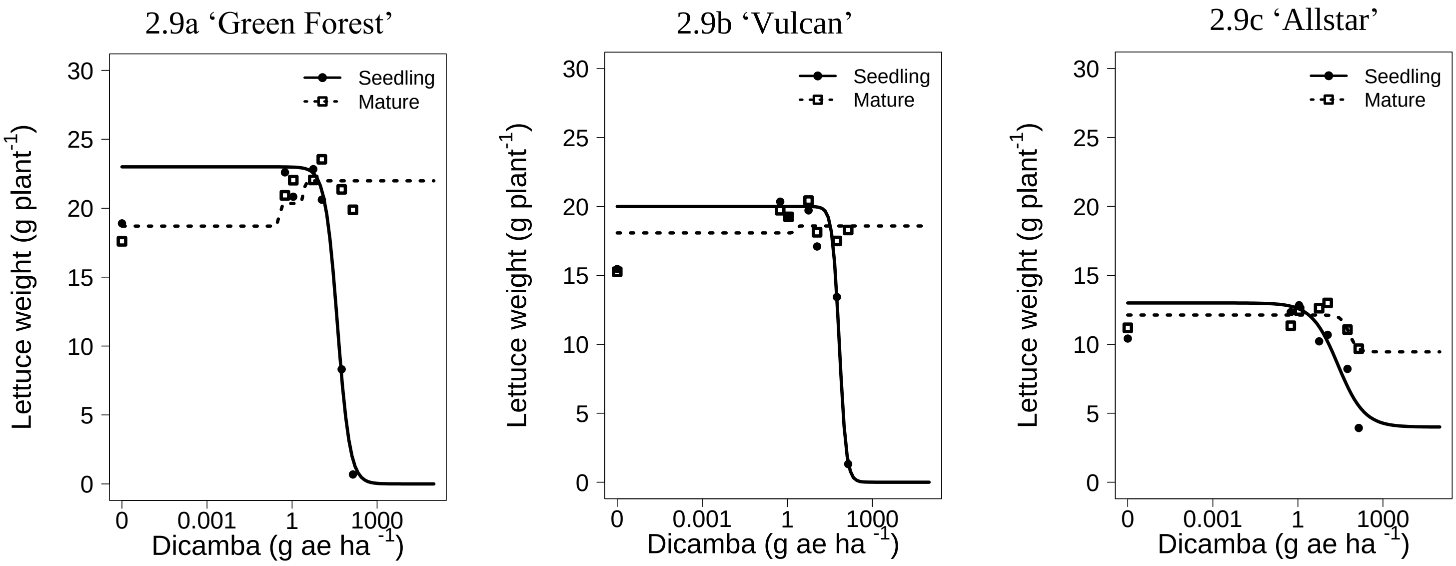


2.8a ‘Green Forest’

2.8b ‘Vulcan’

2.8c ‘Allstar’

Figure 2.8: 2020 Greenhouse mature stage of 3 DAT and 7 DAT for ‘Green Forest’ (2.8a), ‘Vulcan’ (2.8b), and ‘Allstar’ (2.8c) percentage injury non-linear regression curve of XtendiMax® (dicamba). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500; 1/1000.

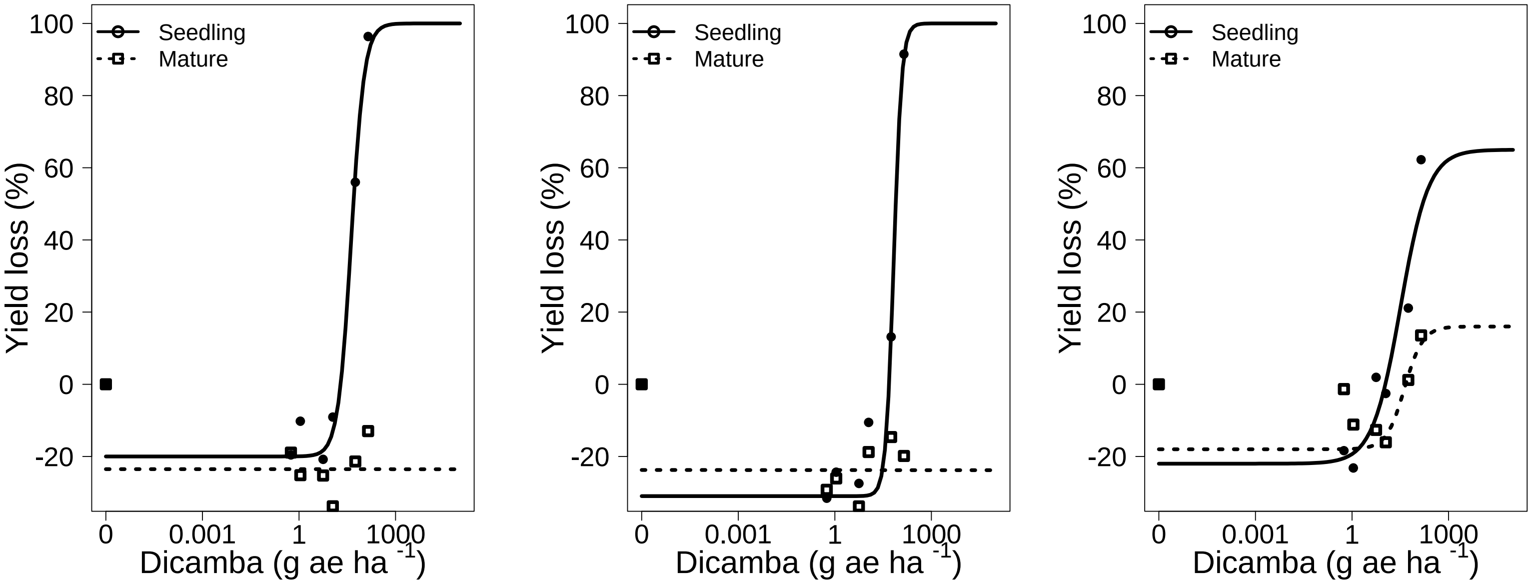


2.9a ‘Green Forest’

2.9b ‘Vulcan’

2.9c ‘Allstar’

Figure 2.9: 2020 Greenhouse yield upon harvest non-linear regression of XtendiMax® (dicamba) on ‘Green Forest’ (2.9a), ‘Vulcan’ (2.9b), and ‘Allstar’ (2.9c) lettuce at both seedling and mature stage. Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500; 1/1000.


2.10a ‘Green Forest’

2.10b ‘Vulcan’

2.10c ‘Allstar’

Figure 2.10: 2020 Greenhouse percentage yield loss upon harvest non-linear regression of XtendiMax® (dicamba) on ‘Green Forest’ (2.10a), ‘Vulcan’ (2.10b), and ‘Allstar’ (2.10c) lettuce at both seedling and mature stage. Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500; 1/1000.

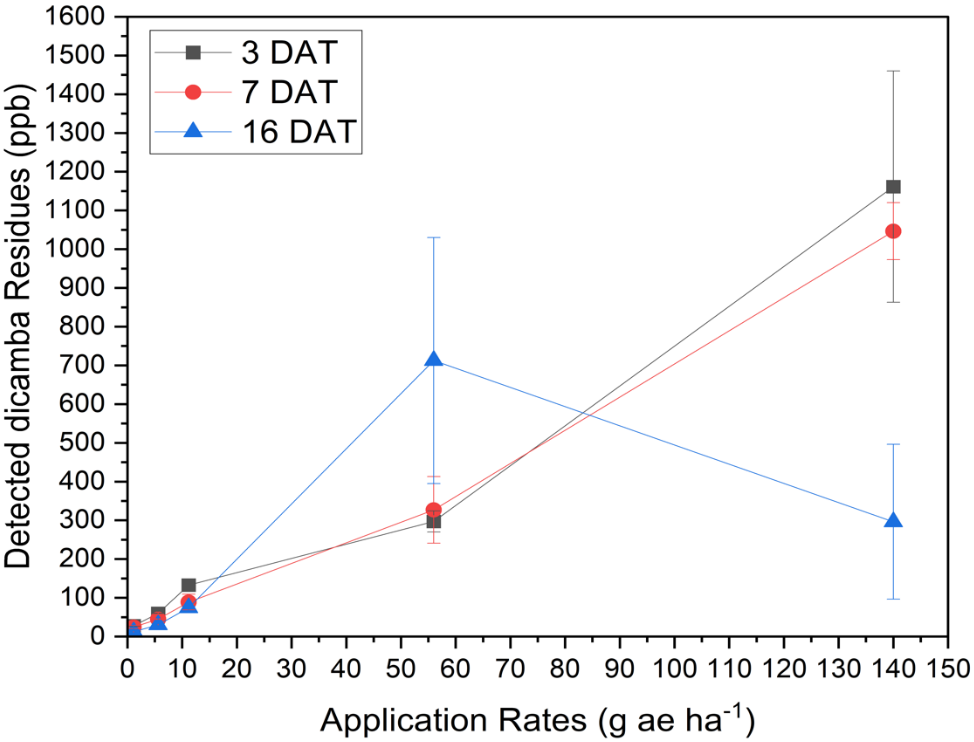
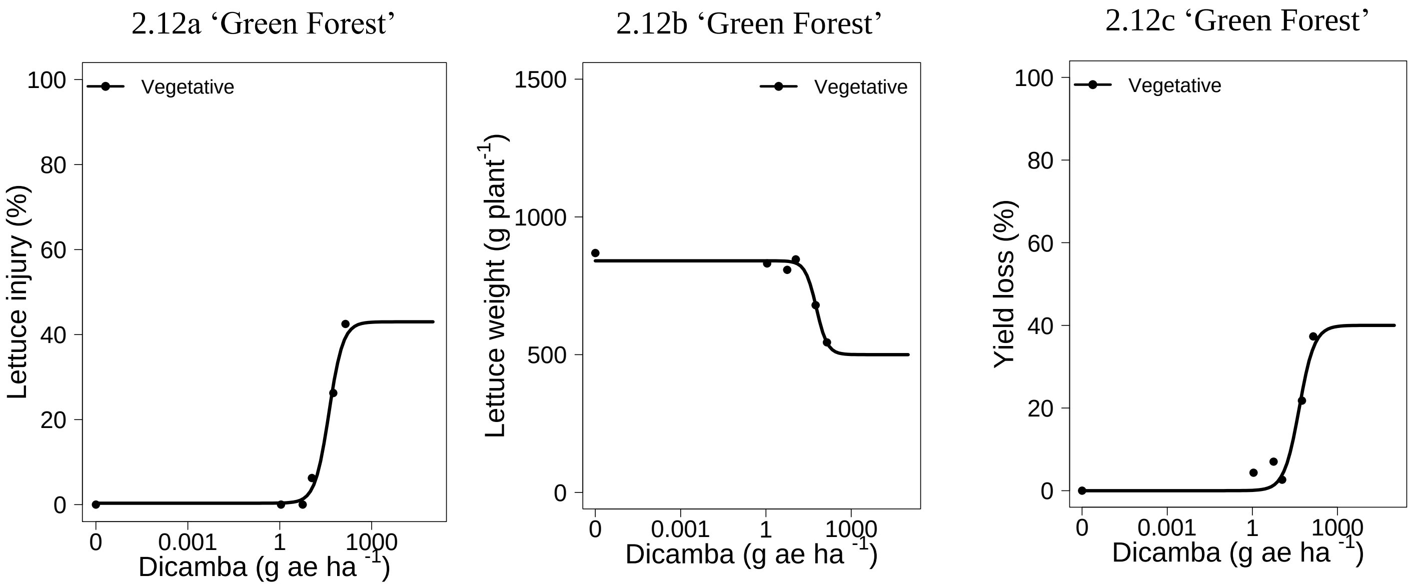


Figure 2.11: 2020 Greenhouse XtendiMax® (dicamba) residue test on mature stage ‘Green Forest’. Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500. Noted dicamba residue results were stable within 7 days after the treatment.

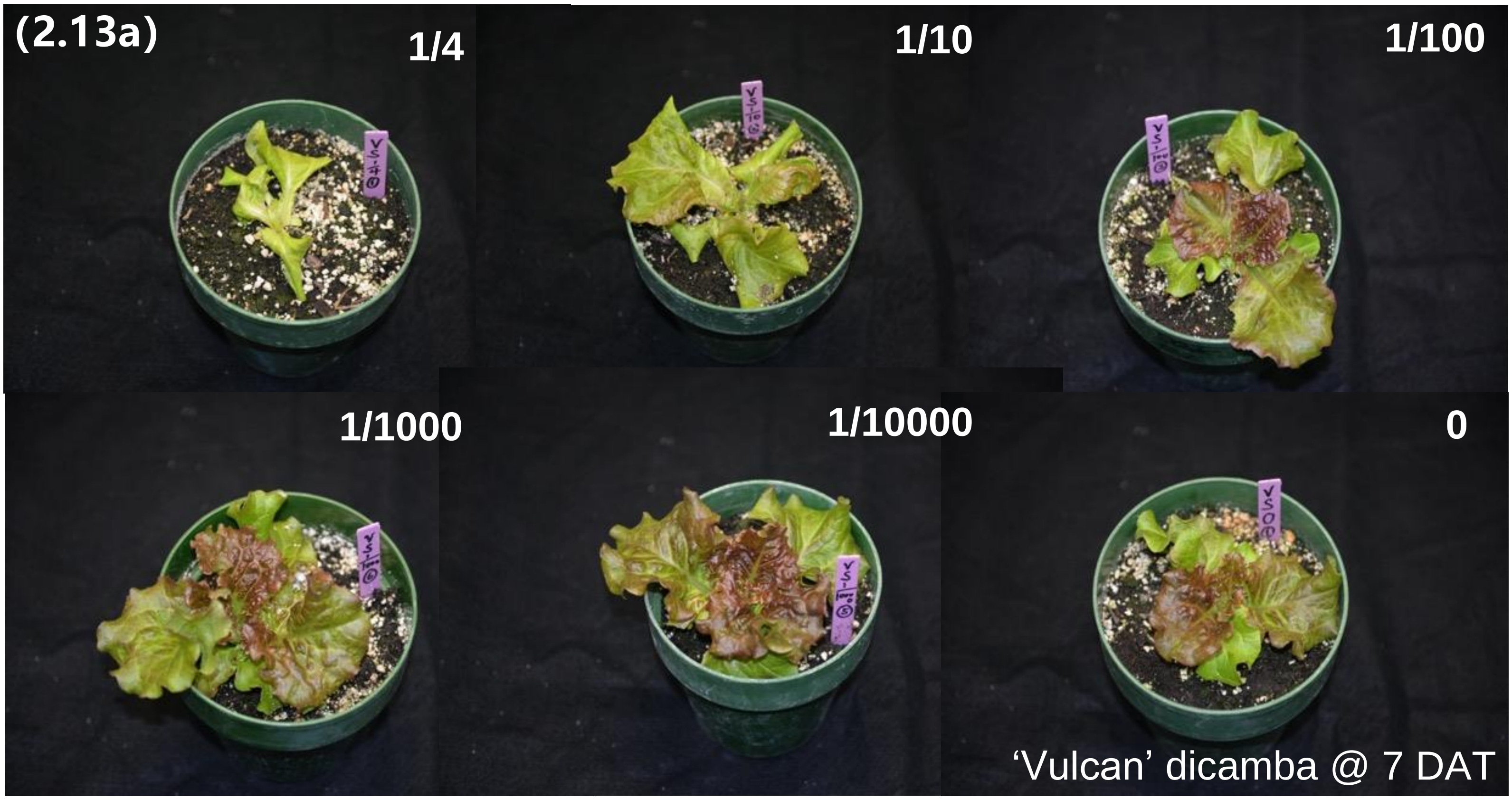


2.12a ‘Green Forest’

2.12b ‘Green Forest’

2.12c ‘Green Forest’

Figure 2.12: 2020 Field 7 DAT at vegetative stage ‘Green Forest’ percentage injury (2.12a), yield (2.12b), and percentage yield loss upon harvest (2.12c) non-linear regression curve of XtendiMax® (dicamba). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500.


(2.13a)
1/4
1/10
1/100
1/1000
1/10000
0
'Vulcan' dicamba @ 7 DAT

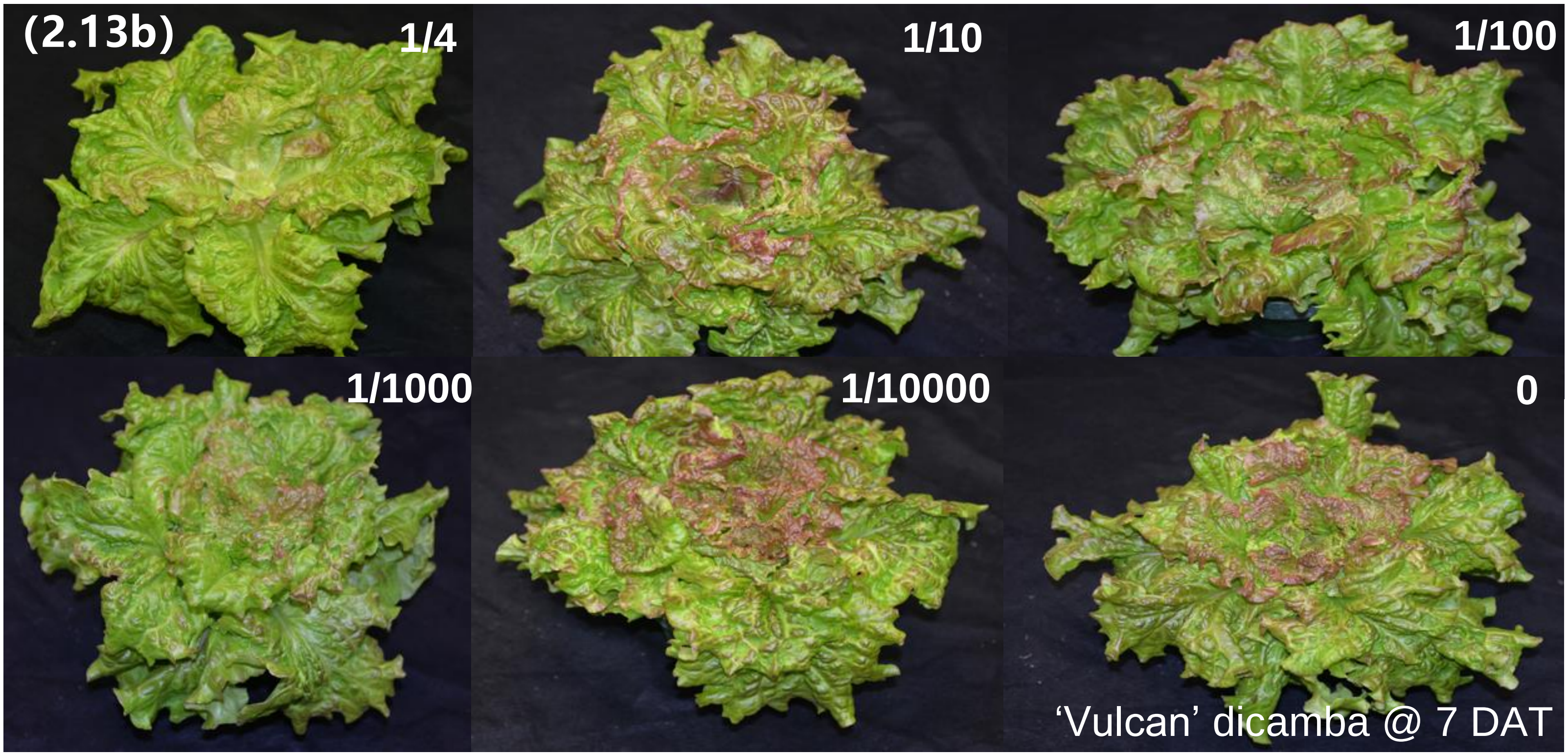
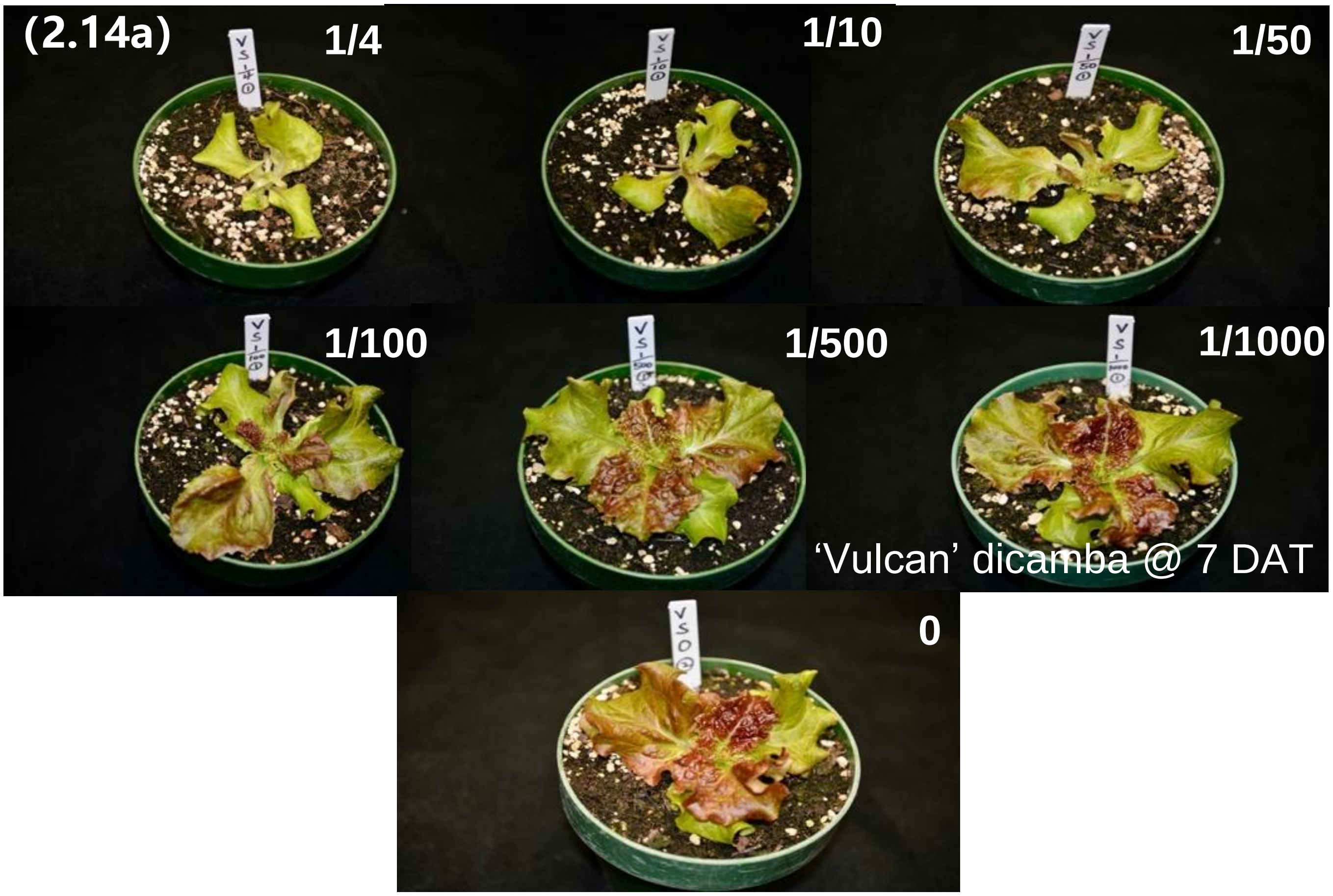

(2.13b)
1/4
1/10
1/100
1/1000
1/10000
0
'Vulcan' dicamba @ 7 DAT

Figure 2.13: 2019 Greenhouse Seedling ‘Vulcan’ (2.13a) and Mature ‘Vulcan’ (2.13b) injury from different sublethal rates of XtendiMax® (dicamba) at 7 DAT. On seedling ‘Vulcan’, dose of 1/4, and1/10 showed signs of chlorosis, curling, and twisting of young leaves as well as stunting, and necrosis. On mature ‘Vulcan’, dose of 1/4 and 1/10 showed backside curling of newly growing leaves.


(2.14a)
1/4
1/10
1/50
1/100
1/500
1/1000
'Vulcan' dicamba @ 7 DAT
0

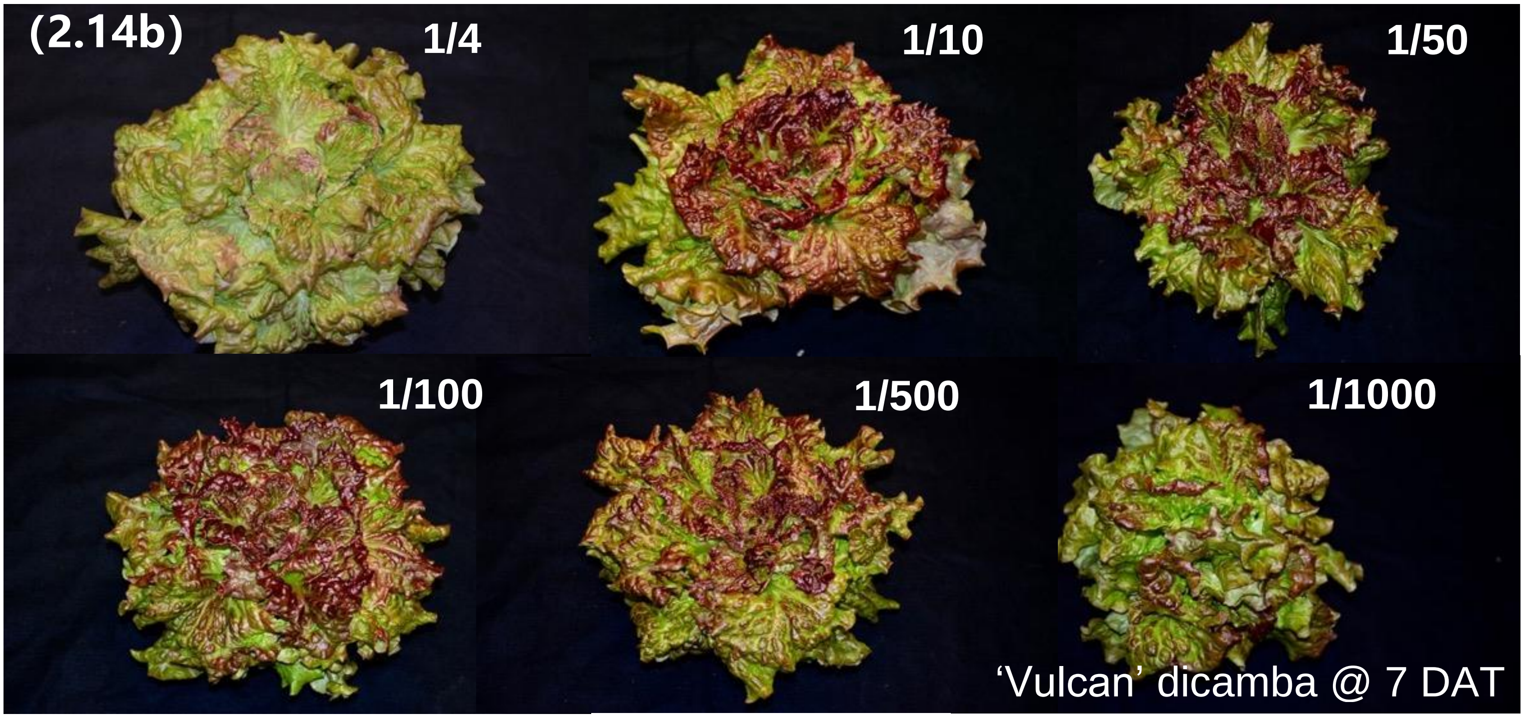
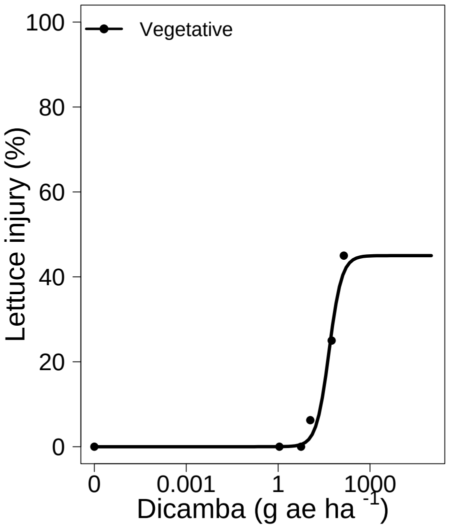
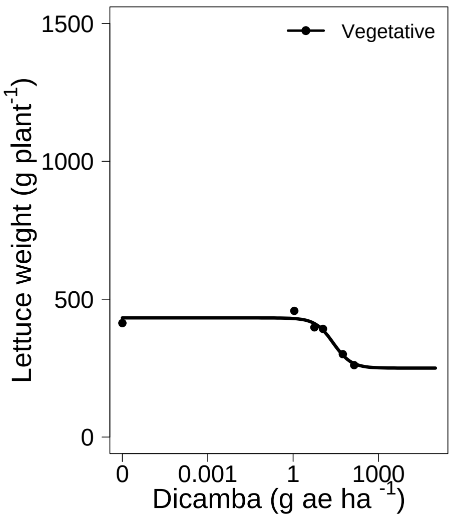

(2.14b)
1/4
1/10
1/50
1/100
1/500
1/1000
'Vulcan' dicamba @ 7 DAT

Figure 2.14: 2020 Greenhouse Seedling ‘Vulcan’ (2.14a) and Mature ‘Vulcan’ (2.14b) injury from different sublethal rates of XtendiMax® (dicamba) at 7 DAT. On seedling ‘Vulcan’, dose of 1/4, 1/10, 1/50 and 1/100 showed signs of chlorosis, curling, and twisting of young leaves as well as stunting, and necrosis. On mature ‘Vulcan’, only dose of 1/4 showed backside curling of both old and newly growing leaves.


2.15a ‘Vulcan’


2.15b ‘Vulcan’

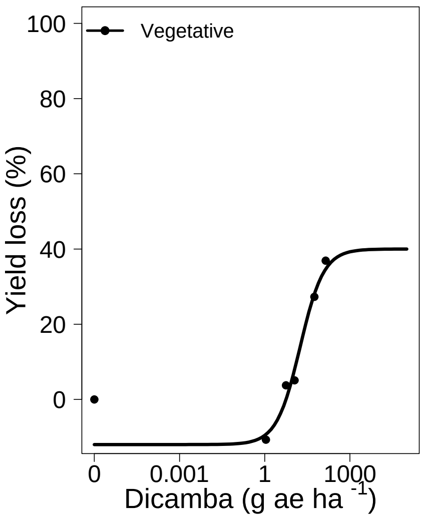
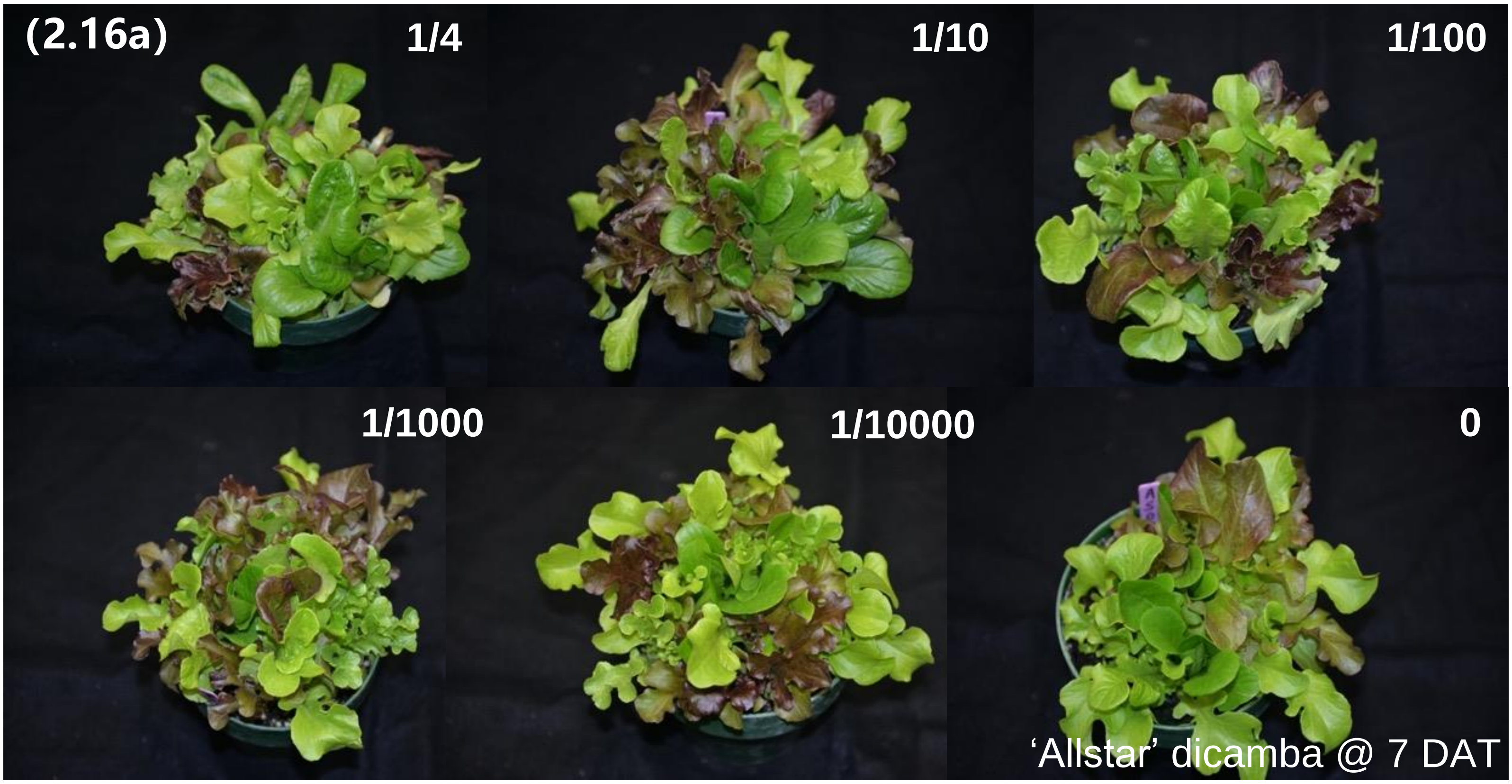
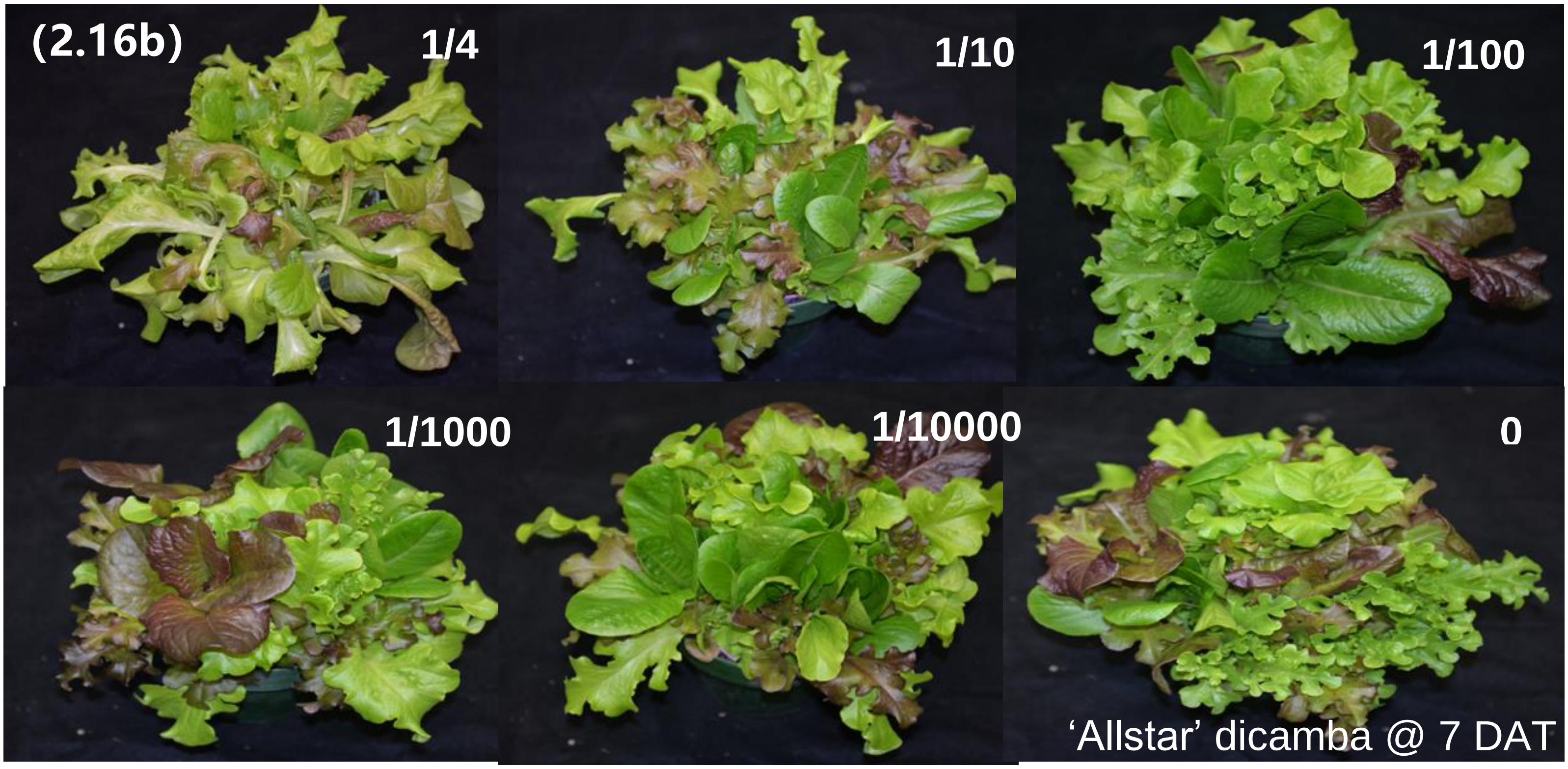

2.15c ‘Vulcan’

Figure 2.15: 2020 Field 7 DAT at vegetative stage ‘Vulcan’ percentage injury (2.15a), yield (2.15b), and percentage yield loss upon harvest (2.15c) non-linear regression curve of XtendiMax® (dicamba). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500. The two highest rate of 1/4 and 1/10 observed injury.

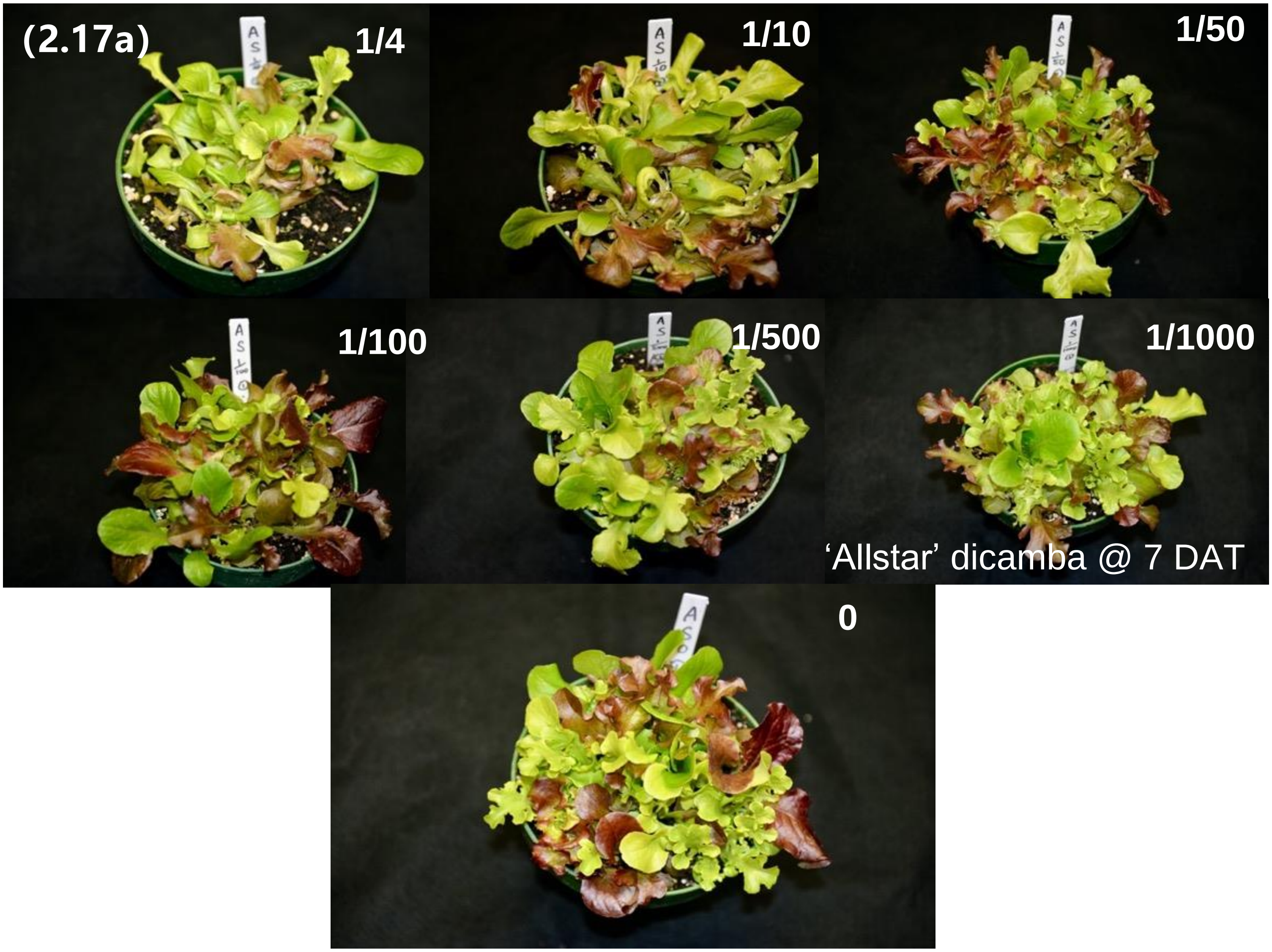


‘Allstar’ dicamba @ 7 DAT



‘Allstar’ dicamba @ 7 DAT

Figure 2.16: 2019 Greenhouse Seedling ‘Allstar’ (2.16a) and Mature ‘Allstar’ (2.16b) injury from different sublethal rates of XtendiMax® (dicamba) at 7 DAT. On seedling ‘Allstar’, dose of 1/4, and 1/10 showed signs of chlorosis, curling, and twisting of young leaves as well as stunting, and necrosis. On mature ‘Allstar’, dose of 1/4 and 1/10 showed backside curling of both old and newly growing leaves.



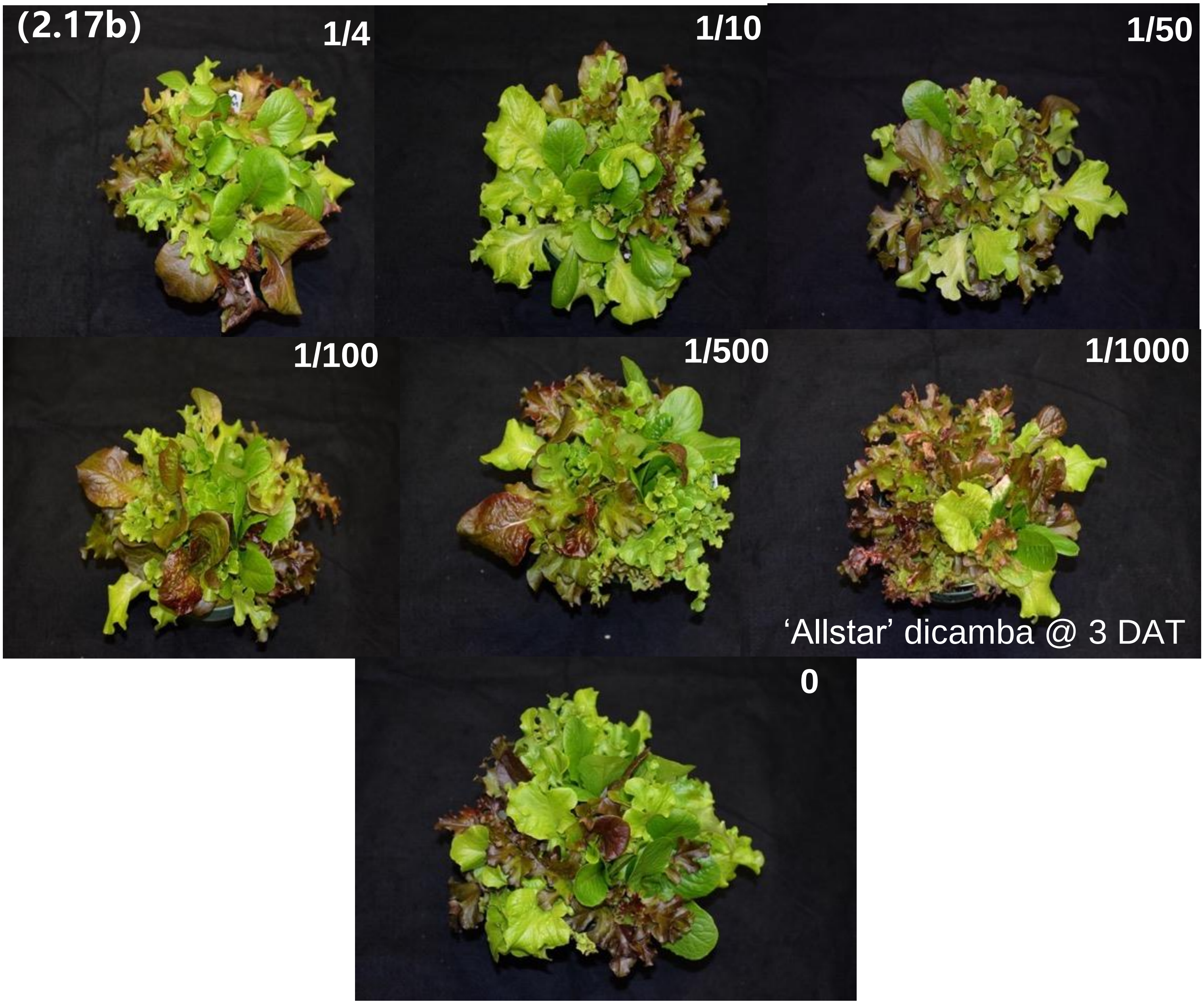

(2.17b)
1/4
1/10
1/50
1/100
1/500
1/1000
‘Allstar’ dicamba @ 3 DAT
0

Figure 2.17: 2020 Greenhouse Seedling ‘Allstar’ (2.17a) and Mature ‘Allstar’ (2.17b) injury from different sublethal rates of XtendiMax® (dicamba) at 7 DAT. On seedling ‘Allstar’, dose of 1/4, 1/10, and 1/50 showed signs of chlorosis, curling, and twisting of young leaves as well as stunting, and necrosis. On mature ‘Allstar’, dose of 1/4, and 1/10 showed backside curling of both old and newly growing leaves.

Table 2.1: 2019 greenhouse dose of XtendiMax® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on ‘Green Forest’, ‘Vulcan’, and ‘Allstar’ at SEEDLING stage

Lettuce Variety	**Measurement**	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
‘Green Forest’	**Percentage Injury**	**7 DAT**	0.91 (0.40)	0.16%	1.58 (0.48)	0.28%	2.86 (0.48)	0.51%	7.87 (1.26)	1.4%
		22 DAT	8.84 (3.15)	1.58%	12.11 (3.53)	2.16%	17.06 (3.79)	3.05%	30.63 (3.41)	5.47%
	Yield	**Seedling**	0.44 (0.48)	0.08%	1.11 (0.97)	0.20%	2.99 (2.00)	0.53%	16.31 (6.28)	2.91%
	Percentage Yield loss	**Seedling**	0.44 (0.47)	0.08%	1.11 (0.95)	0.20%	2.99 (1.94)	0.53%	16.31 (6.11)	2.91%
‘Vulcan’	**Percentage Injury**	**7 DAT**	0.65 (0.32)	0.12%	1.43 (0.58)	0.26%	3.38 (1.06)	0.60%	14.70 (2.98)	2.63%
		22 DAT	89.65 (15.54)	16.00%	95.76 (14.14)	17.10%	102.86 (12.32)	18.37%	116.24 (8.40)	20.76%
	Yield	**Seedling**	23.05 (10.98)	4.12%	28.80 (10.48)	5.14%	36.66 (8.93)	6.55%	55.41 (4.42)	9.89%
	Percentage Yield loss	**Seedling**	23.03 (9.65)	4.11%	28.78 (9.21)	5.14%	36.65 (7.86)	6.54%	55.41 (3.90)	9.89%
‘Allstar’	**Percentage Injury**	**3 DAT**	0.23 (0.31)	0.04%	0.64 (0.69)	0.11%	1.93 (1.58)	0.34%	12.88 (6.02)	2.30%
		12 DAT	1.83 (0.38)	0.33%	3.00 (0.50)	0.54%	5.15 (0.66)	0.92%	12.92 (1.42)	2.30%
	Yield	**Seedling**	0.07 (0.21)	0.01%	0.30 (0.91)	0.05%	1.41 (5.24)	0.25%	19.71 (110.40)	3.52%
	Percentage Yield loss	**Seedling**	0.01 (0.01)	0.002%	0.03 (0.05)	0.005%	0.17 (0.20)	0.03%	3.27 (2.35)	0.58%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 2.2: 2019 greenhouse dose of XtendiMax® that resulted in 5%, 10%, 20%, and 50% injury at 7 DAT and yield loss on ‘Green Forest’, ‘Vulcan’, and ‘Allstar Gourmet’ at MATURE stage

Lettuce Variety	**Measurement**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
‘Green Forest’	**Percentage Injury**	23.00 (2.48)	4.11%	30.00 (2.48)	5.36%	40.01 (2.29)	7.14%	65.50 (2.14)	11.70%
	Yield	0.85 (49.53)	0.15%	0.88 (51.94)	0.16%	0.94 (56.03)	0.17%	1.72 (66.85)	0.31%
	Percentage Yield loss	NA	NA	NA	NA	NA	NA	NA	NA
‘Vulcan’	**Percentage Injury**	57.74 (7.27)	10.31%	59.44 (5.47)	10.61%	61.35 (8.40)	10.96%	64.76 (18.38)	11.56%
	Yield	1.14 (8.28)	0.20%	1.19 (8.71)	0.21%	1.28 (9.38)	0.23%	1.72 (7.80)	0.31%
	Percentage Yield loss	26.30 (21.03)	4.70%	34.08 (21.02)	6.09%	45.16 (19.71)	8.06%	73.05 (21.52)	13.00%
‘Allstar Gourmet’	**Percentage Injury**	29.28 (3.09)	5.23%	36.31 (2.88)	6.48%	45.87 (2.46)	8.19%	68.38 (2.73)	12.21%
	Yield	7.85 (24.69)	1.40%	10.21 (28.52)	1.82%	13.56 (33.78)	2.42%	22.04 (49.47)	3.94%
	Percentage Yield loss	0.06 (0.22)	0.01%	0.20 (0.57)	0.04%	0.67 (1.49)	0.12%	5.72 (6.82)	1.02%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 2.3: 2020 greenhouse dose of XtendiMax® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on ‘Green Forest’, ‘Vulcan’, and ‘Allstar Gourmet’ at SEEDLING stage

Lettuce Variety	**Measurement**	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
‘Green Forest’	**Percentage Injury**	**7 DAT**	0.49 (0.16)	0.09%	1.09 (0.28)	0.19%	2.58 (0.48)	0.46%	11.30 (1.55)	2.02%
		27 DAT	25.06 (75.15)	4.48%	28.94 (71.06)	5.17%	33.84 (63.12)	6.04%	44.19 (37.91)	7.89%
	Yield	**Seedling**	9.09 (5.51)	1.62%	13.23 (6.53)	2.36%	19.90 (7.44)	3.55%	39.97 (8.02)	7.14%
	Percentage Yield loss	**Seedling**	10.46 (5.68)	1.87%	14.86 (6.54)	2.65%	21.75 (7.19)	3.88%	41.71 (6.98)	7.45%
‘Vulcan’	**Percentage Injury**	**7 DAT**	1.26 (0.71)	0.23%	2.47 (1.11)	0.44%	5.11 (1.74)	0.91%	17.74 (4.08)	3.17%
		27 DAT	33.08 (3.56)	5.91%	39.89 (3.14)	7.12%	48.88 (2.51)	8.73%	69.18 (3.22)	12.35%
	Yield	**Seedling**	29.82 (8.06)	5.33%	36.79 (7.38)	6.57%	46.21 (6.06)	8.25%	68.23 (6.35)	12.18%
	Percentage Yield loss	**Seedling**	29.23 (7.89)	5.22%	36.16 (7.24)	6.46%	45.56 (5.95)	8.14%	67.63 (5.92)	12.08%
‘Allstar Gourmet’	**Percentage Injury**	**7 DAT**	3.14 (1.73)	0.56%	5.73 (2.59)	1.02%	11.02 (3.86)	1.97%	33.74 (7.41)	6.03%
		16 DAT	28.57 (4.85)	5.10%	35.32 (4.46)	6.31%	44.47 (3.67)	7.94%	65.92 (3.52)	11.77%
	Yield	**Seedling**	1.13 (0.92)	0.20%	2.50 (1.61)	0.45%	5.93 (2.80)	1.06%	25.92 (7.51)	4.63%
	Percentage Yield loss	**Seedling**	1.68 (1.27)	0.30%	3.56 (2.13)	0.64%	8.02 (3.54)	1.43%	32.28 (8.62)	5.76%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 2.4: 2020 greenhouse dose of XtendiMax® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on 'Green Forest', 'Vulcan', and 'Allstar Gourmet' at MATURE stage

Lettuce Variety	**Measurement**	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
'Green Forest'	**Percentage Injury**	**3 DAT**	5.28 (1.69)	0.94%	8.66 (2.28)	1.55%	14.81 (3.02)	2.64%	37.04 (4.38)	6.61%
		7 DAT	4.36 (0.75)	0.78%	6.98 (0.99)	1.25%	11.64 (1.30)	2.08%	27.89 (2.14)	4.98%
	Yield	**Mature**	NA	NA	NA	NA	NA	NA	NA	NA
	Percentage Yield loss	**Mature**	NA	NA	NA	NA	NA	NA	NA	NA
'Vulcan'	**Percentage Injury**	**3 DAT**	43.80 (58.96)	7.82%	46.79 (46.07)	8.36%	50.26 (29.77)	8.98%	56.81 (4.57)	10.14%
		7 DAT	26.01 (2.60)	4.64%	32.57 (2.44)	5.82%	41.56 (2.06)	7.42%	63.05 (1.73)	11.26%
	Yield	**Mature**	1.58 (1.37)	0.28%	1.64 (1.49)	0.29%	1.74 (2.02)	0.31%	1.97 (6.05)	0.35%
	Percentage Yield loss	**Mature**	NA	NA	NA	NA	NA	NA	NA	NA
'Allstar Gourmet'	**Percentage Injury**	**3 DAT**	77.31 (153.08)	13.81%	83.16 (144.51)	14.85%	90.02 (132.79)	16.08%	103.08 (106.07)	18.41%
		7 DAT	27.72 (4.31)	4.95%	34.64 (4.05)	6.19%	44.11 (3.46)	7.88%	66.68 (3.52)	11.91%
	Yield	**Mature**	24.88 (27.45)	4.44%	31.71 (25.07)	5.66%	41.27 (23.20)	7.37%	64.73 (49.81)	11.56%
	Percentage Yield loss	**Mature**	6.42 (16.19)	1.15%	10.41 (20.84)	1.86%	17.58 (25.45)	3.14%	43.09 (26.00)	7.69%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 2.5: 2020 field study dose of XtendiMax® that resulted in 5%, 10%, 20%, and 50% injury at 7 DAT and yield loss on 'Green Forest' and 'Vulcan' at VEGETATIVE stage

Lettuce Variety	**Measurement**	**Observation time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Perce nt of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
'Green Forest'	**Percentage Injury**	**7 DAT**	8.61 (4.01)	1.54%	12.75 (4.88)	2.28%	19.53 (5.73)	3.49%	40.45 (6.21)	7.22%
	Yield	**Vegetative**	15.80 (11.23)	2.82%	22.07 (12.28)	3.94%	31.73 (12.52)	5.67%	59.01 (10.92)	10.54%
	Percentage Yield loss	**Vegetative**	7.35 (7.70)	1.31%	11.63 (9.75)	2.08%	19.15 (11.76)	3.42%	44.92 (12.55)	8.02%
'Vulcan'	**Percentage Injury**	**7 DAT**	11.40 (6.48)	2.04%	16.25 (7.45)	2.90%	23.85 (8.12)	4.26%	46.00 (6.87)	8.21%
	Yield	**Vegetative**	2.96 (2.02)	0.53%	5.13 (2.88)	0.92%	9.30 (4.09)	1.66%	25.72 (7.59)	4.59%
	Percentage Yield loss	**Vegetative**	1.06 (0.66)	0.19%	2.17 (1.05)	0.39%	4.71 (1.66)	0.84%	17.65 (4.12)	3.15%

REFERENCES

A Final Report on Dicamba-injured Soybean Acres (Kevin Bradley) (n.d.) . https://ipm.missouri.edu/IPCM/2017/10/final_report_dicamba_injured_soybean/. Accessed May 14, 2021

Baker EA, Hunt GM (1981) Developmental Changes in Leaf Epicuticular Waxes in Relation to Foliar Penetration. New Phytologist 88:731–747

Behrens MR, Mutlu N, Chakraborty S, Dumitru R, Jiang WZ, LaVallee BJ, Herman PL, Clemente TE, Weeks DP (2007) Dicamba Resistance: Enlarging and Preserving Biotechnology-Based Weed Management Strategies. Science 316:1185–1188

Bentley B (2019) Investigating Dicamba Tolerance in Grapevine Cultivars through Drift Simulation Assays. MSU Graduate Theses

Byker HP, Soltani N, Robinson DE, Tardif FJ, Lawton MB, Sikkema PH (2013) Control of Glyphosate-Resistant Horseweed (Conyza canadensis) with Dicamba Applied Preplant and Postemergence in Dicamba-Resistant Soybean. wete 27:492–496

Cao M, Sato SJ, Behrens M, Jiang WZ, Clemente TE, Weeks DP (2011) Genetic Engineering of Maize (Zea mays) for High-Level Tolerance to Treatment with the Herbicide Dicamba. J Agric Food Chem 59:5830–5834

Chahal GS, Johnson WG (2012) Influence of Glyphosate or Glufosinate Combinations with Growth Regulator Herbicides and Other Agrochemicals in Controlling Glyphosate-Resistant Weeds. wete 26:638–643

Cliff M (2002) Development of a model for prediction of consumer liking from visual attributes of new and established apple cultivars. Journal- American Pomological Society 4:223–229

Culpepper S (n.d.) Dicamba and 2,4-D Visual Sensitivity Scale for Georgia in 2018 – Laminated Handout | UGA Cotton News

Duke SO (2005) Taking stock of herbicide-resistant crops ten years after introduction. Pest Management Science 61:211–218

Egan JF, Barlow KM, Mortensen DA (2014) A Meta-Analysis on the Effects of 2,4-D and Dicamba Drift on Soybean and Cotton. Weed Science 62:193–206

Egan JF, Mortensen DA (2012) Quantifying vapor drift of dicamba herbicides applied to soybean. Environmental Toxicology and Chemistry 31:1023–1031

Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992. Phytochemistry 55:481–504

Hartzler B (2017) Dicamba: Past, present, and future. Proceedings of the Integrated Crop Management Conference

He J, Giusti MM (2010) Anthocyanins: natural colorants with health-promoting properties. Annu Rev Food Sci Technol 1:163–187

Hock B, Elstner EF (2004) Plant Toxicology. CRC Press. 664 p

Ji X-H, Wang Y-T, Zhang R, Wu S-J, An M-M, Li M, Wang C-Z, Chen X-L, Zhang Y-M, Chen X-S (2015a) Effect of auxin, cytokinin and nitrogen on anthocyanin biosynthesis in callus cultures of red-fleshed apple (Malus sieversii f.niedzwetzkyana). Plant Cell Tiss Organ Cult 120:325–337

Ji X-H, Zhang R, Wang N, Yang L, Chen X-S (2015b) Transcriptome profiling reveals auxin suppressed anthocyanin biosynthesis in red-fleshed apple callus (Malus sieversii f. niedzwetzkyana). Plant Cell Tiss Organ Cult 123:389–404

Jones GT, Link to external site this link will open in a new window, Norsworthy JK, Barber T, Gbur E, Kruger GR (2019a) Off-target Movement of DGA and BAPMA Dicamba to Sensitive Soybean. Weed Technology 33:51–65. 51–65 p

Jones GT, Norsworthy JK, Barber T (2019b) Off-Target Movement of Diglycolamine Dicamba to Non-dicamba Soybean Using Practices to Minimize Primary Drift. Weed Technol 33:24–40

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R Software Package for Dose-Response Studies: The Concept and Data Analysis. wete 21:840–848

Lettuce I, Way JM (1962) The Effects of Sub-Lethal Doses of Mcpa on the Morphology and Yield of Vegetable Crops. Weed Research 2:233–246

Marple ME, Al-Khatib K, Peterson DE (2008) Cotton Injury and Yield as Affected by Simulated Drift of 2,4-D and Dicamba. Weed Technology 22:609–614

Masiunas J (University of I (1991) Herbicide drift injury to high value ornamentals, fruits, and vegetables

Meyer HJ, Van Staden J (1995) The in vitro production of an anthocyanin from callus cultures of Oxalis linearis. Plant Cell Tiss Organ Cult 40:55–58

Mou B (2008) Lettuce. Pages 75–116 *in* J Prohens, F Nuez, eds. Vegetables I: Asteraceae, Brassicaceae, Chenopodicaceae, and Cucurbitaceae. New York, NY: Springer

Mou B (2009) Nutrient Content of Lettuce and its Improvement. Current Nutrition & Food Science 5:242–248

Ozeki Y, Komamine A (1986) Effects of Growth Regulators on the Induction of Anthocyanin Synthesis in Carrot Suspension Cultures. Plant and Cell Physiology 27:1361–1368

Peterson MA, McMaster SA, Riechers DE, Skelton J, Stahlman PW (2016) 2,4-D Past, Present, and Future: A Review. Weed Technology 30:303–345

Powles SB (2008) Evolved glyphosate-resistant weeds around the world: lessons to be learnt. Pest Management Science 64:360–365

Roesler GD, Jonck LCG, Silva RP, Jeronimo AV, Hirata ACS, Monquero PA (2020) Decontamination methods of tanks to spray 2,4-D and dicamba and the effects of these herbicides on citrus and vegetable species. Aust J Crop Sci:1302–1309

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-Logistic Analysis of Herbicide Dose-Response Relationships. Weed Technology 9:218–227

Vink JP, Soltani N, Robinson DE, Tardif FJ, Lawton MB, Sikkema PH (2012) Glyphosate-Resistant Giant Ragweed (Ambrosia trifida) Control in Dicamba-Tolerant Soybean. wete 26:422–428

What Have We Learned from Four Years of Studying Temperature Inversions? (n.d.) . https://ipm.missouri.edu/IPCM/2019/4/inversion/. Accessed May 14, 2021

Zhou L-L, Zeng H-N, Shi M-Z, Xie D-Y (2008) Development of tobacco callus cultures over expressing Arabidopsis PAP1/MYB75 transcription factor and characterization of anthocyanin biosynthesis. Planta 229:37

Heap, I. The International Herbicide-Resistant Weed Database. Online. Tuesday, June 29, 2021 . Available www.weedscience.org

Senseman SA, ed. (2007) Herbicide Handbook. 9th ed. Lawrence, KS: Weed Science Society of America. Pp 335–338

CHAPTER 3, IMPACT OF MICRO-RATES 2,4-D CHOLINE ON PUMPKINS INJURY AND YIELD LOSS

The release of 2,4-D tolerant crops has increased the number of options for controlling resistant weeds and this technology has been widely adopted throughout the United States. However, improper application of 2,4-D is a risk to specialty crop like pumpkins. The objective of this study was to determine the effect of simulated sublethal drift rates of 2,4-D on visual injury and crop yield loss in pumpkins at the vegetative and flowering growth stages. Rates of 2,4-D ranged from 1/500 to 1/4 of the labeled rate (1066 g ae ha^{-1}) for choline salt. Visual injury ratings were recorded every 7 days and pumpkins were harvested and weighed fresh throughout the growing season. In both years, injuries on pumpkins peaked at the window of 14 to 21 days after treatment (DAT) with the highest of 55% in 2020 vegetative stage. In 2020, vegetative stage pumpkins sprayed at the rate of 266.4 g ae ha^{-1} (25% labeled rate) showed significant yield reduction of approximately 32%. Both years, flowering stage pumpkins were less susceptible to 2,4-D and yield loss was observed at rates between 106.6 (10% labeled rate) to 266.6 g ae ha^{-1}(25% labeled rate). In the north central region, drift events are most likely to occur in May and June which corresponds to the vegetative stage for pumpkins; thus, we conclude that pumpkin growers are at high risk of economic loss from 2,4-D drift and off-target injury if care is not taken to minimize off-target chemical movement.

3.1. INTRODUCTION

Pumpkin production is common in the United States. In 2019, Illinois farmers grew 10,900 acres of pumpkins, followed by California, Indiana, Michigan, and Virginia where pumpkin acres ranged from 4,700 and 5,600 acres (USDA National Agricultural Statistics Service's 2017-2019 Vegetable Annual Survey and QuickStats). In 2019, the value of pumpkin sales in the U.S. was measured at $180,190,000 (USDA National Agricultural Statistics Service Pumpkins QuickStats). In Nebraska, Census in 2017 showed approximate 141 acres pumpkins (USDA National Agricultural Statistics Service Quick Stats). Pumpkins growing in the north central region are grown in close proximity to corn and soybean and are at high risk of potential herbicide drift. Herbicide tolerant crop traits have been developed recently in response to recent growth in the number of glyphosate-resistant weeds such as *Ambrosia artemissifolia*, *Ambrosia trifida*, *Amaranthus palmeri*, *Amaranthus rudis*, *Amaranthus tuberculatus*, and various *Conyza* and *Lolium* spp. (Powles 2008). For example, Enlist^{TM} corn$^{®}$, Enlist^{TM} E3 soybeans$^{®}$, and Enlist^{TM} cotton$^{®}$ were developed by Dow AgroSciences (Indianapolis, IN, USA) and these crops are tolerant to the mixture of glyphosate with 2,4-D choline.

The Association of American Pesticide Control Officers (2005) reported that 2,4-Dichlorophenoxyacetic acid (2,4-D) and 3,6-dichloro-o-anisic acid (dicamba) ranked first and third, respectively, on the list of herbicide active ingredients in confirmed drift occurrences (Mohseni-Moghadam et al. 2015). Total applications of 2,4-D in the Midwest was estimated in 2017 at more than 28 pounds per square mile, which is two to three times higher than the average of 3 to 11 pounds per square mile in the north and northeastern regions of the U.S. (USGS, Estimated Annual Agricultural Pesticide Use for 2,4-D, 2017). The volume of 2,4-D applied – and associated drift events – is likely to increase as 2,4-D tolerant crops become more common.

2,4-D is a synthetic auxinic herbicide belong to phenoxyalkanoic acids group that was first marketed under the brand name “Weedone” (Peterson 1967). This original form was modified further to improve dispersal and form a suitable mixture with water (Peterson et al. 2016). Two basic forms of 2,4-D included amine salts and esters. Amine formulations are readily soluble in water (greater than 50% by weight) and form a true solution (Peterson et al. 2016). Esters were formed by reacting 2,4-D acid with an alcohol. The alcohol with longer carbon chain forms 2,4-D ester with lower volatility (Peterson et al. 2016). Butyl ester has a shorter carbon chain which results in a drift potential that is 8 to 10 times greater than the dimethylamine formulation (Grover et al. 1972). The ester formulation also has greater activity than the salt formulation as it quickly penetrates the leaf surface and is converted to acid (Peterson et al. 2016). However, this makes it even more destructive to sensitive off-target plants when vaporized and drifted. Several low dose application studies have been done using the forms of 2,4-D amine salts and esters on specialty crops. A study on watermelon using sublethal rates of 2,4-D (Weedar 64®; 1,120 g ae ha^{-1}) and dicamba (Clarity®; 560 g ae ha^{-1}) at three time points showed higher visual injury and reductions in vine growth when herbicide applications were made before flowering (Culpepper et al. 2018). Similar results were found in a cucumber study where application of 2,4-D at the vegetative growing stage were most injurious (Hand et al. 2020). Culpepper et al. (2018) found that melon injury at 20, 40, and 60 days after planting was 40%, 16%, and 11%, respectively, when treated with a rate of 1/75× of 2,4-D. Similar trends were observed at the rate of 1/250× where injury symptoms decreased over time (Culpepper et al. 2018). In cucumber, a sublethal application of 2,4-D using aqueous solutions of the dimethylamine salt (1040 g ae ha^{-1}) showed that when treated at first bloom 2.1 to 20.8 g ae ha^{-1} caused mild epinasty but fruit yield and shape were unaffected at 2.1 g ae ha^{-1} (Hemphill and

Montgomery 1981). Hemphill and Montgomery (1981) found cucumber yield was reduced when treated with 104 g ae ha^{-1}.

Many other dicotyledon specialty crops have demonstrated susceptibility and similar responses to 2,4-D. Injury percentages from 2,4-D dimethylamine salt (label rate of 840 g ae ha^{-1}) averaged over grape cultivars Riesling, Chardonnay, Chardonel, Vidal blanc, and Traminette showed injury peaking at 42 DAT. Rates of 2.8, 8.4, and 28 g ae ha^{-1} caused 37, 29, and 66% injury, respectively (Mohseni-Moghadam et al. 2015). In peanuts, 2,4-D Amine® (label rate of 1,120 g ae ha^{-1}) at sublethal rates from 70 to 1,120 g ae ha^{-1} on peanuts showed yield reduction at all rates and the highest yield reduction of 41% at the rate of 1,120 g ae ha^{-1} (Leon et al. 2014). Interestingly, Leon et al. (2014) noted that rates of 70, 140, and 280 g ae ha^{-1} showed no injury but still resulted in 11-19% peanut yield loss. A 2,4-D visual sensitivity study in Georgia showed pepper, tomato, and watermelon were severely susceptible to 2,4-D at rates of 1/300 to 1/800 of the labeled rate (Culpepper, 2018).

Despite recent research on the effects of 2,4-D on cucurbits and other dicotyledonous crops, information about pumpkins is lacking. The drift incidents reported to the Nebraska Department of Agriculture noted most drift incidents occurred during the month of May and June (33 out of 38 incidents), which corresponds to typical herbicide application periods in corn and soybeans (Personal communication with Rick Leonard, Committee Research Analyst). These applications correspond to the vegetative and early flowering growth stages of pumpkins and may results in injury and subsequent yield loss. The objective for this study was to determine the effect of low dose application of 2,4-D choline on visual injury and crop yield loss in pumpkins at the vegetative (prior to flower) and flowering growth stages.

3.2 MATERIAL AND METHODS

3.2.1 FIELD STUDY EXPERIMENTAL DESIGN AND TREATMENT APPLICATIONS

Field experiments were conducted in 2019 and 2020 using the pumpkin variety ‘Orange Smoothie’ (F1) (Johnny’s Selected Seeds Company; Winslow, ME). This pumpkin variety is known for its nice handle, medium size, and semi-bush growth habit with less vining (ideal of collecting visual injury and yield data).

In 2019, pumpkin seeds were planted flats in the greenhouse on 13 May for seedling plugs. On 31 May, pumpkins seedings were transplanted to the field located at the UNL Havelock Research Farm in Lincoln, NE (40°51’ 7.008” N, 96°36’ 52.980” W). Before transplanting, the field was prepared with rotary tillage. In a single field pass of the bed-shaper/mulch-layer (RB448; Nolt’s Produce Supplies), raised beds were shaped, and drip irrigation line was laid beneath a white on black plastic film. Each plot was 3.7 meters long by 1.2 meters wide and five pumpkins were planted in a single row within each pot pumpkins were spaced 0.7 meters. The gap between plots was 2.4 meters to prevent herbicide movement between treatments. Treatments included two growth stage treatments (vegetative vs. flowering) and six sublethal rates of 2,4-D including 0 (control), 1/4, 1/10, 1/50, 1/100, and 1/500 of the label rate (1066 g ae ha^{-1} 2,4-D choline salt). There were four replications of all of possible combinations of growth stage by rate treatments. Plants were fertigated two times during the growing season – once before and once after herbicide treatment using calcium nitrate fertilizer (15N–0P–0K, YaraLiva Tropicote 15–0–0; Yara North America, Tampa, FL). Fertilizer injected into the drip irrigation line to deliver 44.8 kg ha^{-1} N in each application.

In 2020, pumpkin was direct seeded on 15 May into a field at the UNL East Campus Research Farm (40° 50’ 10.890” N, 96° 39’ 45.162” W). Planting method was changed in 2020 to avoid transplant shock observed in 2019 that delayed crop growth and development. Before planting seed, the field was prepared with rotary tillage. All plots received an application of 112 kg ha^{-1} N with granular urea (46N-0P-0K; PRO-AP, Wawaka, IN) applied as preplant broadcast fertilizer and incorporated into the soil. Plot setup and dimensions and treatment structure were otherwise identical to 2019.

Herbicide was applied using a CO_2-pressurized tank sprayer with a two-nozzle boom and nozzles spaced 51 cm apart. The sprayer was calibrated to deliver 140 L ha^{-1} at 276 kPa through a TeeJet 8001E nozzle (TeeJet Technologies, Spraying systems Co., Wheaton, IL). Travel speed of the nozzle was based on the walking speed of approximate 4.8 km/hr. The vegetative stage treatment was applied on 20 June 2019 and 12 June 2020 before pumpkin plants had produced any flowers. The flowering stage treatments were applied on 11 July 2019 and 23 June 2020. At this stage, each pumpkin plant was presenting two or more flowers and had begun vining out. The later application in 2019 can be attributed to delayed growth due to transplant shock and less fertilizer N compared to 2020. The application for the vegetative stage was conducted in June to simulate when 2,4-D is typically applied to Enlist Corn® and Enlist E3 soybean® for post-emergence weed control. The application window for Enlist corn is before V8 stage or 30 inches tall. For Enlist E3 soybean®, application can be done before R2 or full flowering stage (Enlist 2021 Product Use Guide). Depending on planting date, region, and cultural practices, it is possible that pumpkin would reach the flowering stage during these 2,4-D application windows, which is why we compared the two growth stages.

Visual injury ratings were conducted every seven days until harvest. Visual injury ratings were based on the percentage scale of 0 (no injury) to 100 (death of the plant) relative to the nontreated control (Appendix 1). Visual injuries included chlorosis, leaf malformation, and epinasty. The rating protocol was adapted from Frans et al. (1986). In 2019, pumpkins were harvested on 2 September and 30 October. In 2020, pumpkins were harvested on 4 August, 18 August, and 3 September. Pumpkin yield at each harvest event were pooled for a season total and adjusted for stand density prior to analysis.

3.2.2 STATISTICAL ANALYSIS

Yield data were based on fresh weight and percentage yield loss relative to the controls. Due to the non-linear nature of plant response to sublethal rates of herbicide, a four-parameter log-logistic regression model was used to analyze the relationship between sublethal rates of 2,4-D with visual injury, average yield and percentage yield loss utilizing approach described in Knezevic et al. (2007).

The four parameter model was defined by the equation

$Y = c + \{ d - c / 1 + \exp [b(\log x - \log e)]\}$

where c is lower limit, d is upper limit, b is slope and e is the ED 50 (dose giving 50% response) (Knezevic et al. 2007, Seefeldt et al. 1995). The regression analyses helped estimate rates of 2,4-D, or effective doses (ED values), that would cause different levels of injury or yield loss. Regression analyses were conducted using the *drc* package in R version 3.4.1 (R Core Team, 2019). Injury ratings within time intervals and applications stage treatments were averaged across the four replications and fit to the response across the six sublethal application rates, as described in Knezevic et al. (2007).

Percentage yield loss was calculated using the equation:

$Y = [(C – T) / C]\ 100$

where *Y* represents the percentage yield loss compared to the nontreated control plot in the corresponding replicate block, *C* represents the biomass of the nontreated control plot, and *T* represents the biomass of the treated plot.

3.3 RESULTS

3.3.1 2019 SUMMER FIELD STUDY VISUAL INJURY

In 2019, pumpkins treated at the vegetative stage showed injury symptoms – including leaves and stems curling and epinasty – as early as 1 day on the 2 highest rates of 1/4 and 1/10. Injury symptoms was stable 14 days after treatment (DAT) and peaked at 21 DAT and all 3 highest rates of 1/4, 1/10, and 1/50 showed injury ranging from 25 to 35% (Figure 3.1a). Estimated drift rate by the dose response model did not have good fit at both 14 and 21 DAT due to variation in percentage injury with no significant differences (Figure 3.1a).

At 14 DAT, dose-response model estimated rate of 0.21 ± 0.53 g ae ha^{-1} caused 5% injury (ED5) and rate of 4.16 ± 4.14 g ae ha^{-1} caused 50% injury (ED 50) (Table 3.1). Pumpkins treated at the flowering stage showed injury peaking at 14 DAT (Figure 3.1b). The 1/4 rate caused 38% injury and 1/10 caused 16% injury (Figure 3.1b). Pumpkins recovered by 28 DAT as only the highest rate of 1/4 showed injury (Figure 3.1b). Dose response model had a good fit at 14 DAT and pumpkins experience 5% injury in response to 51.17 ± 14.01 g ae ha^{-1} and 50% injury at 126.66 ± 10.90 g ae ha^{-1} (Table 3.2).

3.3.2 2019 SUMMER FIELD STUDY YIELD

No significant yield reduction was observed when ‘Orange Smoothie’ was treated at the vegetative stage (Figure 3.2a). Dose response model converged but with high standard error with no reasonable estimation (Table 3.1). Flowering stage ‘Orange Smoothie’ showed yield reduction at the two highest rate (Figure 3.2a). Due to no significant differences among rates, dose response model failed to converged (Table 3.2).

3.3.3 2019 SUMMER FIELD STUDY YIELD LOSS

Despite greater injury symptoms when sprayed at the vegetative stage, yield loss was greater when pumpkin was sprayed at the flowering stage (Figure 3.2b). When treated at vegetative stage, 2,4-D did not decrease yield; in fact, yield may have increased in response to all rates besides 1/500 (Figure 3.2b). Due to this dose response model converged but with high standard error with no reasonable estimation (Table 3.1). When treated at flowering stage, the top two rates of 1/4 and 1/10 caused approximately 30% yield loss and 1/50 and 1/500 rates caused approximately 10% loss (Figure 3.2b). Even though the dose response model were able to converge but dose also showed high standard error. The dose response model suggests a rate of 9.28 ± 19.88 g ae ha^{-1} caused 5% yield loss and a rate of 41.95 ± 54.16 g ae ha^{-1} caused 50% yield loss.

3.3.4 2020 SUMMER FIELD STUDY VISUAL INJURY

In 2020, injury symptoms after treatment at the vegetative stage peaked at 14 DAT, and rates of 1/4, 1/10 and 1/50 caused approximately 58%, 45%, and 10% injury, respectively (Figure 3.3a). As in 2019, injury symptoms declined over time after treatment during the vegetative stage from injury incurred during the vegetative stage (Figure 3.3a). Dose response model of vegetative stage at 14 DAT showed good model fitting. A rate of 10.98 ± 2.41 g ae ha^{-1} resulted in 5% injury and a rate of 45.28 ± 5.41 g ae ha^{-1} resulted in 50% yield loss (Table 3.3).

When pumpkins were treated at flowering stage, 14 DAT showed as rate increases, percentage injury increases with the highest injury of 30% caused by the rate of 1/4 (Figure 3.3b). Due to no significant differences in percentage injury among rates, dose response model showed high standard error with no reasonable suggestions (Table 3.4). Estimated rate by the dose response model on flowering stage 21 DAT showed a rate of 6.27 ± 6.82 g ae ha^{-1} caused 5% injury and the rate of 9.14 ± 3.37 g ae ha^{-1} caused 50% injury (Table 3.4).

3.3.5 2020 SUMMER FIELD STUDY YIELD

Both vegetative and flowering stage ‘Orange Smoothie’ showed yield reduction at the two highest rates (Figure 3.4a). However, other rate do not have significant differences compared with the control (Figure 3.4a). Dose response model for vegetative stage ‘Orange Smoothie’ converged but with no reasonable suggestions. Model suggests a rate of 32.98 ± 89.91 g ae ha^{-1} caused 5% yield reduction and rate of 71.11 ± 69.48 g ae ha^{-1} caused 50% yield reduction (Table 3.3). Flowering stage ‘Orange Smoothie’ showed high variation on yield (Figure 3.4b) which caused dose response model failed to converge (Table 3.4).

3.3.6 2020 SUMMER FIELD STUDY YIELD LOSS

Yield loss trend for the 2020 date showed the opposite results as pumpkins treated at the vegetative stage was more susceptible to 2,4-D yield loss compared with treated at the flowering stage (Figure 3.4b). Pumpkins treated at vegetative stage showed rate of 1/4 and 1/10 resulted in highest yield loss of 30% (Figure 3.4b). It was observed the rate of 1/50 resulted approximate 13% yield loss but the rate of 1/100 resulted relative 4% of yield increase (Figure 3.4b). The lowest rate of 2.1 g ae ha^{-1} had more yield loss compared with the rate of 10.7 and 21.3 g ae ha^{-1}

(Figure 3.4b). This contradict higher rate would cause higher yield loss and caused dose response model for vegetative stage with high standard error. Estimated yield loss for vegetative stage showed rate of 0.29 ± 1.89 g ae ha^{-1} caused 5% yield loss and rate of 22.05 ± 29.00 g ae ha^{-1} caused 50% yield loss (Table 3.3). For flowering stage, only the two highest rates of 1/4 and 1/10 caused yield loss which is approximately 5 to 10% yield loss (Figure 3.4b). Rate of 1/50 and rate of 1/500 both showed no yield loss but rate of 1/100 showed approximate 20% yield increase (Figure 3.4b). Due to the high variation and only two highest rate showed yield loss, the response model field to converge with no estimated value produced (Table 3.4).

3.4. DISCUSSION

3.4.1 VEGETATIVE STAGE PUMPKINS WERE MORE SUSCEPTIBLE TO 2,4-D AND PUMPKINS WERE GENERALLY LESS SUSCEPTIBLE TO 2,4-D INJURY

Results in 2019 suggested that ‘Orange Smoothie’ may be more sensitive to 2,4-D at the flowering stage. This was showed 14 DAT injury was higher in flowering than vegetative stage. Also in 2019, yield loss was observed in flowering but not vegetative pumpkins. However, the opposite results were observed in 2020 as ‘Orange Smoothie’ may be more sensitive to 2,4-D at the vegetative stage. In 2020, the 14 DAT injury was higher in vegetative stage than flowering stage. In addition, yield loss was higher at the vegetative stage and we observed huge yield reduction on the rate of 266.4 g ae ha^{-1} when compared with control. This corelated with several other cucurbits crops watermelon (*Citrullus lanatus*) (Culpepper et al. 2018), cucumber (*Cucumis sativus*) (Gilreath et al. 2001), and cantaloupe (*Cucumis melo var. cantalupo*) (Hand et al. 2020) that observed vegetative stage was more susceptible compared with flowering stage. Hemphill and Montgomery (1981) observed mild epinasty with 2.1 to 20.8 g ae ha^{-1} of 2,4-D dimethylamine salt when treated on cucumber at the first bloom stage but with yield not affected. Rate of 104 g ae ha^{-1} reduced yield by 35% while rate of 208 g ae ha^{-1} reduced yield by 72%. In our study, the highest rate of 266.4 g ae ha^{-1} caused about 30% yield loss which showed pumpkins were not as sensitive to 2,4-D. No significant differences from the dose response model estimation on yield loss in 2019 at both stage and 2020 flowering stage also proved pumpkins were not as susceptible to 2,4-D injury. There were research demonstrated rapid metabolism of 2,4-D in cucumber (Schroeder 1998) and this might be the reason of the tolerance of pumpkins to low rates of 2,4-D.

3.4.2 PUMPKINS RECOVER OVERTIME WITH ALL SUBLETHAL RATES

Trend of recovery were observed in both year for all sublethal rates at both vegetative and flowering stages. However, even with the recovery, pumpkins at the flowering stage in 2019 showed high yield loss at the three highest rates. This showed plant recovery can be deceptive and cannot be used to directly estimate yield loss. We observed some flower abortion when pumpkins were treated at the flowering stage with the 1/4 and 1/10 rates in both years; however, aborted flowers had less effect on yield in 2020. Culpepper et al. (2018) also observed the trend of recovery when treated with 1/75x of 2,4-D. Our study showed trend of recovery on pumpkins at both vegetative and flowerings stage at even higher rate of 1/50, 1/10 and 1/4x.

3.4.3 CUCURBITS PLANTS TOOK LONGER TO SHOW THE HERBICIDE INJURY PEAK

Pumpkins treated with a low dose of 2,4-D at the vegetative stage exhibited peak injury symptoms at 21 DAT in 2019 and 14 DAT in 2020. When treated at flowering stage, injury also peaked at approximately 14 DAT in both years. Lettuce as a leafy crop when treated with sublethal rates of 2,4-D showed injury peaked at 7 DAT (Roesler et al. 2020). This suggests it may take longer for cucurbit crops to show 2,4-D injury. Possible reason for this delayed in injury could likely due to the natural defense of pumpkins which slow down the translocation of the herbicides (DEXTER 1969). Gallup and Gustafson (1952) showed translocation of ^{14}C labelled 2,4-dichloro-5-iodophenoxyacetic acid were slower in corn, oats, and wheat. Same results was found by Fang and Butts (1954) which showed translocation of ^{14}C was very low in the apical region of corn and wheat. Although pumpkins is a broadleaf crop, it could alters 2,4-D which change the rate of translocation.

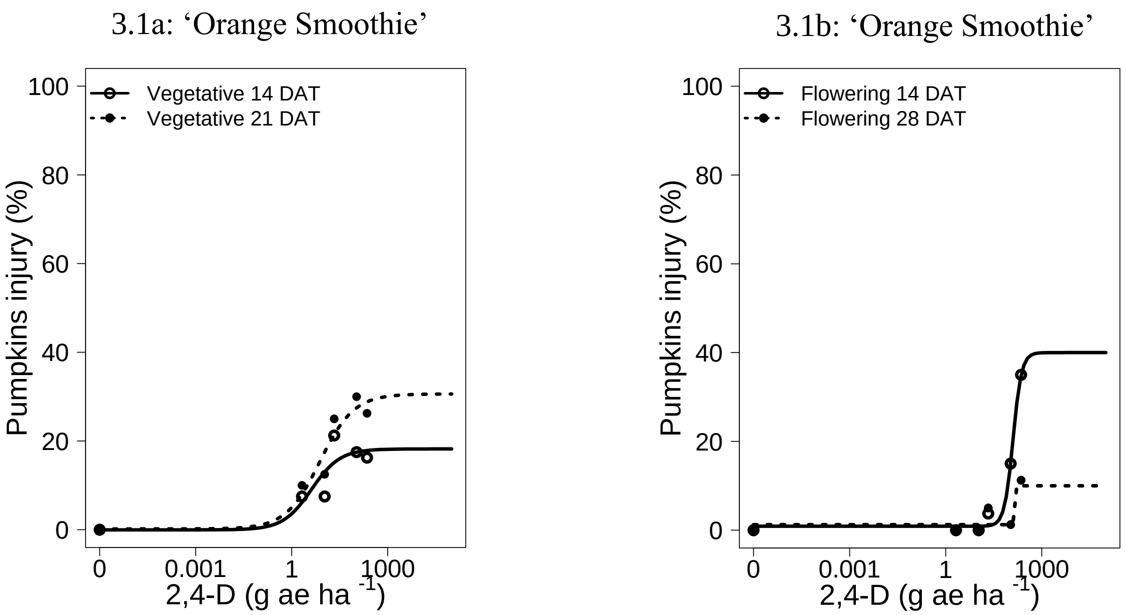
3.4.4 RECOMMENDATIONS ON MITIGATING HERBICIDE DRIFT

Newly developed crops with resistance to auxin herbicides have opens the window to control post emergence weeds that are resistant to glyphosate (Foster 2017). Research by Egan and Mortensen (2012) estimated planted auxin-resistant varieties will likely increase and this could increase the incidence for off-target movement. An early study conducted by Ozkan et al. (1997) in the wind tunnel with nine different shield design have showed effectively reduced particles drift by redirecting small droplets into the ground. Even the porous shield reduced drift by 13% whereas double-foil shield performed the best result of reducing drift by 59% (Ozkan et al. 1997). There is no single nozzle that perform the best in all conditions (Creech et al. 2015). It is always important to consult the pesticide label first for specific nozzle types, carrier rates, droplet size, and drift precautions. Select nozzles that create larger droplets which are not as easy to carry by winds.

3.5. CONCLUSION

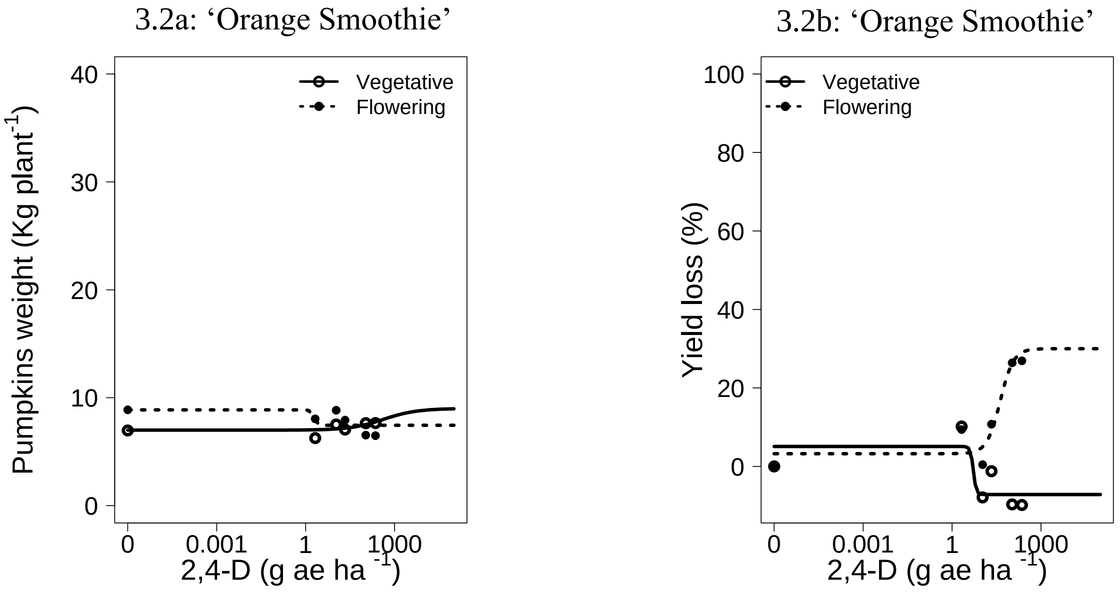
Results of this experiment confirm that pumpkin is not as highly susceptible to 2,4-D injury. Pumpkin sprayed at the vegetative stage prior to flowering was more sensitive to injury and yield loss compared to the flowering growth stage, except in 2019 when herbicide injury symptoms were less severe due to rainfall after application. Our dose response model failed to make prediction on the rate that caused yield reduction but rate of 106.6 (10% of the labeled rate) and 266.5 (25% of the labeled rate) g ae ha^{-1} caused the highest injury, even able to recover, the yield reduction is in the range of 30 to 40%. Peak injury on pumpkin occurred generally between 14 to 21 DAT, which is important to consider when scouting for injury after a suspected drift event.

Figures and Tables


3.1a: ‘Orange Smoothie’

3.1b: ‘Orange Smoothie’

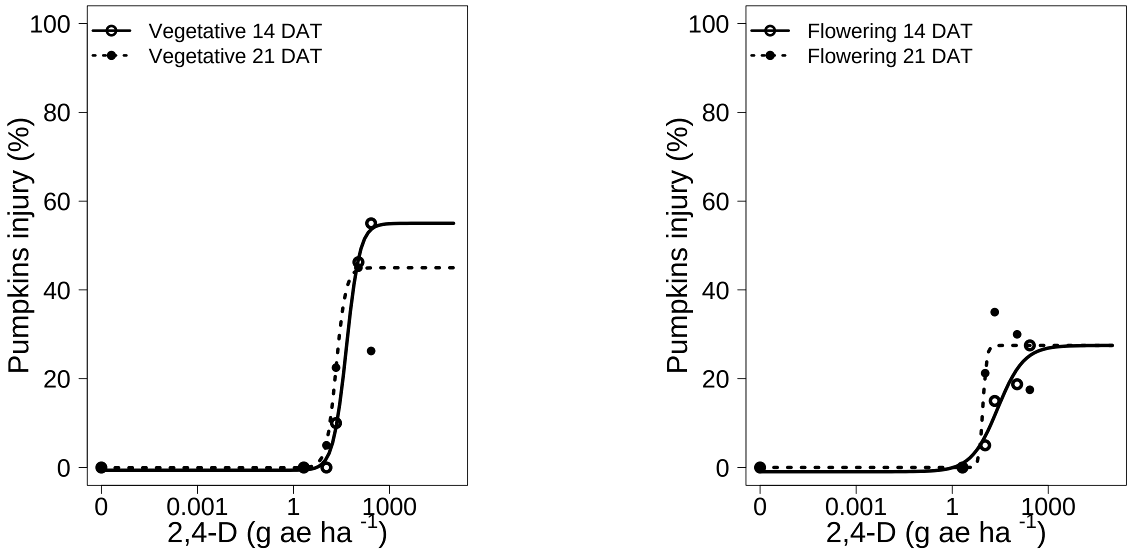
Figure 3.1: 2019 Field vegetative stage percentage injury dose-response comparison of 14 DAT and 21 DAT (3.1a) and flowering stage percentage injury comparison of 14 DAT and 28 DAT (3.1b) for ‘Orange Smoothie’ pumpkin treated with Enlist one® (2,4-D). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500.



3.2a: ‘Orange Smoothie’

3.2b: ‘Orange Smoothie’

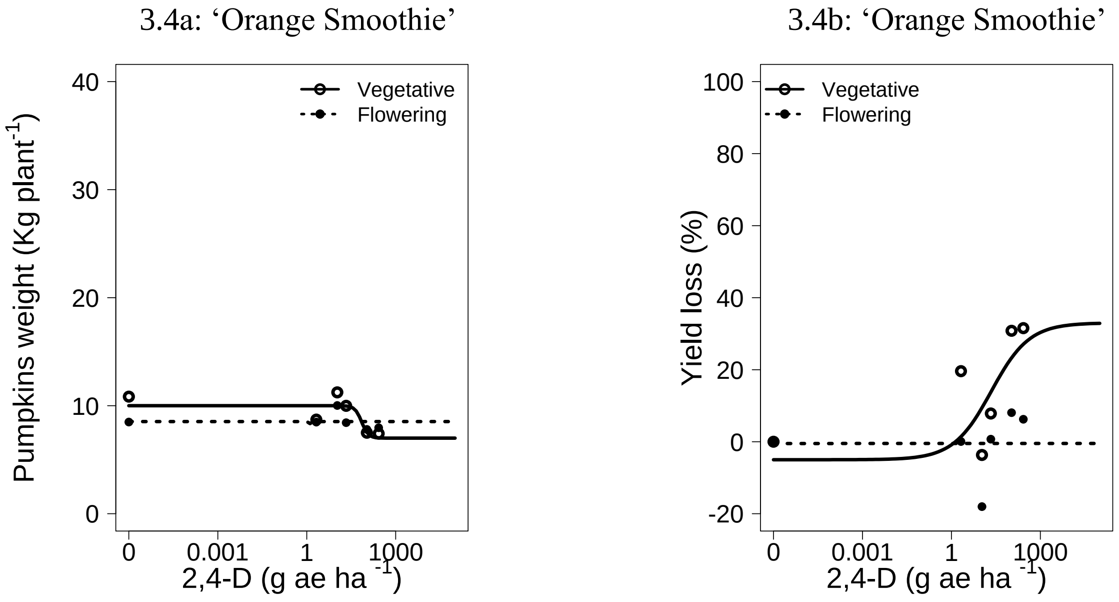
Figure 3.2: 2019 Field yield (3.2a) and percentage yield loss (3.2b) based on the yield from the whole growing season non-linear regression of Enlist one® (2,4-D) on ‘Orange Smoothie’ pumpkins when treated at vegetative and flowering stage. Each point from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500.



3.3a: ‘Orange Smoothie’

3.3b: ‘Orange Smoothie’

Figure 3.3: 2020 Field vegetative stage percentage injury dose-response comparison of 14 DAT and 21 DAT (3.3a) and flowering stage percentage injury comparison of 14 DAT and 21 DAT (3.3b) for ‘Orange Smoothie’ pumpkin treated with Enlist one® (2,4-D). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500.


3.4a: ‘Orange Smoothie’

3.4b: ‘Orange Smoothie’

Figure 3.4: 2020 Field yield (3.4a) and percentage yield loss (3.4b) based on the yield from the whole growing season non-linear regression of Enlist one® (2,4-D) on ‘Orange Smoothie’ pumpkins when treated at vegetative and flowering stage. Each point from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500.

Table 3.1: 2019 Field Study dose of Enlist one® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on ‘Orange Smoothie’ at Vegetative Stage

Measurement	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
Percentage Injury	**14 DAT**	0.21 (0.53)	0.02%	0.44 (0.93)	0.04%	1.01 (1.62)	0.09	4.16 (4.14)	0.4%
	21 DAT	0.21 (0.58)	0.02%	0.53 (1.11)	0.05%	1.41 (2.09)	0.13%	7.65 (6.76)	0.72%
Yield	**Vegetative**	9.33 (56.78)	0.88%	24.54 (104.76)	2.30%	70.13 (189.94)	6.58%	422.21 (1449.76)	39.61%
Percentage Yield loss	**Vegetative**	3.76 (42.73)	0.35%	4.08 (45.77)	0.38%	4.46 (49.90)	0.42%	5.20 (59.48)	0.49%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 3.2: 2019 Field Study dose of Enlist one® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on 'Orange Smoothie' at Flowering Stage

Measurement	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
Percentage Injury	**14 DAT**	51.17 (14.01)	4.8%	64.41 (13.65)	6.0%	82.67 (12.32)	7.8%	126.66 (10.90)	11.9%
	28 DAT	125.16 (2183.50)	11.7%	128.34 (2219.83)	12.0%	131.88 (2273.00)	12.4%	138.16 (2400.51)	13.0%
Yield	**Flowering**	NA	NA	NA	NA	NA	NA	NA	NA
Percentage Yield loss	**Flowering**	9.28 (19.88)	0.87%	13.61 (23.64)	1.28%	20.62 (28.81)	1.93%	41.95 (54.16)	3.94%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 3.3: 2020 Field Study dose of Enlist one® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on 'Orange Smoothie' at Vegetative Stage

Measurement	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
Percentage Injury	**14 DAT**	10.98 (2.41)	1.03%	15.74 (2.89)	1.48%	23.24 (3.49)	2.18%	45.28 (5.41)	4.25%
	21 DAT	7.66 (5.67)	0.72%	9.95 (5.46)	0.93%	13.20 (4.61)	1.24%	21.41 (3.54)	2.0%
Yield	**Vegetative**	32.98 (89.91)	3.09%	40.08 (90.75)	3.76%	49.52 (87.67)	4.65%	71.11 (69.48)	6.67%
Percentage Yield loss	**Vegetative**	0.29 (1.89)	0.03%	0.87 (4.49)	0.08%	2.86 (10.58)	0.27%	22.05 (29.00)	2.07%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 3.4: 2020 Field Study dose of Enlist one® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on ‘Orange Smoothie’ at Flowering Stage

Measurement	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
Percentage Injury	**14 DAT**	1.59 (3.02)	0.15%	3.24 (4.92)	0.30%	7.02 (8.00)	0.66%	26.30 (20.17)	2.47%
	21 DAT	6.27 (6.82)	0.59%	6.90 (6.22)	0.65%	7.65 (5.36)	0.72%	9.14 (3.37)	0.86%
Yield	**Flowering**	NA	NA	NA	NA	NA	NA	NA	NA
Percentage Yield loss	**Flowering**	NA	NA	NA	NA	NA	NA	NA	NA

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

REFERENCES

Creech CF, Henry RS, Fritz BK, Kruger GR (2015) Influence of Herbicide Active Ingredient, Nozzle Type, Orifice Size, Spray Pressure, and Carrier Volume Rate on Spray Droplet Size Characteristics. Weed Technology 29:298–310

Culpepper AS, Sosnoskie LM, Shugart J, Leifheit N, Curry M, Gray T (2018) Effects of Low-Dose Applications of 2,4-D and Dicamba on Watermelon. Weed Technology 32:267–272

Culpepper S (n.d.) Dicamba and 2,4-D Visual Sensitivity Scale for Georgia in 2018 – Laminated Handout | UGA Cotton News

Delbert D. Hemphill Jr, Montgomery ML (1981) Response of Vegetable Crops to Sublethal Application of 2,4-D. Weed Science 29:632–635

DEXTER AG (n.d.) Fate of 2,4-Dichlorophenoxyacetic Acid in Several Plant Species. Ph.D. United States -- Illinois: University of Illinois at Urbana-Champaign. 93 p

Egan JF, Barlow KM, Mortensen DA (2014) A Meta-Analysis on the Effects of 2,4-D and Dicamba Drift on Soybean and Cotton. Weed Science 62:193–206

Egan JF, Mortensen DA (2012) Quantifying vapor drift of dicamba herbicides applied to soybean. Environmental Toxicology and Chemistry 31:1023–1031

Fang SC, Butts JS (1954) Studies in Plant Metabolism. III. Absorption, Translocation and Metabolism of Radioactive 2,4-D in Corn and Wheat Plants. 123. Plant Physiol 29:56–60

Foster HC (2017) The effect of droplet size and sprayer type on physical drift. M.S. United States -- Mississippi: Mississippi State University. 45 p

Gallup AH, Gustafson FG (1952) ABSORPTION AND TRANSLOCATION OF RADIOACTIVE 2,4-DICHLORO-5-IODO131-PHENOXYACETIC ACID BY GREEN PLANTS 1. Plant Physiol 27:603–612

Gilreath JP, Chase CA, Locascio SJ (2001) Crop Injury from Sublethal Rates of Herbicide. II. Cucumber. HortScience 36:674–676

Grover R, Maybank J, Yoshida K (1972) Droplet and Vapor Drift from Butyl Ester and Dimethylamine Salt of 2,4-D. Weed Science 20:320–324

Hand LC, Vance JC, Randell TM, Shugart J, Gray T, Luo X, Culpepper AS (undefined/ed) Effects of low-dose applications of 2,4-D and dicamba on cucumber and cantaloupe. Weed Technology:1–6

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R Software Package for Dose-Response Studies: The Concept and Data Analysis. wete 21:840–848

Leon RG, Ferrell JA, Brecke BJ (2014) Impact of Exposure to 2,4-D and Dicamba on Peanut Injury and Yield. Weed Technology 28:465–470

Mohseni-Moghadam M, Wolfe S, Dami I, Doohan D (2015) Response of Wine Grape Cultivars to Simulated Drift Rates of 2,4-D, Dicamba, and Glyphosate, and 2,4-D or Dicamba Plus Glyphosate. wete 30:807–815

Ozkan H, Miralles A, Sinfort C, Zhu H, Fox R (1997) Shields to Reduce Spray Drift. Journal of Agricultural Engineering Research 67:311–322

Peterson GE (1967) The Discovery and Development of 2,4-D. Agricultural History 41:243–254

Peterson MA, McMaster SA, Riechers DE, Skelton J, Stahlman PW (2016) 2,4-D Past, Present, and Future: A Review. Weed Technology 30:303–345

Powles SB (2008) Evolved glyphosate-resistant weeds around the world: lessons to be learnt. Pest Management Science 64:360–365

Roesler GD, Jonck LCG, Silva RP, Jeronimo AV, Hirata ACS, Monquero PA (2020) Decontamination methods of tanks to spray 2,4-D and dicamba and the effects of these herbicides on citrus and vegetable species. Aust J Crop Sci:1302–1309

Schroeder J (1998) Cucumber (Cucumis sativus) Response to Selected Foliar- and Soil-Applied Sulfonylurea Herbicides. Weed Technology 12:595–601

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-Logistic Analysis of Herbicide Dose-Response Relationships. Weed Technology 9:218–227

CHAPTER 4, IMPACT OF MICRO-RATES OF DICAMBA ON PUMPKINS INJURY AND YIELD LOSS

The newly released herbicide tolerant crops Enlist^{TM} with Colex-D^{TM} and Xtend® technology with VaporGrip® increased weed control efficacy for glyphosate resistant weeds. However, both Enlist^{TM} and Xtendimax® are auxinic herbicides that tend to vaporize or cause particle drift with improper application. The unintended off-target herbicide drift could injure specialty crops that are highly susceptible. Pumpkins are a high value crop with known susceptibility to auxinic herbicides but most research has been limited to measurements of injury, not yield loss. With an increase in the adoption of herbicide tolerant crops, pumpkins are at higher risk of potential off-target injury. The objective of this study was to determine the effects of low dose simulated drift rates of dicamba on visual injury and crop yield loss in pumpkins at vegetative and flowering growth stages. In 2019 and 2020, pumpkins were treated with dicamba rates ranging from 1/500 to 1/4 of the labeled rate at each growth stage. Visual injury ratings were recorded every seven days and pumpkins were harvested and weighed fresh throughout the growing season. A simulated drift rate of 139.8 g ae ha^{-1} (1/4x) consistently reduced yield regardless of growth stage. In 2019 and 2020, pumpkins sprayed with the rate of 139.8 g ae ha^{-1} at the vegetative stage showed consistent yield reduction compared with all the other sublethal rates. Pumpkins sprayed at the flowering stage showed similar trends but only the rate of 139.8 g ae ha^{-1} reduced yield compared with the control. Results suggest pumpkins are most susceptible to dicamba damage during the vegetative stage of growth prior to flowering. The prevalence of dicamba applications in May and June throughout the U.S. Midwest correlates to the vegetative stage of pumpkin growth and represents a significant economic risk to specialty crop growers.

4.1. Introduction

Glyphosate is a non-selective herbicide that was introduced in the early 1970s that can control a wide spectrum of weeds. The high efficacy, cheaper price, and reduced need for tank-mixing has saved farmers time and labor inputs, which has increased its popularity. Several glyphosate tolerant (GT) crops have been developed including GT soybean introduced in 1996, GT cotton in 1997, and GT corn in 1998 (Givens et al. 2009). This technology opened a wider herbicide application window and allowed for controlling weeds without damaging the target crops. In 2014, farmers sprayed approximately 1 kg ha^{-1} on every hectare of U.S. cultivated cropland and about 0.53 kg ha^{-1} on all cropland worldwide (Benbrook 2016), which makes glyphosate one of the most commonly used herbicides. However, the repetitive use of glyphosate has selectively increased resistance in many weeds. To date, fifty-three glyphosate resistant weed species have been reported globally including twenty-six dicots and twenty-seven monocots (Heap, 2021). Compounding this problem is the fact that the development of herbicides with new active ingredient has slowed down due to the high research expenses with much stringent requirement for the toxicological and environmental regulations to ensure product and produce safety (Peters and Strek 2018). Dayan (2019) showed an increase cost to develop a single new active ingredient from $184 million in 2000 to almost $286 million in 2016. Because of this, major agriculture chemical companies started to invested money in developing herbicide tolerant crops using herbicide modes of action already available. Among the herbicides, 2,4-D and dicamba were the two auxinic herbicides successfully deployed for use with genetically engineered crop tolerance traits. Enlist^{TM} with Colex-D^{TM} by Dow AgroSciences LLC (Indianapolis, IN) is a2,4-D tolerant corn, soybean, and cotton system. Bayer Crop Science (St. Louis, MO) developed the dicamba tolerant Xtend® technology with VaporGrip®. This included

Roundup Ready 2 Xtend® soybeans with tolerance to dicamba and glyphosate and Xtendflex® soybeans and Xtendflex® cotton that were “triple-stacked” to include tolerance to dicamba, glyphosate, and glufosinate (Bayer Crop Science, St. Louis, MO). These technologies provided alternative tools and expanded windows to manage existing glyphosate resistant weeds (Behrens et al. 2007). This not only improved weed management but also saved money for farmers and increased crop yield (Duke 2015). A survey conducted in Nebraska for the adoption of dicamba-resistant (DR) soybean showed DR soybeans were planted on 20% of hectares in 2017 and the number increased to 50% of the hectares in 2018 (Werle et al. 2018). Xtendimax® with Vapor Grip® technology and Engenia® were formulized to dissociate the parent acid of dicamba which reduces the likelihood of vaporization (Hartzler 2017). Many independent researchers have verified these formulations do reduce the volatilization when compared with the old dicamba formulations but potential drift can still occur (Hartzler 2017). Data in 2018 showed approximately 2,700 cases reported by various state Department of Agriculture with approximately 3.6 million acres of dicamba-injured soybeans acres reported (Bradley, 2018). Roesler et al. (2020) showed dicamba can drift up to 152 m from the target application area and the yield loss reduction was noted in non-dicamba-resistant soybean at the R1 reproductive stage located 42.8 m from the application area. Increased adoption of DR soybeans and potential off-target drift raised concerns about off-target injury on sensitive specialty crops.

Research on watermelon using sublethal rates of dicamba (Clarity®, 560 g ae ha^{-1}) at three time intervals showed higher visual injury and reductions in vine growth when herbicide applications were made before flowering (Culpepper et al. 2018). The 1/75 and 1/250 rate reduced marketable fruit numbers 13 to 20% but only when plants were injured 20 days after planting while other rates did not showed significant of marketable fruit numbers (Culpepper et al. 2018). Total biomass of the marketable melons was 69, 89, and 103 kg plot $^{-1}$ when treated with the 1/75 rate dicamba applied at 20, 40, and 60 days after planting; 81, 103, and 105 kg plot $^{-1}$ of melons were produced treated with the rate of 1/250 dicamba (Culpepper et al. 2018). This showed the high sensitivity of watermelon to dicamba at the early growth stage. Another study on cucumbers treated with sublethal rates of dicamba reported greater injury when plants were treated at the vegetative growth stage (Hand et al. 2020). Total fruit number and relative weights were reduced by 19% when dicamba was applied at the 1/75 rate 26 days before harvest (Hand et al. 2020). A simulated drift study on eight species of flowering bedding plants showed foliar injury occurred on all species with 28 g ha^{-1} dicamba (Hatterman-Valenti and Mayland 2005). Pumpkin is a high value specialty crop in the U.S. with total value measured in 2019 at $180,190,000 (USDA NASS). The growing season for pumpkins in the Midwest U.S. is typically May through October, which overlaps with the growing season for corn and soybean and increases the likelihood of an economically damaging dicamba drift event.

Previously discussed studies on watermelon(*Citrullus lanatus*) (Culpepper et al. 2018), cucumber (*Cucumis sativus*) and cantaloup (*Cucumis melo var. cantalupo*) (Hand et al. 2020) also compared dicamba with 2,4-D and found dicamba injury and yield loss was greater. The objective of this study was to determine susceptibility of pumpkins to sublethal rates of dicamba at the vegetative and flowering growth stages.

4.2. Material and Methods

4.2.1 Field study experimental design and treatment applications

Field experiments were conducted in 2019 and 2020 using the pumpkin variety ‘Orange Smoothie’ (F1) (Johnny’s Selected Seeds Company; Winslow, ME). This pumpkin variety is known for its nice handle, medium size, and semi-bush growth habit with less vining (ideal of collecting visual injury and yield data).

In 2019 summer, pumpkin seeds were planted flats in the greenhouse on 13 May for seedling plugs. On 31 May, pumpkins seedings were transplanted to the field located at the UNL Havelock Research Farm in Lincoln, NE (40°51’ 7.008” N, 96°36’ 52.980” W). Before transplanting, the field was prepared with rotary tillage. In a single field pass of the bed-shaper/mulch-layer (RB448; Nolt’s Produce Supplies), raised beds were shaped, and drip irrigation line was laid beneath a white on black plastic film. Each plot was 3.7 meters long by 1.2 meters wide and five pumpkins were planted in a single row within each pot pumpkins were spaced 0.7 meters. The gap between plots was 2.4 meters to prevent herbicide movement between treatments. Treatments included two growth stage treatments (vegetative vs. flowering) and six sublethal rates of dicamba (Xtendimax® Bayer CropScience, St. Louis, MO) including 0 (control), 1/4, 1/10, 1/50, 1/100, and 1/500 of the label rate (560 g ae ha^{-1} dicamba diglycolamine salt). There were four replications of all of possible combinations of growth stage by rate treatments. Plants were fertigated two times during the growing season – once before and once after herbicide treatment using calcium nitrate fertilizer (15N–0P–0K, YaraLiva Tropicote 15–0–0; Yara North America, Tampa, FL). Fertilizer injected into the drip irrigation line to deliver 44.8 kg ha^{-1} N in each application.

In 2020, pumpkin was direct seeded on 15 May into a field at the UNL East Campus Research Farm (40° 50’ 10.890” N, 96° 39’ 45.162” W). Planting method was changed in 2020 to avoid transplant shock observed in 2019 that delayed crop growth and development. Before planting seed, the field was prepared with rotary tillage. All plots received an application of 112 kg ha^{-1} N with granular urea (46N-0P-0K; PRO-AP, Wawaka, IN) applied as preplant broadcast fertilizer and incorporated into the soil. Plot setup and dimensions and treatment structure were otherwise identical to 2019.

Herbicide was applied using a CO_2-pressurized tank sprayer with a two-nozzle boom and nozzles spaced 51 cm apart. The sprayer was calibrated to deliver 140 L ha^{-1} at 276 kPa through a TeeJet 8001E nozzle (TeeJet Technologies, Spraying systems Co., Wheaton, IL). Travel speed of the nozzle was based on the walking speed of approximate 4.8 km/hr. The vegetative stage treatment was applied on 20 June 2019 and 12 June 2020 before pumpkin plants had produced any flowers. The flowering stage treatments were applied on 11 July 2019 and 23 June 2020. At this stage, each pumpkin plant was presenting two or more flowers and had begun vining out. The later application in 2019 can be attributed to delayed growth due to transplant shock and less fertilizer N compared to 2020. The application for the vegetative stage was conducted in June to simulate when dicamba was typically apply to XtendFlex® Soybeans, XtendFlex® cotton, and dicamba tolerant corn as POST emergence weed control. Depending on planting date, region, and cultural practices, it is possible that pumpkin would reach the flowering stage during these dicamba application windows, which is why we compared the two growth stages.

Visual injury ratings were conducted every seven days until harvest. Visual injury ratings were based on the percentage scale of 0 (no injury) to 100 (death of the plant) relative to the nontreated control (Appendix 1). Visual injuries included chlorosis, leaf malformation, and epinasty. The rating protocol was adapted from Frans et al. (1986). In 2019, pumpkins were harvested on 2 September and 30 October. In 2020, pumpkins were harvested on 4 August, 18 August, and 3 September. Pumpkin yield at each harvest event were pooled for a season total and adjusted for stand density prior to analysis.

4.2.2 Statistical analysis

Yield data were based on fresh weight and percentage yield loss relative to the controls. Due to the non-linear nature of plant response to sublethal rates of herbicide, a four-parameter log-logistic regression model was used to analyze the relationship between sublethal rates of 2,4-D with visual injury, average yield and percentage yield loss utilizing approach described in Knezevic et al. (2007).

The four parameter model was defined by the equation

$Y = c + \{ d - c / 1 + \exp [b(\log x - \log e)]\}$

where c is lower limit, d is upper limit, b is slope and e is the ED 50 (dose giving 50% response) (Knezevic et al. 2007, Seefeldt et al. 1995). The regression analyses helped estimate rates of 2,4-D, or effective doses (ED values), that would cause different levels of injury or yield loss. Regression analyses were conducted using the *drc* package in R version 3.4.1 (R Core Team, 2019). Injury ratings within time intervals and applications stage treatments were averaged across the four replications and fit to the response across the six sublethal application rates, as described in Knezevic et al. (2007).

Percentage yield loss was calculated using the equation:

$Y= [(C – T) / C]\ 100$

where Y represents the percentage yield loss compared to the nontreated control plot in the corresponding replicate block, C represents the biomass of the nontreated control plot, and T represents the biomass of the treated plot.

4.3. Results

4.3.1 2019 Summer field study visual injury

The symptoms of dicamba injury on pumpkins included stunting, leaf cupping, vine twisting, epinasty, and bubbled leaf texture. In 2019, pumpkins treated at the vegetative stage showed injury only at the highest rate of 1/4 (Figure 4.1a). Observed injury progressed from 40% at 14 DAT to a peak of 65% at 21 DAT (Figure 4.1a). Because only one sublethal rate showed injury, dose response model fit was poor and standard errors of parameter estimates were high (Table 4.1). Pumpkins treated at the flowering stage exhibited peak injury symptoms by 14 DAT. The 1/4 rate caused 50% injury and 1/10 caused 32% injury (Figure 4.1b). Pumpkins recovered over time, but the two highest rates were still visibly injured at 42 DAT (Figure 4.1b). The dose response model suggests that 42 DAT pumpkins treated at the flowering stage experience 5% injury in response to 27.20 ± 11.32 g ae ha^{-1} and 50% injury in response to 57.72 ± 3.52 g ae ha^{-1} (Table 4.2).

4.3.2 2019 Summer field study yield

Vegetative stage ‘Orange Smoothie’ showed yield reduction as sublethal rate increase (Figure 4.2a). Dose response model had good model fitting and suggest a rate of 46.74 ± 30.03 g ae ha^{-1} resulted 5% yield reduction and rate of 182.85 ± 43.35 resulted 50% yield reduction (Table 4.1). Flowering stage ‘Orange Smoothie’ showed high variability in yield (Figure 4.2a) which resulted dose response model produced unreasonable estimation with high standard error (Table 4.2).

4.3.3 2019 Summer field study yield loss

Despite greater injury symptoms when sprayed at the vegetative stage, yield loss was greater when pumpkin was sprayed at the flowering stage (Figure 4.2b). At the flowering stage, the rate of 1/4 and 1/10 caused 40% and 25% yield loss, respectively. Comparing with the vegetative stage, the rate of 1/4 caused 30% yield loss and the rate of 1/10 caused 2% yield loss. Either of the growth stages due to variability within rates and limited responses beyond the two highest rates resulted dose response model produced unreasonable estimation with high standard error. The dose response model for yield loss at vegetative stage showed a rate of 45.16 ± 43.86 g ae ha^{-1} resulted 5% yield loss and rate of 65.11 ± 45.19 g ae ha^{-1} resulted 50% yield loss (Table 4.1). At flowering stage, dose response model estimated rate of 19.39 ± 34.57 g ae ha^{-1} resulted 5% yield loss and rate of 47.75 ± 18.04 resulted 50% yield loss (Table 4.2).

4.3.4 2020 Summer field study visual injury

In 2020, injury symptoms on vegetative stage pumpkins stabled at 14 DAT. Pumpkins treated with the highest rate of 1/4 did not recover by season end. Injury progressed from 80% at 14 DAT to 100% (mortality) at 45 DAT (Figure 4.3a). The rate of 1/10 showed slight recovery from 65% injury at 14 DAT to 50% injury by 45 DAT (Figure 4.3a). The dose response model for vegetative pumpkins suggests that a rate of 15.51 ± 5.10 g ae ha^{-1} results in 5% pumpkin injury and 35.58 ± 4.17 g ae ha^{-1} results in 50% injury by 14 DAT (Table 4.3). When treated at the flowering stage, injury from the 1/4 rate peaked at 21 DAT with approximately 75% damage (Figure 4.3b). The dose response model at 14 DAT for flowering pumpkin showed a rate of 43.97 ± 19.63 g ae ha^{-1} results in 20% injury and a rate of 56.90 ± 3.56 g ae ha^{-1} results in 50%

injury (Table 4.4). The estimated dose required for 50% injury at the flowering stage was higher than at the vegetative stage.

4.3.5 2020 Summer field study yield

A significant yield reduction was observed when ‘Orange Smoothie’ was treated at the vegetative stage (Figure 4.4a). Dose response model had good model fitting and suggest a rate of 22.29 ± 13.83 g ae ha^{-1} resulted 5% yield reduction and rate of 70.48 ± 13.82 g ae ha^{-1} resulted 50% yield reduction (Table 4.3). High variation in yield was observed when ‘Orange Smoothie’ was treated at the flowering stage (Figure 4.4a). This resulted dose response model produce unrealistic estimation (Table 4.4).

4.3.6 2020 Summer field study yield loss

The highest simulated drift rate of 1/4, with 95% visible injury, was able to produce some yield but much it was small and deformed. The 1/4 rate showed approximate 85% yield loss compared with the control. From the comparision, ‘Orange Smoothie’ showed high susceptibility at the vegetative stage compared with the flowering stage (Figure 4.4b). At the vegetative stage, the rate of 1/10 reduced yield by 35% (Figure 4.4b). Dose response model for vegetative stage showed rate of 32.76 ± 41.38 g ae ha^{-1} caused 5% yield loss and rate of 59.09 ± 9.80 g ae ha^{-1} caused 50% yield loss (Table 4.3). Due to the high variability in yield loss and rate of 1/10, 1/50, and 1/100 showed yield increase, dose response model did not produce realistic estimation with high standard error (Table 4.4).

4.4. Discussion

4.4.1 Rate of 1/4 consistently showed yield reduction at both year both growth stage

Although pumpkins were able to recover somewhat from peak injury symptoms, yield loss was typically observed at the two highest rates of 1/4 and 1/10. Rate of 1/4 despite of years and stage of treatment consistently reduced yield compared with control. Other study on similar cucurbit crops like watermelon (*Citrullus lanatus*) (Culpepper et al. 2018), cucumber (*Cucumis sativus*) and cantaloupe (*Cucumis melo var. cantalupo*) (Hand et al.2020) also found that plants at the flowering stage were more tolerant to dicamba compared with the vegetative stage. All pumpkins treated with the 1/4 rate in both years, regardless of growth stage, were more likely to die and rarely produced marketable fruit. The symptoms of the injury were observed mainly in the new growing region which made plants unable to recover. This is because dicamba translocate to newly formed meristematic tissues (Senseman 2007). Similar effects were observed in soybean new growth leaves (Jones et al. 2019) and cotton (Marple et al. 2008).

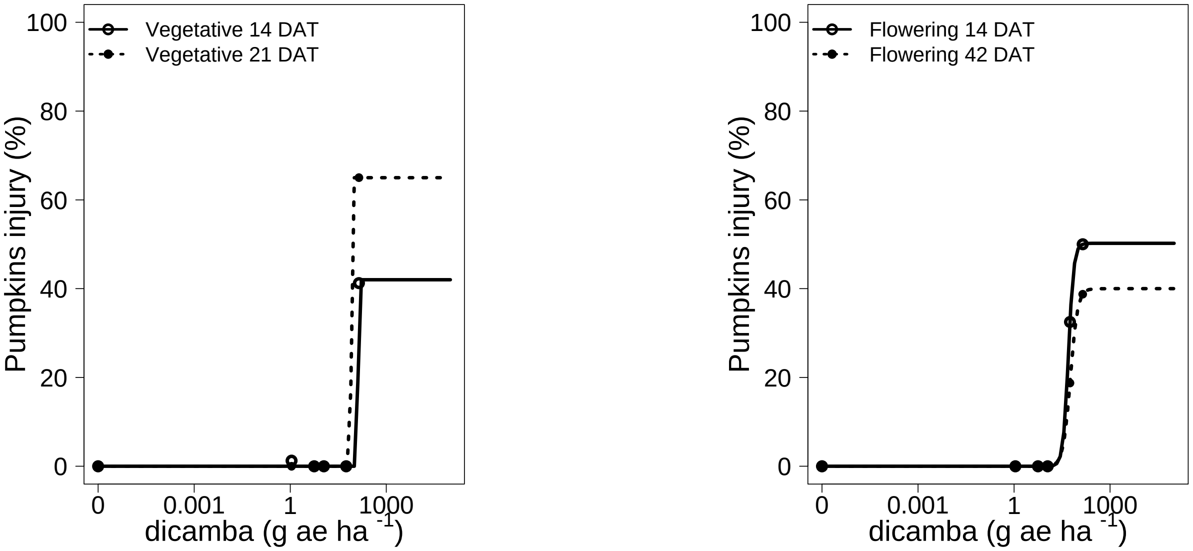
4.4.2 Cucurbits plants took longer to show the herbicide injury peak

In our study, pumpkins treated with low doses of dicamba exhibited peak injury symptoms at 14 to 21 DAT. Only the highest rate of 1/4 in 2020 at the vegetative stage showed injury worsened over time. This suggests it might take longer for cucurbit crops to show dicamba injury. Herbicide selectivity were previously described on the different ability of plant species to metabolically detoxify herbicide (Cole 1994). Especially higher plants species have the innate versatile system to protect them from the potentially phytotoxic actions of xenobiotics (Kreuz et al. 1996). We suspect pumpkins could slow down the translocation of the herbicides (DEXTER 1969).

4.5 Conclusion

In conclusion, pumpkins were not as susceptible to dicamba compared to 2,4-D evaluated in chapter 3. In both years, regardless of growth stages, only the rate of 139.8 and 55.9 g ae ha^{-1} showed injuries and yield loss. Pumpkins at the vegetative stage showed higher susceptibility with more severe injury symptoms. At the vegetative stage, the highest rate of 139.8 g ae ha^{-1} showed stunted growth and pumpkins failed to vine out and produce quality fruits. When pumpkins were at the flowering stage, very similar stunted growth was observed but less severe as pumpkins were able to recover with less yield reductions. Pumpkins farmers should be more cautious of the potential dicamba drift especially at the early growth season. Our research as well as several other research on cucurbits demonstrated the high injury and yield reduction when plant were at the early growth stage.

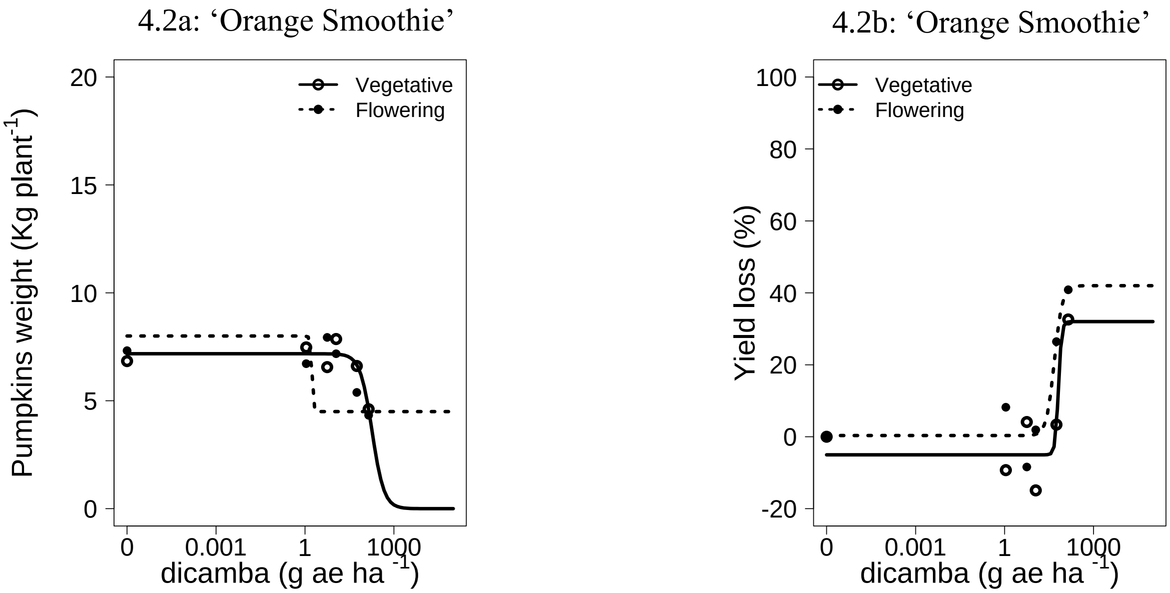
Figures and Tables



4.1a: ‘Orange Smoothie’

4.1b: ‘Orange Smoothie’

Figure 4.1: 2019 Field vegetative stage percentage injury dose-response comparison of 14 DAT and 21 DAT (4.1a) and flowering stage percentage injury comparison of 14 DAT and 42 DAT (4.1b) for ‘Orange Smoothie’ pumpkin treated with XtendiMax® (dicamba). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500.



4.2a: ‘Orange Smoothie’

4.2b: ‘Orange Smoothie’

Figure 4.2: 2019 Field yield (4.2a) and percentage yield loss (4.2b) based on the yield from the whole growing season non-linear regression of XtendiMax® (dicamba) on ‘Orange Smoothie’ pumpkins when treated at vegetative and flowering stage. Each point from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500.

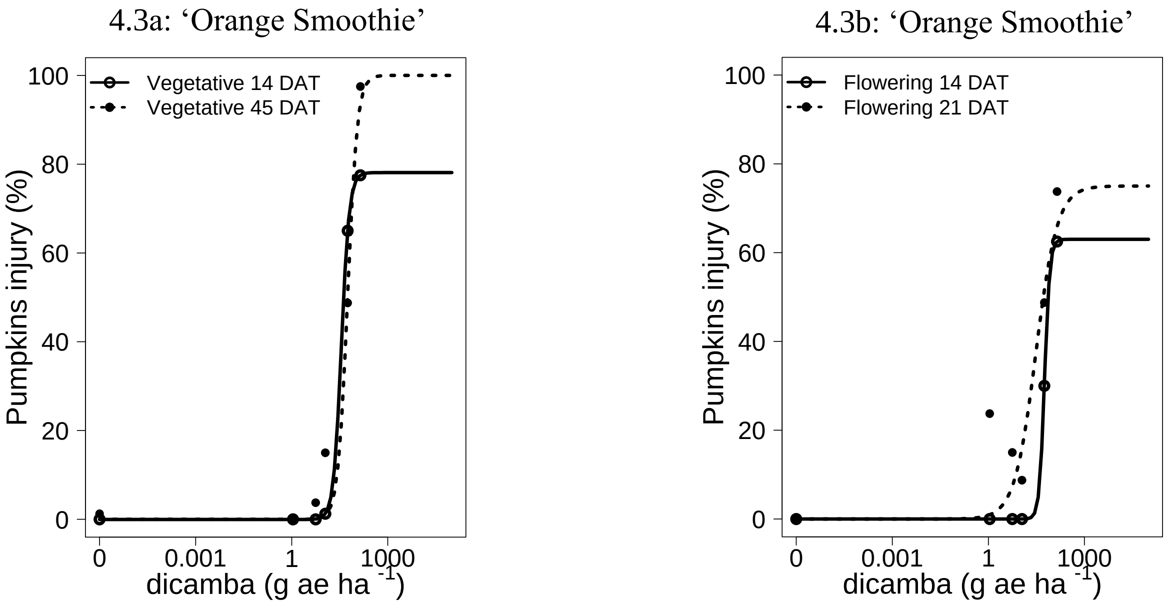


Figure 4.3: 2020 Field vegetative stage percentage injury dose-response comparison of 14 DAT and 45 DAT (4.3a) and flowering stage percentage injury comparison of 14 DAT and 21 DAT (4.3b) for ‘Orange Smoothie’ pumpkin treated with XtendiMax® (dicamba). Each dot from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500.

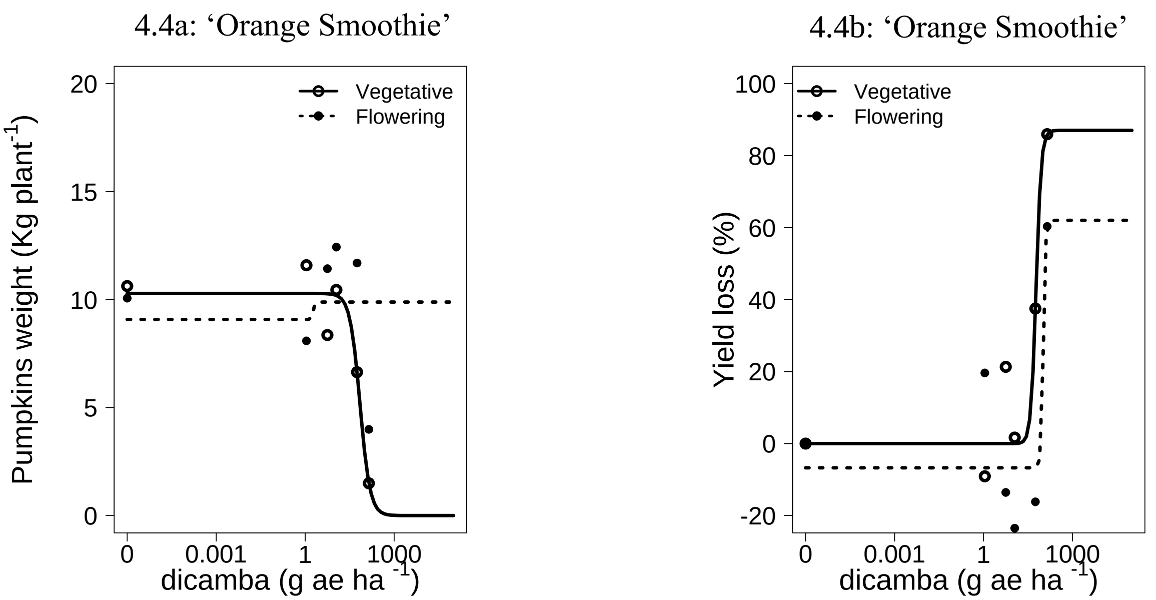


Figure 4.4: 2020 Field yield (4.4a) and percentage yield loss (4.4b) based on the yield from the whole growing season non-linear regression of XtendiMax® (dicamba) on 'Orange Smoothie' pumpkins when treated at vegetative and flowering stage. Each point from left to right represents the fraction rate of the labeled rate, 0 (control); 1/4; 1/10; 1/50; 1/100; 1/500.

Table 4.1: 2019 Field Study dose of Xtendimax® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on 'Orange Smoothie' at Vegetative Stage 14 DAT, 21 DAT, and 49 DAT

Measurement	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
Percentage Injury	**14 DAT**	122.67 (226.02)	21.91%	124.40 (204.49)	22.21%	126.31 (180.39)	22.56%	129.65 (137.37)	23.15%
	21 DAT	73.69 (63185.60)	13.16%	75.14 (69612.94)	13.42%	76.75 (77025.69)	13.71%	79.58 (90699.42)	14.21%
Yield	**Vegetative**	46.74 (30.03)	8.35%	66.07 (29.67)	11.80%	96.20 (24.60)	17.18%	182.85 (43.35)	32.65%
Percentage Yield loss	**Vegetative**	45.16 (43.86)	8.06%	49.55 (28.11)	8.85%	54.81 (10.31)	9.79%	65.11 (45.19)	11.63%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 4.2: 2019 Field Study dose of Xtendimax ® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on 'Orange Smoothie' at Flowering Stage 14 DAT, 42 DAT, and 56 DAT

Measurement	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
Percentage Injury	**14 DAT**	28.46 (95.86)	5.08%	32.81 (86.84)	5.86%	38.27 (71.37)	6.83%	49.81 (26.27)	8.89%
	42 DAT	27.20 (11.32)	4.86%	32.93 (10.22)	5.88%	40.51 (7.99)	7.23%	57.72 (3.52)	10.30%
Yield	**Flowering**	1.36 (338.93)	0.24%	1.42 (686.86)	0.25%	1.50 (1759.74)	0.27%	1.72 (8427.09)	0.31%
Percentage Yield loss	**Flowering**	19.39 (34.57)	2.39%	24.37 (34.41)	4.35%	31.24 (31.65)	5.58%	47.75 (18.04)	8.53%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 4.3: 2020 Field Study dose of Xtendimax ® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on ‘Orange Smoothie’ at Vegetative Stage

Measurement	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
Percentage Injury	**14 DAT**	15.51 (5.10)	2.77%	19.15 (5.24)	3.42%	24.07 (5.16)	4.30%	35.58 (4.17)	6.35%
	45 DAT	20.10 (19.40)	3.59%	26.03 (19.45)	4.65%	34.46 (17.63)	6.15%	55.68 (7.29)	9.94%
Yield	**Vegetative**	22.29 (13.83)	3.98%	29.85 (14.40)	5.33%	40.99 (14.01)	7.32%	70.48 (13.82)	12.59%
Percentage Yield loss	**Vegetative**	32.76 (41.38)	5.85%	38.05 (34.80)	6.79%	44.76 (24.13)	7.99%	59.09 (9.80)	10.55%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

Table 4.4: 2020 Field Study dose of Xtendimax ® that resulted in 5%, 10%, 20%, and 50% injury and yield loss on 'Orange Smoothie' at Flowering Stage

Measurement	**Observation Time**	**ED 5 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 10 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 20 (SE) g ae ha^{-1}**	**Percent of Label Rate**	**ED 50 (SE) g ae ha^{-1}**	**Percent of Label Rate**
Percentage Injury	**14 DAT**	32.90 (32.16)	5.88%	37.81 (27.32)	6.75%	43.97 (19.63)	7.85%	56.90 (3.56)	10.16%
	21 DAT	3.20 (3.07)	0.57%	5.68 (4.33)	1.01%	10.57 (5.90)	1.89%	30.59 (8.72)	5.46%
Yield	**Flowering**	1.58 (1.29)	0.28%	1.64 (1.36)	0.29%	1.72 (1.61)	0.31%	1.89 (3.15)	0.34%
Percentage Yield loss	**Flowering**	79.94 (130.86)	14.28%	85.22 (126.18)	15.22%	91.34 (120.80)	16.31%	102.85 (112.54)	18.37%

ED is effective dosage value which is the same as percentage. For example, ED(10) is 10%
SE was generated from the four parameter model by calculating the data of 6 replications

References

Behrens MR, Mutlu N, Chakraborty S, Dumitru R, Jiang WZ, LaVallee BJ, Herman PL, Clemente TE, Weeks DP (2007) Dicamba Resistance: Enlarging and Preserving Biotechnology-Based Weed Management Strategies. Science 316:1185–1188

Benbrook C (2016) Trends in glyphosate herbicide use in the United States and globally. Environmental Sciences Europe 28

Cole DJ (1994) Detoxification and activation of agrochemicals in plants. Pesticide Science 42:209–222

Culpepper AS, Sosnoskie LM, Shugart J, Leifheit N, Curry M, Gray T (2018) Effects of Low-Dose Applications of 2,4-D and Dicamba on Watermelon. Weed Technology 32:267–272

Dayan FE (2019) Current Status and Future Prospects in Herbicide Discovery. Plants 8:341

DEXTER AG (n.d.) Fate of 2,4-Dichlorophenoxyacetic Acid in Several Plant Species. Ph.D. United States -- Illinois: University of Illinois at Urbana-Champaign. 93 p

Dicamba injury mostly confined to specialty crops, ornamentals and trees so Far (2018) . Corn and Soybean Digest

Duke SO (2015) Perspectives on transgenic, herbicide-resistant crops in the United States almost 20 years after introduction. Pest Management Science 71:652–657

Givens WA, Shaw DR, Johnson WG, Weller SC, Young BG, Wilson RG, Owen MDK, Jordan D (2009) A Grower Survey of Herbicide Use Patterns in Glyphosate-Resistant Cropping Systems. wete 23:156–161

Hand LC, Vance JC, Randell TM, Shugart J, Gray T, Luo X, Culpepper AS (undefined/ed) Effects of low-dose applications of 2,4-D and dicamba on cucumber and cantaloupe. Weed Technology:1–6

Hartzler B (2017) Dicamba: Past, present, and future. Proceedings of the Integrated Crop Management Conference

Hatterman-Valenti H, Mayland P (2005) Annual Flower Injury from Sublethal Rates of Dicamba, 2,4-D, and Premixed 2,4-D + Mecoprop + Dicamba. HortScience 40:680–684

Jones GT, Norsworthy JK, Barber T (2019) Off-Target Movement of Diglycolamine Dicamba to Non-dicamba Soybean Using Practices to Minimize Primary Drift. Weed Technol 33:24–40

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R Software Package for Dose-Response Studies: The Concept and Data Analysis. wete 21:840–848

Kreuz K, Tommasini R, Martinoia E (1996) Old Enzymes for a New Job (Herbicide Detoxification in Plants). Plant Physiol 111:349–353

Marple ME, Al-Khatib K, Peterson DE (2008) Cotton Injury and Yield as Affected by Simulated Drift of 2,4-D and Dicamba. Weed Technology 22:609–614

Peters B, Strek HJ (2018) Herbicide discovery in light of rapidly spreading resistance and ever-increasing regulatory hurdles. Pest Management Science 74:2211–2215

Roesler GD, Jonck LCG, Silva RP, Jeronimo AV, Hirata ACS, Monquero PA (2020) Decontamination methods of tanks to spray 2,4-D and dicamba and the effects of these herbicides on citrus and vegetable species. Aust J Crop Sci:1302–1309

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-Logistic Analysis of Herbicide Dose-Response Relationships. Weed Technology 9:218–227

Werle R, Oliveira MC, Jhala AJ, Proctor CA, Rees J, Klein R (2018) Survey of Nebraska Farmers' Adoption of Dicamba-Resistant Soybean Technology and Dicamba Off-Target Movement. Weed Technology 32:754. 754

Appendix 1

ECW/CWSS Ratings (%)	Phytotoxicity Ratings
0	No injury evident
2	Very slight, hardly noticeable ("I think I see injury")
4	Negligible: discoloration, distortion and/or stunting barely seen
6	
8	Slight: discoloration, distortion and/or stunting clearly seen
10	Injury is noticeable, but would be considered "just acceptable"
15	Moderate damage: moderate injury, recovery is expected
20	
25	Substantial damage: much discoloration, distortion, stunting. Some damage irreversible
30	
40	Majority of plants damaged: some plants (<40%) killed; substantial necrosis and distortion. Biomass reduced by 40%
50	Nearly all plants damaged: most irreversibly, 40-50% killed
70	Severe: 50-60% killed
80	Very Severe: most plants killed (60-80%)
90	Remaining Live plants (<20%); remainder have much injury
100	Complete loss of plants and/or crop yield