Absorption and translocation of dicamba in dicamba-tolerant wild tomato

Rouzbeh Zangoueinejad, Mohammad Taghi Alebrahim, and Te-Ming Tseng

Abstract: Herbicide tolerance is commonly associated with reduced absorption and translocation of the herbicide; we hypothesized that the mechanism of dicamba tolerance in wild tomato (Solanum lycopersicum L.) accessions is due to these characteristics. The absorption and translocation of dicamba were investigated at a drift rate of 2.8 g a.e. ha\(^{-1}\) in three predetermined dicamba-tolerant (DT) wild accessions (TOM199, TOM198, and TOM300) and compared with two dicamba-susceptible (DS) commercial tomato cultivars [Money Maker (MM) and Better Boy (BB)]. Dicamba was quantified in three different parts of the tomato plant: two upper leaves, two lower leaves, and the roots at 1, 3, and 7 d after treatment. Both MM and BB absorbed more dicamba than all the three DT accessions. The overall translocation pattern of dicamba was similar between DS cultivars and DT accessions, thus suggesting that tolerance to dicamba in wild accessions may not be associated with reduced translocation but instead with reduced uptake of the herbicide. Additionally, reduced dicamba absorption in DT accessions may be attributed to their leaf characteristics, such as the presence of narrower leaves (3.42 leaf length/width ratio) and higher trichome density (20 no. mm\(^{-2}\)) in DT accessions, than compared with DS cultivars (1.92 leaf length/width ratio and 8 no. mm\(^{-2}\) trichome density).

Key words: auxin herbicide, wild tomato, herbicide tolerance, herbicide translocation, herbicide absorption.

Introduction

The mode of action of auxin herbicides is different in dicotyledonous and monocotyledonous plant species (Grossmann and Scheltrup 1997; Grossmann 2003). In susceptible (S) dicotyledonous plant species, numerous molecular mechanisms are influenced by the high...
concentration of auxin herbicides, resulting in uncontrolled growth (Grossman 2003, 2009). Abnormal growth signs such as epinasty, abnormal stem tip growth, and leaf stunting occur (Sterling and Jochem 1995), followed by inhibition of plant growth (Grossmann and Scheltrup 1997; Grossmann 2003). Finally, chlorosis and necrosis progress, leading to plant death (Grossmann and Scheltrup 1997; Grossmann 2003, 2009). Plants that are either resistant (R) or tolerant to auxin herbicide, however, often do not exhibit any symptoms (Sterling and Jochem 1995).

Tomato (Solanum lycopersicum L.) plants are highly sensitive to auxinic herbicides (Jordan and Romanowski 1974; Hemphill and Montgomery 1981; Fagliari et al. 2005). Hemphill and Montgomery (1981) investigated sublethal doses of 2,4-D to determine its effects on yield and quality of tomato and root crops, and they observed distorted fruit shape and elongated roots in tomato and radish (Raphanus sativus L.), respectively. A 92% and 93% reduction in tomato fruit number and yield, respectively, was caused by 2,4-D applied at 13.44 g a.e. ha$^{-1}$ (0.05X) (Fagliari et al. 2005). To understand the mechanism of resistance to dicamba, the pattern of herbicide absorption was studied in R and S populations of common lambsquarters (Chenopodium album L.); however, no difference in absorption by the two populations was identified (Ghanizadeha et al. 2018). Similarly, Penuik et al. (1992) observed no difference in dicamba uptake, translocation, and metabolism between the R and S biotypes of charlock mustard (Sinapis arvensis L.), and they concluded that the primary basis of auxin resistance in R biotype was due to differential sensitivity at the target sites of action. Several lines of cucumber (Cucumis sativus L.) were studied to investigate their differential susceptibility against chloramphen, and it was concluded that the R and S lines showed differences in herbicide uptake and translocation pattern (Miller et al. 1973). A study to identify dicamba-resistance mechanisms in an inbred dicamba-resistant kochia [Kochia scoparia (L.) Schrad.] line found no difference in dicamba absorption and metabolism when compared with an S line (Pettinga et al. 2018); however, a significantly higher translocation of dicamba was observed in the S (inbred dicamba-resistant kochia) than in the R (dicamba-resistant kochia) population.

In the postemergence application of herbicides, leaves are the primary parts exposed to herbicides (Wang et al. 2015; Zimdahl 2018). Additionally, leaf area has a direct correlation with herbicide absorption (Hess and Falk 1990; Knoche 1994), where an increase in leaf area increases the amount of herbicide received by the plant (Liu et al. 1996). Broad-leaved plant species with flat, large leaves oriented parallel to the ground have a greater tendency of being exposed to herbicide droplets and can retain spray droplets better than grass leaves (Hess and Falk 1990; Knoche 1994; Wang et al. 2015; Zimdahl 2018). In addition to the leaf area, the presence and density of trichomes on the leaf surface play an essential role in the absorption of herbicides into the leaf (Hess and Falk 1990; Wang and Liu 2007). Trichomes have several functions such as leaf cooling, defense against herbivores, reflecting sunlight, reducing transpiration, and affecting leaf wettability (Gutschick 1999; Haworth and McElwain 2008; Wang et al. 2015); however, in relation to herbicide application, the most critical role of trichome is in reducing leaf wettability (the affinity for water on leaf surfaces) (Brewer et al. 1991; Wang et al. 2015). It is reported that trichomes suspend water spray droplets on leaf surfaces (Xu et al. 2011), and a positive correlation was found between increasing trichome density on the leaf surface of Brassica juncea (L.) Czern. and reduction in herbicide efficacy (Huangfu et al. 2009). Thus, investigating these leaf characteristics may help determine the basis behind herbicide absorption in plants.

From our previous field and greenhouse studies (Zangoueinejad et al. 2019), it was found that TOM199, TOM198, and TOM300 as wild tomato accessions are tolerant in their response to dicamba application at the drift rate (2.8 g a.e. ha$^{-1}$). We hypothesized that (i) tolerance to dicamba is associated with differential absorption and (or) translocation of the herbicide in dicamba-tolerant (DT) phenotypes and (ii) lower leaf length/width ratio and lower trichome number in leaves are associated with increased dicamba absorption. The goal of this study was to measure the quantity of absorbed dicamba and monitor dicamba distribution in dicamba-susceptible (DS) tomato cultivars (as susceptible plants) and DT accessions (as tolerant plants), over time. Additionally, the effects of leaf length/width ratio and trichome density on dicamba absorption were determined.

**Materials and Methods**

**Greenhouse study**

A greenhouse assay was conducted to study the absorption and distribution of dicamba in three DT wild tomato accessions (TOM199, TOM198, and TOM300) and two DS tomato cultivars [Money Maker (MM) and Better Boy (BB)] at the R.R. Foil Plant Science Research Center, Mississippi State, MS, USA. The average greenhouse temperature, photoperiod, and light quantity on top of the tomato canopy during both experiments conducted in spring 2018 was 24–26 °C/19–20 °C (day/night), 11.5–13.5 (h d$^{-1}$), and 120 μmol s$^{-1}$ m$^{-2}$, respectively.

Tomato seeds were planted in 72-cell trays (size of each tray: 50.8 cm × 25.4 cm) filled with potting media (SunGro® Horticulture Sunshine Mix No. 2 Basic, QC, Canada). Each experimental unit consisted of 10 plants. Plants were irrigated once a day to field capacity, and irrigation was avoided a day before herbicide application, after which plants were subirrigated without disturbing the foliage. Fertigation was performed twice a week using a 20:20:20 (nitrogen:phosphorous:...
potassium) formulated fertilizer (Scotts Miracle-Gro Company, Marysville, OH, USA) from seedling emergence until the herbicide application. The dimethylamine salt formulation of dicamba (Clarity®, BASF, Florham Park, NJ, USA) was applied at a rate of 2.8 g a.e. ha\(^{-1}\) (Kruger et al. 2012) when plants were at the four-leaf stage, using a CO\(_2\) pressurized backpack sprayer equipped with a two-nozzle boom with TP8002VS Flat spray tip (Teejet Technologies, Wheaton, IL, USA). The spray boom was calibrated to deliver 186 L ha\(^{-1}\) at 275.79 kPa with a constant speed of 4.8 kph. At the time of herbicide application, the surface of the tray was covered with aluminum foil to prevent the adsorption of herbicide onto the soil surface. The visual injury was determined at 1, 3, and 7 d after treatment (DAT) using a scale of 0% (no injury) to 100% (plant death). To measure the plant dry biomass, all aboveground tomato plant parts were harvested at 7 DAT, oven-dried at 50 °C for 72 h, and then weighed.

Absorption and translocation experiment
To study the dicamba absorption and translocation, plants were divided into three sections (two upper leaves, two lower leaves, and the roots), and tissues from each part were harvested at 1, 3, and 7 DAT. The residue of dicamba on the surface of the two upper and lower leaves was washed off using 50% ethanol to determine the amount of unabsorbed dicamba. The extraction process was performed according to Shin et al. (2011) after some alterations. Briefly, a subsample (0.5 g) of the tissues from each part was individually homogenized with distilled water and transferred into 1.5 mL tubes. The tubes were mixed for 20 min and centrifuged at 16 100g for 25 min at room temperature. The resulting supernatant was filtered (0.2 μm pore size; VWR Scientific, Seattle, WA, USA), and air-evaporated overnight at room temperature. The dried aliquot was then transferred into a 25 mL vial and mixed with 20 mL of 0.6 mol L\(^{-1}\) hydrochloric acid – ACN (5:95, v/v) by gently shaking for 60 min. The aliquot was evaporated to 10 mL and pipetted into a separatory funnel containing 2 g of sodium chloride. Alkalinizing and partitioning were performed using 20 mL of 5 mol L\(^{-1}\) sodium hydroxide and 10 mL of n-hexane, respectively. After pouring off the upper hexane layer, the partitioning was repeated with an additional 10 mL of n-hexane. The aqueous layer was acidified with 2 mL of 6 mol L\(^{-1}\) sulfuric acid, and the partitioning of the acidified aqueous layer was performed twice using 10 mL dichloromethane for each partitioning. The water content of the extract was eliminated by passing the organic layer through anhydrous sodium sulfate. The hydrated sodium sulfate was combined with the dehydrated original extract after rinsing with 7 mL of dichloromethane. The final extract was evaporated to a volume of 1 mL under a vacuum evaporator and then dissolved in 2 mL of the mobile phase [acetic acid 0.2% and acetonitrile (60:40, v/v)]. The standard solution of dicamba was prepared by dissolution of 10.1 mg of dicamba in 100 mL of acetonitrile to achieve a final concentration of 100 μg mL\(^{-1}\). An Agilent 1100 series high-performance liquid chromatography (Agilent, Santa Clara, CA, USA) was used to analyze dicamba in the tissue extracts. The Agilent Chemstation A.10.02 software with a spectral module (Agilent Technologies Inc., Wilmington, DE, USA) was used to process the data. The separation was achieved on an Alltech Adsorbosphere reverse phase C18 column (150 mm × 4.6 mm, Dr. A. Maisch High-Performance LC GmbH, Germany) with particle size 3 μm. Injection volume with wash was 5 μL, and the mobile phase included a 59.8%, 0.2%, and 40% mixture of water, acetic acid, and acetonitrile, respectively. The flow rate, stop time, and post time was 0.5 mL min\(^{-1}\), 10 min, and 2 min, respectively. The temperature was maintained at 25 °C, and the diode-array detector detection was set at 280 nm; the fluorescence detection was not specifically set. To construct the calibration curve, six dicamba standard solutions (0.05, 0.1, 0.5, 1, 5, and 10 mg L\(^{-1}\)) were prepared. The dicamba peak was found at 7.25 min (retention time), and the peak height was used to make the calibration curve with \(R^2 = 0.999\).

The amount of intercepted herbicide washed off from the two upper and two lower leaves of each tomato plant were measured in mg g\(^{-1}\) at 0 DAT to determine the uniformity of the herbicide application. Also, at 1, 3, and 7 DAT, the amount of absorption was calculated by subtracting the amount of absorbed herbicide at each time-point from the quantity of absorbed herbicide at the previous time-point. Finally, the values of herbicide absorption were reported as a percentage of applied herbicide. The translocation of herbicide was calculated by comparing the percentage of absorbed dicamba among different parts of each plant (two upper leaves, two lower leaves, and the roots) during three time-points, 1, 3, and 7 DAT. The concentration of dicamba was converted from mg L\(^{-1}\) to mg g\(^{-1}\) of fresh tissue.

Leaf length/width ratio and trichome density
The length and width of two upper and two lower leaves of each tomato plant were measured using a ruler, and the length/width ratio was calculated based on the two upper, two lower, and total leaves (average of two upper and lower leaves) of five tomato plants. A light microscope (BX51, Olympus, Japan) equipped with a cube consisting of D360/40×, 400dclp, and ET560lp filters was used to count the number of trichomes on the adaxial surface of the leaves. The microscope was coupled to a computer with DP Controller and DP Manager Software. For trichome density, the number of trichomes on the upper and lower leaves of five plants was counted, and mean trichome density was calculated as the number of trichomes per square millimetre.
**Statistical analysis**

The bioassay experiment to detect the level of visual injury, dry biomass, and dicamba absorption and translocation was set up as a 2 (herbicide treatments) × 5 (tomato lines) factorial arrangement in a completely randomized design. Treatments were replicated three times, and the experiment was repeated twice. To investigate the leaf length/width ratio and trichome density, a completely randomized design experiment was conducted with four replicates and repeated twice. The leaf length/width ratio and trichome density were evaluated four times per plant across all tomato lines (including wild accessions and cultivars) before dicamba application. All analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). All the data were tested for normality using the PROC UNIVARIATE procedure, and natural logarithmic transformations were performed when necessary. Homogeneity of the variances was verified by performing the Levene’s test (Joley et al. 1992).

Additionally, because the experimental run or run × factor interaction was not significant for either experiment, data were combined across the experiments. All data were subjected to ANOVA using PROC MIXED SAS version 9.4. Herbicide treatments were considered as a fixed factor, and tomato lines, as well as the interaction between herbicide treatment and tomato lines, were considered as random factors. Also, means were separated using Fisher’s protected LSD test at the 5% level of probability. The concentration of absorbed dicamba was plotted against length/width leaf ratio and trichome density on leaves of each accession and cultivar to quantify their relationship. The significance of the slope was tested using a linear regression (eq. 1) for each variable.

\[ Y = ax + b \]

In the linear regression equation, \( Y \) is the concentration of absorbed dicamba by each tomato accession or cultivar. Independent variable \( x \) is the variation of length/width leaf ratio and trichome density on leaves of each accession and cultivar. Furthermore, \( a \) and \( b \) were slope and intercept of the equation, respectively. The GraphPad Prism version 8 (GraphPad Software, La Jolla, CA, USA) was used to plot graphs.

**Results and Discussion**

**Visual injury and dry biomass**

The DS cultivars showed higher injury than the DT accessions at 1, 3, and 7 DAT (Fig. 1A). Although no visual injury was observed on the foliage of DT accessions, the injury level on MM and BB was 35% and 40%, respectively, at 1 DAT (Fig. 1A). Herbicide injury on DT accessions appeared 3 DAT, where leaves showed slight signs (<5% injury symptom) of epinasty and twisting, while injury in both cultivars MM and BB increased up to 47% and 45%, respectively (Fig. 1A). Dicamba injury on the foliage of all three DT accessions did not exceed 5% at 7 DAT, while the recorded visual injury for MM and BB raised up to 52% and 54%, respectively (Figs. 1A and 1B). According to Fig. 2, no difference in the dry biomass among TOM199, TOM198, TOM300, MM, and BB in control (without herbicide) plots was observed, and it was about 3.7 g plot\(^{-1}\) at 7 DAT. Under the application of dicamba, the amount of plant dry matter of DT accessions was approximately 3.2-fold higher than DS cultivars (Fig. 2). Noticeably, TOM199 and TOM198 did not display different dry biomass values, whereas this was different for TOM300 under both herbicide treatments. Based on both visual injury and dry biomass results, all three DT wild accessions confirmed their tolerance to the simulated drift rate (2.8 g a.e. ha\(^{-1}\)) of dicamba (Figs. 1 and 2).

**Dicamba absorption and translocation**

The average quantity of applied dicamba was approximately 0.003 mg g\(^{-1}\) on all four leaves (including two upper and two lower leaves) of TOM199, TOM198, TOM300, MM, and BB at 0 DAT (data not shown). There was no difference in the amount of dicamba intercepted by each plant based on the value of intercepted herbicide in mg g\(^{-1}\) of leaf tissue sampled (data not shown), thus suggesting that all plants received the same dose of the herbicide. As expected, no dicamba was recovered from the leaf surface of all tomato lines in the control plots (data not shown). Therefore, the control plots do not show any evidence of dicamba injury (Fig. 1A), absorption, and translocation for both DT accessions and DS cultivars; hence, only data from the treated plots are presented.

In general, the DS cultivars absorbed more dicamba than the wild DT accessions at all time-points. At 1 DAT, the amount of herbicide absorbed by MM was 4.5-, 3.3-, and 3.3-fold higher than TOM199, TOM198, and TOM300, respectively. Also, although this quantity was lower for BB, it was still approximately two times greater compared with DT accessions at 1 DAT (Table 1). At 3 DAT, the DS cultivars demonstrated about 1.7- to 2-fold higher dicamba absorption compared with DT accessions. Dicamba absorption continued to increase in MM and BB at 7 DAT so that the level of herbicide absorbed by MM and BB was 1.6 and 1.8 times higher than TOM198, respectively. Kohler et al. (2004) observed that 2,4 D-susceptible ground ivy (Glechoma hederacea L.) absorbed 37% more C\(^{14}\)-labeled 2,4-D than the 2,4-D-tolerant ground ivy, while the translocation pattern was similar between the R and S populations. Similarly, diclofop-methyl resistant (R1) and S populations of ryegrass (Lolium multiflorum Lam.) indicated a significant difference in herbicide absorption, with R1 absorbing lesser herbicide than others at 3, 6, 12, 24, and 48 h after application of diclofop-methyl (De Prado et al. 2005). Likewise, the glyphosate spray retention by...
the glyphosate-susceptible ryegrass population was 35% higher than the R plants (Michitte et al. 2007). Vila-Aiub et al. (2012) suggested that among the four glyphosate-resistant Johnsongrass [Sorghum halepense (L.) Pers.] populations, R2 (a Johnsongrass accession) absorbed glyphosate nearly 20% less than individuals of the S Johnsongrass. In terms of dicamba distribution in different plant parts, most of the dicamba was initially present (at 1 DAT) in the upper leaves, followed by the lower leaves, and then the roots (Table 2). This trend was consistent throughout the DT accessions and DS cultivars. At a later time-point (at 7 DAT), some amount of dicamba distribution was observed in the roots.
dicamba in the upper leaves translocated to the lower sections of the plant in all DT accessions and DS cultivars (Table 2). No distinct difference in distribution pattern was observed between DT accessions and DS cultivars. Among all accessions and cultivars, MM and TOM198 distributed the most and least percentage of absorbed dicamba, respectively, into the two lower leaves over time. In other words, MM translocated the absorbed herbicide up to 3-, 4.2-, and 2-fold more than TOM198 into the two lower leaves at 1, 3, and 7 DAT, respectively (Table 2).

Interestingly, all tomato DS cultivars and DT accessions distributed a similar percentage of absorbed dicamba to the roots at all three time-points (1, 3, and 7 DAT), which was lower compared with the other parts (upper and lower leaves) (Table 2). Although the pattern of dicamba movement was the same between DT accessions and DS cultivars, the concentration of dicamba present in the upper and lower leaves of both cultivars was higher than in accessions (Table 2). This pattern suggests that the reason for the difference in herbicide concentration between the upper and lower leaves of DS cultivars and DT accessions may be primarily due to their difference in dicamba absorption (Tables 1 and 2). Our findings are contrary to Ou et al. (2018), where differential translocation, and not absorption, was found to be associated with dicamba resistance. Dicamba resistance in kochia was due to a reduction in intercellular transport and vascular loading of the herbicide, thus resulting in reduced translocation of dicamba. Cranston et al. (2001), on the other hand, found no difference in both dicamba absorption and translocation between R and S kochia.

### Leaf morphological characteristics

In accordance with the results of visual injury and herbicide absorption, the DS cultivars, in general, showed a lower leaf length/width ratio than the DT accessions and DS cultivars. Among all accessions and cultivars, MM and TOM198 distributed the most and least percentage of absorbed dicamba, respectively, into the two lower leaves over time. In other words, MM translocated the absorbed herbicide up to 3-, 4.2-, and 2-fold more than TOM198 into the two lower leaves at 1, 3, and 7 DAT, respectively (Table 2).

Interestingly, all tomato DS cultivars and DT accessions distributed a similar percentage of absorbed dicamba to the roots at all three time-points (1, 3, and 7 DAT), which was lower compared with the other parts (upper and lower leaves) (Table 2). Although the pattern of dicamba movement was the same between DT accessions and DS cultivars, the concentration of dicamba present in the upper and lower leaves of both cultivars was higher than in accessions (Table 2). This pattern suggests that the reason for the difference in herbicide concentration between the upper and lower leaves of DS cultivars and DT accessions may be primarily due to their difference in dicamba absorption (Tables 1 and 2). Our findings are contrary to Ou et al. (2018), where differential translocation, and not absorption, was found to be associated with dicamba resistance. Dicamba resistance in kochia was due to a reduction in intercellular transport and vascular loading of the herbicide, thus resulting in reduced translocation of dicamba. Cranston et al. (2001), on the other hand, found no difference in both dicamba absorption and translocation between R and S kochia.

### Table 1. The values of absorbed dicamba (% of applied dicamba) in tomato accessions (TOM199, TOM198, and TOM300) and cultivars [Money Maker (MM) and Better Boy (BB)] at 1, 3, and 7 d after treatment (DAT).

| Variety | % of applied herbicide | 1 DAT | 3 DAT | 7 DAT |
|---------|------------------------|-------|-------|-------|
| TOM199  | 9.49 (±0.03)e           | 26.51 (±0.02)e | 26.51 (±0.02)e |
| TOM198  | 13.05 (±0.01)d          | 30.09 (±0.01)d | 39.57 (±0.06)c |
| TOM300  | 13.15 (±0.02) c         | 33.86 (±0.04) c | 33.86 (±0.04) d |
| MM      | 42.78 (±0.03)a          | 57.04 (±0.02)a | 63.92 (±0.07)b |
| BB      | 26.32 (±0.01)b          | 56.91 (±0.01)b | 70.4 (±0.09)a |

Note: Different letters indicate significant differences within each column at $P \leq 0.05$ using Fisher's protected LSD test.

### Table 2. Distribution of dicamba, based on the percentage of absorbed dicamba, into three plant parts (two upper leaves, two lower leaves, and the roots) of tomato accessions (TOM199, TOM198, and TOM300) and cultivars [Money Maker (MM) and Better Boy (BB)] at 1, 3, and 7 d after treatment (DAT).

| Variety | % of absorbed herbicide | 1 DAT | 3 DAT | 7 DAT |
|---------|------------------------|-------|-------|-------|
|         | Two upper leaves | Two lower leaves | Roots | Two upper leaves | Two lower leaves | Roots | Two upper leaves | Two lower leaves | Roots |
| TOM199  | 68c | 30b | 2a | 73b | 24d | 3a | 56c | 42b | 2a |
| TOM198  | 86a | 13d | 1a | 86a | 11e | 3a | 74a | 25d | 1a |
| TOM300  | 74b | 25c | 1a | 68c | 29c | 3a | 49d | 49a | 2a |
| MM      | 60d | 39a | 1a | 52e | 46a | 2a | 46d | 51a | 3a |
| BB      | 74b | 25c | 1a | 58d | 40b | 2a | 61b | 37c | 2a |

Note: Different letters indicate significant differences within each column at $P \leq 0.05$ using Fisher's protected LSD test.
TOM199, TOM198, and TOM300 was higher than MM and BB by more than 2-fold (Fig. 5). Additionally, TOM199 and TOM300 showed higher trichome density on their upper leaves than TOM198. No difference in trichome density on the lower leaves was observed among the DT accessions. Similar to the leaf length/width ratio, the applied model to predict the relationship between dicamba absorption and the trichome density was significant ($P < 0.01$). Trichome density was negatively correlated with dicamba absorption (Fig. 4B). It was shown that large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and velvetleaf (*Abutilon theophrasti* Medik.) could prevent instant and direct contact of herbicide droplets with their leaf surface due to the dense number of trichomes on their leaf surface (Zimdahl 2018). Trichomes cause a reduction in optimal epidermal herbicide coverage by intervening the herbicide droplets before they reach the surface of the epiderm (Hess and Falk 1990).

**Conclusion**

Taking everything into account, the leaf length/width ratio of both DS tomato cultivars was found to be lower than all the three DT accessions. This suggests that the DS cultivars with higher leaf area may be able to intercept a more significant amount of dicamba spray droplets than the DT accessions, thus allowing greater absorption of the herbicide in time. Furthermore, the trichome density on the leaves of wild DT accessions (TOM199, TOM198, and TOM300) was greater than the DS cultivars. Therefore, in comparison to MM and BB that have lesser trichome density, TOM199, TOM198, and TOM300 may have prevented dicamba from coming in contact with their leaf surfaces, resulting in reduced dicamba uptake as compared with the DS cultivars. Also, as the dicamba translocation pattern was not different between DT accessions and DS cultivars, it is apparent that the higher amount of dicamba absorption in the DS cultivars was the reason these plants showed higher injury than the wild DT accessions, which subsequently resulted in plant death. It should also not be overlooked that there may be other potential mechanisms for dicamba tolerance in the DT accessions, such as differential gene expression or gene mutation associated with target-site tolerance. It is, therefore, crucial to study the molecular mechanisms involved in dicamba tolerance in these DT accessions in future studies.

**Acknowledgements**

Funding for this project was provided by the Specialty Crop Block Grant sponsored by the Mississippi Department of Agriculture and Commerce/U.S. Department of Agriculture — Agriculture Marketing Service, and the study presented here is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project under accession number 230060. We also thank...
Fig. 5. Comparison of the trichome density on the adaxial surface of the leaves among all cultivars [Money Maker (MM) and Better Boy (BB)] and accessions (TOM199, TOM198, and TOM300). Different letters indicate significant differences within each leaf type at $P \leq 0.05$ using Fisher’s protected LSD test.

Brooklyn Schumaker, Swati Shrestha, Edicarlos Castro, and Ziming Yue for their assistance throughout the research. We declare that there is no conflict of interest.

References

Brewer, C.A., Smith, W.K., and Vogelmann, T.C. 1991. Functional interaction between leaf trichomes, leaf wettability and the optical properties of water droplets. Plant Cell Environ. 14: 955–962. doi:10.1111/j.1365-3040.1991.tb00965.x.

Cranston, H.J., Kern, A.J., Hackett, J.L., Miller, E.K., Maxwell, B.D., and Dyer, W.E., 2001. Dicamba resistance in kochia. Weed Sci. 49(2): 164–170. doi:10.1614/0043-1745(2001)049[0164:DRIK]2.0.CO;2.

De Prado, J.L., Osuna, M.D., Heredia, A., and De Prado, R. 2005. Lolium rigidum, a pool of resistance mechanisms to ACCCase inhibitor herbicides. J. Agric. Food Chem. 53(6): 2185–2191. doi:10.1021/jf049481m. PMID:15769155.

Fagliari, J.R., Oliveira, R.S.D., Jr., and Constantin, J. 2005. Impact of sublethal doses of 2, 4-D, simulating drift, on tomato yield. J. Environ. Sci. Health B. 40(1): 201–206. doi:10.1081/PFC-200034327. PMID:15656182.

Ghanizadeha, H., Harringtona, K.C., and James, T.K. 2018. Micromorphology. Palaeogeogr. Palaeoclimatol. Palaeoecol. 262: 79–90. doi:10.1016/j.palaeo.2008.02.009.

Hemphill, D.D., Jr., and Montgomery, M.L. 1981. Response of vegetable crops to sublethal application of 2,4-D. Weed Sci. 29: 632–635. doi:10.1614/0043174500040182.

Hess, F.D., and Falk, R.H. 1990. Herbicide deposition on leaf surfaces. Weed Sci. 38(3): 280–288. doi:10.1093/004317450005654X.

Huangfu, C.H., Song, X.L., and Qiang, S. 2009. Morphological disparities in the epidermal and anatomical features of the leaf among wild Brassica juncea populations. Weed Biol. Manag. 9(3): 234–242. doi:10.1111/j.1445-6664.2009.00344.x.

Joley, D.B., Maddox, D.M., Supkoff, D.M., and Mayfield, A. 1992. Dynamics of yellow starthistle (Centaurea solstitialis) achenes in field and laboratory. Weed Sci. 40(2): 190–194. doi:10.1017/S0043174500057209.

Jordan, T.N., and Romanowski, R.R. 1974. Comparison of dicamba and 2, 4-D injury to field grown tomato. HortScience, 9: 74–75.

Knoch, M. 1994. Effect of droplet size and carrier volume on performance of foliace-applied herbicides. Crop Prot. 13(3): 163–178. doi:10.1016/02612194(94)90075-2.

Kohler, E.A., Throssell, C.S., and Reicher, Z.L. 2004. 2, 4-D rate response, absorption, and translocation of two ground ivy (Glechoma hederacea) populations. Weed Technol. 18(4): 917–923. doi:10.1614/WT-03-089R1.

Kruger, G.R., Johnson, W.G., Doohan, D.J., and Weller, S.C. 2012. Dose response of glyphosate and dicamba on tomato (Lycopersicon esculentum) injury. Weed Technol. 26(2): 256–260. doi:10.1614/WT-D-11-00073.1.

Li, S.H., Campbell, R.A., Studens, J.A., and Wagner, R.G. 1996. Absorption and translocation of glyphosate in aspen (Populus tremuloides Michx.) as influenced by droplet size, droplet number, and herbicide concentration. Weed Sci. 44:482–488. doi:10.1614/0043174500094224.

Michitte, P., De Prado, R., Espinoza, N., Ruiz-Santaella, J.P., and Gaurvitt, C. 2007. Mechanisms of resistance to glyphosate in a ryegrass (Lolium multiflorum) biotype from Chile. Weed Sci. 55(5): 435–440. doi:10.1614/WS-06-1671.

Miller, J.C., Jr., Baker, L.R., and Penner, D. 1973. Inheritance of tolerance to chloramben methyl ester in cucumber. J. Am. Soc. Hortic. Sci. 98: 386–389.

Ou, J., Stahlman, P.W., and Jugulam, M., 2018. Reduced absorption of glyphosate and decreased translocation of dicamba contribute to poor control of kochia (Kochia scoparia) at high
temperature. Pest Manag. Sci. 74(5): 1134–1142. doi:10.1002/ps.4463. PMID:27766747.
Penuik, M.G., Romano, M.L., and Hall, J.C. 1992. Absorption, translocation and metabolism are not the basis for differential selectivity of wild mustard (Sinapis arvensis L.) to auxinic herbicides. Weed Sci. Soc. Am. Abstr. 32: 55.
Pettinga, D.J., Ou, J., Patterson, E.L., Jugulam, M., Westra, P., and Gaines, T.A. 2018. Increased chalcone synthase (CHS) expression is associated with dicamba resistance in Kochia scoparia. Pest Manag. Sci. 74(10): 2306–2315. doi:10.1002/ps.4778. PMID:29083527.
Shin, E.H., Choi, J.H., Abd El-Aty, A.M., Khay, S., Kim, S.J., Im, M.H., et al. 2011. Simultaneous determination of three acidic herbicide residues in food crops using HPLC and confirmation via LC–MS/MS. Biomed. Chromatogr. 25(1–2): 124–135. doi:10.1002/bmc.1513. PMID:20842699.
Sterling, T.M., and Jochem, H.S. 1995. Uptake, translocation, and metabolism of picloram and metsulfuron methyl by two loco weed species. Weed Sci. 43: 13–17. doi:10.1017/S0043174500080759.
Vila-Aiub, M.M., Balbi, M.C., Distéfano, A.J., Fernández, L., Hopp, E., Yu, Q., and Powles, S.B. 2012. Glyphosate resistance in perennial Sorghum halepense (Johnsongrass), endowed by reduced glyphosate translocation and leaf uptake. Pest Manag. Sci. 68(3): 430–436. doi:10.1002/ps.2286.
Wang, C.J., and Liu, Z.Q. 2007. Foliar uptake of pesticides—present status and future challenge. Pest Biochem. Physiol. 87: 1–8. doi:10.1016/j.pestbp.2006.04.004.
Wang, H., Shi, H., and Wang, Y. 2015. The wetting of leaf surfaces and its ecological significancegs. Pages 295–321 in M. Aliofkhazraei, eds. Wetting and wettability. IntechOpen, London, UK.
Xu, L., Zhu, H., Ozkan, H.E., Bagley, W.E., and Krause, C.R. 2011. Droplet evaporation and spread on waxy and hairy leaves associated with type and concentration of adjuvants. Pest Manag. Sci. 67: 842–851. doi:10.1002/ps.2122. PMID:21370389.
Zangouinejad, R., Alebrahim, M.T., and Tseng, T.M. 2019. Evaluation of auxin tolerance in selected tomato germplasm under greenhouse and field conditions. Weed Technol. 33(6): 815–822. doi:10.1017/wet.2019.51.
Zimdahl, R.L. 2018. Fundamentals of weed science. Academic Press, Cambridge, MA, USA.