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Evaluating the Volatility of Three Formulations of 2,4-D When Applied in the Field

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Cotton genetically engineered to be resistant to topical applications of 2,4-D could provide growers with an additional tool for managing difficult-to-control broadleaf species. However, the successful adoption of this technology will be dependent on the ability of growers to manage off-target herbicide movement. Field experiments were conducted in Moultrie, GA, to evaluate cotton injury resulting from the volatilization of 2,4-D when formulated as an ester, an amine, or a choline salt. Each formulation of 2,4-D (2.24 kg ha^{-1}) was applied in mixture with glyphosate (2.24 kg ha^{-1}) directly to the soil surface (10 to 20% crop residue) in individual square blocks (750 m^2). Following herbicide applications, replicate sets of four potted cotton plants (five- to seven-leaf stage) were placed at distances ranging from 1.5 to 48 m from the edge of each treatment. Plants were allowed to remain in-field for up to 48 h before being removed. Cotton exposed to 2,4-D ester for 48 h exhibited maximum injury ratings of 63, 57, 48, 29, 13, and 2% at distances of 1.5, 3, 6, 12, 24, and 48 m, respectively. Less than 5% injury was noted for the amine and choline formulations at any distance. Plant height was also affected by formulation and distance; plants that were located closest to the ester-treated block were smaller than their more distantly-positioned counterparts. Exposure to the amine and choline formulations did not affect plant heights. Additionally, two plastic tunnels were placed inside of each treated block to concentrate volatiles and maximize the potential for crop injury. Injury ratings of 76, 13, and 5% were noted for cotton exposed to the ester, amine, and choline formulations, respectively when under tunnels for 48 h. Results indicate that the choline formulation of 2,4-D was less volatile and injurious to cotton than the ester under the field conditions in this study.

Nomenclature: 2,4-dichlorophenoxyacetic acid, glyphosate; Palmer amaranth, *Amaranthus palmeri* S. Wats. AMAPA; cotton, *Gossypium hirsutum* L.

Keywords: 2,4-D, auxinic herbicide, cotton, crop injury, herbicide volatility.

El algodón genéticamente diseñado para ser resistente a las aplicaciones tópicas de 2,4-D podría brindar a los productores una herramienta adicional para el manejo de especies de hoja ancha difíciles de controlar. Sin embargo, la adopción exitosa de esta tecnología dependerá de la habilidad de los productores de manejar el movimiento del herbicida a lugares no deseados. Se realizaron experimentos de campo en Moultrie, Georgia, para evaluar el daño en algodón resultante de la volatilización de 2,4-D cuando se formuló como ester, amine, o sal choline. Cada formulación de 2,4-D (2.24 kg ha^{-1}) fue aplicada en mezcla con glyphosate (2.24 kg ha^{-1}) directamente a la superficie del suelo (10 a 20% de residuos de cultivos) en parcelas cuadradas individuales (750 m^2). Seguido de las aplicaciones del herbicida, grupos replicados de cuatro plantas de algodón en contenedores (en el estado de cinco a siete hojas) fueron colocados a distancias que variarían de 1.5 a 48 m del borde de cada tratamiento. Las plantas fueron mantenidas en el campo por períodos de hasta 48 h antes de ser removidas. El algodón expuesto a 2,4-D ester por 48 h mostró evaluaciones de daño máximas de 63, 57, 48, 29, 13, y 2% a distancias de 1.5, 3, 6, 12, 24, y 48 m, respectivamente. Para las formulaciones amine y choline, el daño notado fue menor a 5% en cualquiera de las distancias evaluadas. La altura de planta también fue afectada por la formulación y la distancia; las plantas que estaban más cerca de la parcela tratada con ester fueron más pequeñas que aquellas que estaban a mayor distancia. La exposición a las formulaciones amine y choline no afectó la altura de las plantas. Adicionalmente, se colocaron dos túneles de plástico dentro de cada parcela tratada para concentrar los compuestos volátiles y maximizar el potencial de daño del cultivo. Las evaluaciones de daño de 76, 13, y 5% fueron notadas para el algodón expuesto a las formulaciones ester, amine, y choline, respectivamente, bajo los túneles por 48 h. Los resultados indican que la formulación choline de 2,4-D fue menos volátil y menos dañina al algodón que la formulación ester bajo condiciones de campo en este estudio.

The management of glyphosate-resistant (GR) Palmer amaranth, POST, in cotton is limited by herbicide efficacy and availability. Historically, pyrithiobac, an acetolactate synthase (ALS) inhibitor, has been successful at controlling the species (Burke and Wilcut 2004); however, Palmer amaranth populations with ALS-resistance have been

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identified throughout much of the southern United States (Heap 2014; Wise et al. 2009). Furthermore, Palmer amaranth populations with multiple resistances to both glyphosate and ALS-inhibiting herbicides have been identified in at least seven states (Heap 2014; Sosnoskie et al. 2011). Glufosinate is an additional POST tool for controlling Palmer amaranth, but only if it is applied to smaller weed seedlings (generally less than 10 cm in height) and only in cotton that is resistant to glufosinate (Coetzer et al. 2002; Culpepper et al. 2010). Palmer amaranth seedlings can be successfully eliminated from in between crop rows using cultivation, although growers on sandier profiles might avoid disturbing soils in order to minimize moisture loss. Although expensive, hand-weeding has been utilized by more than 90% of Georgia cotton growers to manage GR Palmer amaranth plants that have escaped control measures (Sosnoskie and Culpepper 2014). Currently, the most effective option for season-long Palmer amaranth control is the sequential use of residual herbicides; however, the efficacies of these products are dependent upon a timely activation and favorable environmental conditions that maximize their retention time in the soil.

To effectively manage Palmer amaranth, growers are in need of additional in-season weed-control tools (Sosnoskie and Culpepper 2014). One such technology might be cotton that is genetically-modified to be resistant to preplant or topical applications of 2,4-D. Resistance to 2,4-D in cotton, which is normally sensitive to the auxinic herbicides, is conferred by the insertion of a gene that codes for an aryloxyalkanoate dioxygenase enzyme (Wright et al. 2010), allowing transgenic plants to metabolize 2,4-D to a nonlethal form (Richburg et al. 2012). At this time, 2,4-D-resistant cultivars of cotton, corn (*Zea mays* L.), and soybean [*Glycine max* (L.) Merr.], which are also resistant to glyphosate and glufosinate, are nearing commercialization.

The adoption of 2,4-D-resistant crops could expand the temporal and spatial use profile of this auxinic herbicide; concerns regarding off-target movement, via spray drift or volatilization, could hinder the acceptance of this technology. Sublethal doses of phenoxy herbicides can be injurious to many commodities, such as row crops that are not engineered with resistance to 2,4-D, and vegetables (Everitt and Keeling 2009; Fagliari et al. 2005; Gilreath et al. 2001a,b; Hemphill and Montgomery 1981; Johnson et al. 2012; Marple et al. 2007; Robinson et al. 2013; Sciumbato et al. 2004a,b). Drift reduction strategies, such as proper nozzle selection, changes to carrier volume, using appropriate drift control agents, adjusting boom height, and restricting applications during adverse environmental conditions, should help to minimize the possibility of 2,4-D spray droplets moving off-site (Felsot et al. 2010). Furthermore, the production of 2,4-D–resistant crops will require the use of a proprietary formulation of glyphosate plus 2,4-D choline, a quaternary ammonium salt, with the potential for reduced odor, reduced fine and driftable spray droplets, and reduced volatility (Richburg et al. 2012). The objective of these field studies was to determine if 2,4-D choline poses a lower volatility threat to nearby sensitive crops when compared to ester or amine formulations.

Materials and Methods

Two field experiments were initiated in recently harvested corn fields on September 9, 2010, and August 30, 2011, at the Sunbelt Agriculture Expo research farm in Moultrie, GA. The experimental location and application timings were selected to spatially and temporally separate the trial from sensitive crops, mostly cotton and vegetables, in the region. Soil at the site was a Dothan loamy sand (88% sand) with 10 to 20% corn stubble (as determined via a visual assessment) remaining on the surface at the time of application each year. Corn was harvested several weeks before the initiation of the studies, after which standing stubble was tilled into the fields. The study site was flat with little to no discernable slope. The experimental plots were pre-irrigated with 1.25 cm of water 24 h prior to trial initiation. The in-field portion of the study was conducted over 3 d each year. With the exception of maximum daily soil temperature, local weather conditions were similar for 2010 and 2011. Daily air temperatures ranged from 21 to 35 C, with a mean daily air temperature of 28 C. Relative humidity values ranged between 38 and 100% with a daily mean of 77%. Wind speeds ranged between 0.5 and 1.6 m s^{-1} with an average daily wind speed of 1.0 m s^{-1}. Maximum daily soil temperatures ranged from 37 to 39 C and 41 and 45 C in 2010 and 2011, respectively.

Figure 1. Aerial photo of the 2011 study showing the approximate locations of the 2,4-D ester, amine, and choline-treated blocks, each of which were 750 m^2 in size, at the Sunbelt Agriculture Expo Research Farm in Moultrie, GA. Drawn squares are not to scale. (Color for this figure is available in the online version of this paper.)

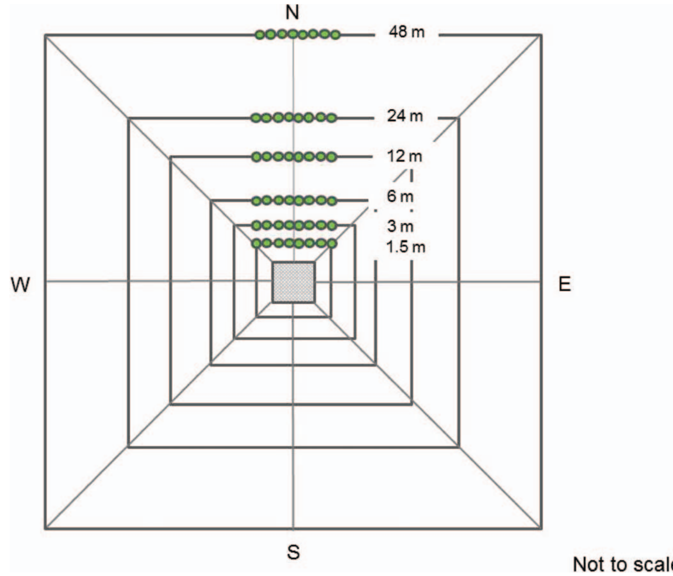

Figure 2. Field trial design for the 2010 and 2011 studies demonstrating the arrangement of cotton indicator plants relative to a 2,4-D treated plot. Plants were placed along transects oriented in each of eight directions (N, NE, E, SE, S, SW, W, NW) at distances of 1.5, 3, 6, 12, 24, and 48 m from the edge of each treatment block, which was 750 m^2 in size. (Color for this figure is available in the online version of this paper.)

Three formulations of 2,4-D, which included an ester, (2,4-D LV 4; Agri Star, Ankeny, IA), an amine salt (Weedar 64; NuFarm, Burr Ridge, IL), and a choline salt (2,4-D choline; Dow AgroSciences, Indianapolis, IN), were evaluated in this study. Each formulation of 2,4-D (2.24 kg ae ha^{-1}) was mixed with glyphosate (2.24 kg ae ha^{-1} Durango; Dow AgroSciences, Indianapolis, IN) and applied to the soil surface in one of three randomly assigned 750 m^2 plots (Figure 1). Treatments were separated from each other by at least 150 m. Herbicides were mixed at a site that was more than 800 m downwind from the treatment blocks. Simultaneous and independent herbicide applications were made using three CO_2-pressurized backpack sprayers equipped with 11002 AIXR nozzles (TeeJet Technologies, Wheaton, IL) calibrated to deliver 140 L ha^{-1} at 165 kPa. Booms were held at a height of 51 cm above the soil surface. Applications were completed by 9:30 A.M. each year.

Because cotton is extremely sensitive to 2,4-D, it was used as an indicator species to evaluate volatility in the field (Sciumbato et al. 2004a). The cotton cultivar 'PHY 499 WRF' (Dow AgroSciences, Indianapolis, IN), which is not resistant to 2,4-D, was planted and grown in 2,650 cm^3 plastic nursery pots containing a peat-based growing media (Premier Tech Horticulture, Quakertown, PA). Plants were maintained outdoors under ambient conditions and watered and fertilized regularly to ensure optimal growth. When plants were between the five- and seven-leaf stages, they were transported to the study site. Following herbicide applications (within 1 h of treatment, but allowing for the dispersal of spray droplets), two sets of four cotton plants were placed directly on the ground along transects oriented in each of eight directions (N, NE, E, SE, S, SW, W, NW) at distances of 1.5, 3, 6, 12, 24, and 48 m from the edge of each treatment block (Figure 2). One set of plants was allowed to remain in-field for 48 h (0 to 48 h), and the second set was removed after 24 h (0 to 24 h). A third set of four plants was also placed at each direction-by-distance location beginning at 24 h after application and allowed to remain on-site until 48 h after application (24 to 48 h), when the in-field portions of the studies were terminated.

Immediately after spraying, two open-ended plastic tunnels (1.6 m tall by 2 m wide by 5 m long) were placed 1 m inside of each treated area and arranged parallel to either the southern and western borders. Tunnels were constructed using 2.5 cm diam PVC pipe (Charlotte Pipe and

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Foundry Company, Charlotte, NC) and clear, 4 mm thick polyethylene film (Blue Hawk, Gilbert, AZ). Ten cotton plants were placed inside of each tunnel for 48 h (0 to 48 h) following application. Special care was taken to minimize disturbing the treated area. Stem and leaf tissue was not allowed to touch the treated soil; no cotton roots extended from the bottom of the pots. The purpose of the tunnels was to trap volatile emissions from the treated soil and concentrate the vapors as an additional, and highly conservative, strategy for evaluating crop injury.

Following removal from the study site, cotton plants were immediately transported 56 km to the University of Georgia's Ponder Farm in Ty Ty, Georgia, where symptom development was evaluated. Care was taken to avoid applying 2,4-D at this site from 1 mo before to 1 mo after the completion of the experiment. The indicator plants were watered daily to promote growth and development. Whole-plant visual estimates of injury were rated on a scale of 0% (no injury) to 100% (complete plant mortality) every 5 to 7 d, for up to 28 d after application (DAA), when the study was terminated (Sciumbato et al. 2004a). Plant heights and node counts were also recorded at 28 DAA in 2011. Additional check plants were evaluated each year. These non-treated plants were placed around the Moultrie field facility (including the mix site and in buffer zones that were equidistant between treatments), in the transport trucks, and at the Ponder farm; their inclusion was designed to ensure that the observed injury resulted from the treatment applications and was not due to external contamination.

Percent injury data were arcsine square-root transformed prior to statistical analysis; height and node count data were log-transformed. Maximum injury, height, and node count data were analyzed using mixed-models analysis of variance (Statistical Analysis Systems Institute Inc., Cary, NC) where 2,4-D formulation and distance from the herbicide source, as well as their interaction, were considered as main effects. Replication (individual plants) and year were considered to be random effects. Transect direction was also considered as a random effect; plants were placed in each of eight orientations to ensure that injury would be observed, regardless of weather conditions. Means separation was accomplished using the Bonferroni adjustment in order to account for numerous multiple pairwise comparisons ($n = 153$). With respect to the tunnel data, formulation was the only main effect evaluated; replication, tunnel orientation and year were considered as random; no adjustments to the Pvalues were applied. Each exposure period (0 to 48 h, 0 to 24 h, and 24 to 48 h) was analyzed separately.

Results and Discussion

Although volatiles were not directly measured, the injury symptoms observed in the study were characteristic of exposure to 2,4-D (e.g., epinasty, leaf deformations, tissue chlorosis, and necrosis of terminal buds), suggesting that the observed damage was the result of the experimental treatments (Sciumbato et al. 2004a,b). Not unlike Sciumbato et al. (2004b), none of the check plants, including those placed halfway between adjacent sprayed plots, exhibited any signs of herbicide injury at any point during the course of the trials.

Injury symptoms on the indicator plants began to develop within 3 DAA for plants exposed to ester volatiles, and within 7 to 14 DAA for plants exposed to the amine and choline formulations (data not shown). Cotton injury increased with time and maximum injury was observed by 28 DAA (data not shown). Analysis of variance indicated that maximum injury ratings were significantly affected by 2,4-D formulation, distance from the treated plot, and the interaction between the two main effects (Figure 3). Mean maximum cotton injury in response to 2,4-D ester (0 to 48 h exposure) was 64, 57, 48, 29, 13, and 2% at distances of 1.5, 3, 6, 12, 24, and 48 m from the edge of the treatment block, respectively. With the exception of the 1.5 m vs. 3 m comparison, ester-related injury decreased significantly with increased distance from the treated plot ($P \leq 0.0003$). Less than 2% maximum visual estimate of injury was detected with the amine formulation (0 to 48 h exposure), and only at distances of 1.5 and 3 m from the treated area. No injury was observed for the choline formulation, except at the 1.5 m distance (< 1%). Statistically, the ester injury ratings differed from those of the amine and choline ($P \leq 0.0003$), except for the 48 m (ester) vs. 1.5 m (amine, choline) and 3 m (amine) comparisons. With the exception of the 1.5 m (amine) vs. 1.5, 3, 6, 12, 24, and 48 m (choline)

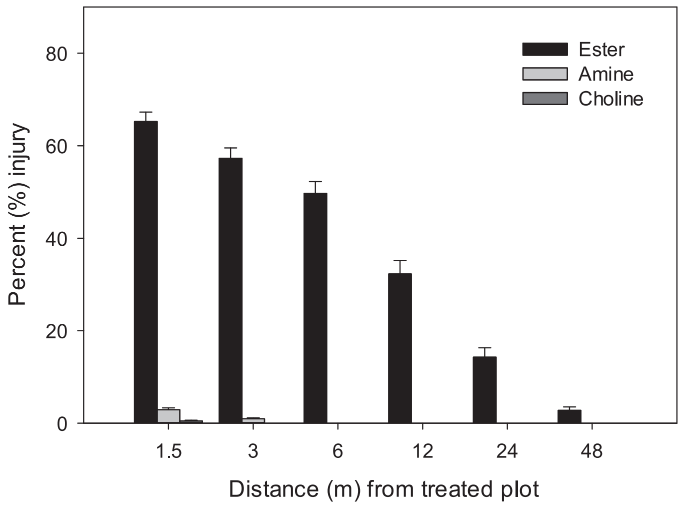

Figure 3. Effects of 2,4-D formulation and distance (m) from the treated plots on maximum cotton injury (%) for the 0 to 48 h exposure period at 21 to 28 d after application. Injury ratings in response to the ester formulation were greater ($P \leq 0.0003$) than those for the amine and choline formulations for almost all comparisons; injury observed for ester-exposed plants at 48 m did not differ from injury observed for amine- and choline-exposed plants at 1.5 to 3 m. The choline formulation did not differ ($P > 0.0003$) from the amine with respect to cotton injury, except when compared to amine-related injury at 1.5 m.

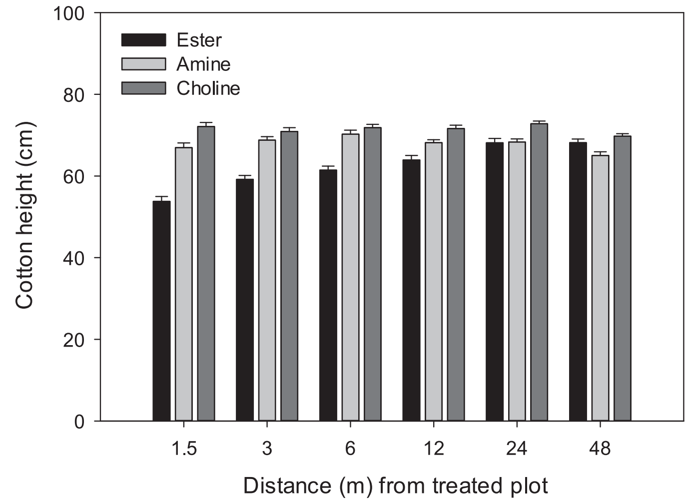

Figure 4. Effects of 2,4-D formulation and distance (m) from the treated plots on mean cotton height (cm) for the 0 to 48 h exposure period at 28 d after application. Statistical analyses showed that plant heights for cotton exposed to the ester formulation were lower ($P \leq 0.0003$) than those for the amine and choline formulations for the 1.5-, 3-, and 12-m distances. In general, the amine- and choline-exposed plants did not differ with respect to size ($P > 0.0003$).

comparisons, there were no differences between the amine and the choline at any distance ($P \geq 0.0003$).

Similar trends in injury ratings were observed for the 0 to 24 h and 24 to 48 h exposure periods. Mean maximum injury for plants exposed to ester volatiles from 0 to 24 h was 58, 55, 43, 23, 8, and < 1% at distances of 1.5, 3, 6, 12, 24, and 48 m from the edge of the treatment block, respectively. For the 24 to 48 h exposure, maximum cotton injury in response to the 2,4-D ester formulation was 23, 18, 14, 7, 2, and < 1% at distances of 1.5, 3, 6, 12, 24, and 48 m from the edge of the treatment block, respectively. No cotton injury (0%) was observed in response to the amine and choline formulations for both the 0 to 24 h and 24 to 48 h exposure periods. Although statistical comparisons were not made across exposure periods, results suggest that the majority of volatiles might have been released within 24 h of application, under the conditions observed during these field trials.

Mean cotton plant heights were also affected by 2,4-D formulation, distance, and the interaction between the main effects (Figure 4). Mean plant heights were negatively associated with plant injury for the ester treatment; in general, plant size increased as injury decreased (e.g., distance from the treated plot increased). Mean heights for plants exposed to 2,4-D ester volatiles for 48 h were 54, 59, 61, 64, 68, and 68 cm at distances of 1.5, 3, 6, 12, 24, and 48 m from the herbicide source, respectively. Plants exposed to the ester formulation were statistically smaller than those exposed to the amine and choline formulations at the 1.5, 3, 6, and 12 m distances ($P \leq 0.0003$). Cotton plants exposed to the amine or choline formulations were, on average, between 67 and 72 cm in height. With few exceptions, no statistical differences ($P \geq 0.0003$) were observed among the amine and choline treatments with respect to height. For reference, non-treated, check plants were, on average, 70 cm in height. Node counts did not differ in response to formulation or distance ($P \geq 0.0003$); mean total node counts ranged from nine to 11 per plant, regardless of treatment.

Formulation significantly ($P \leq 0.05$) affected observed maximum injury for plants that were exposed to volatiles under the open tunnels. Plants that were placed under plastic tunnels in the ester-, amine- and choline-treated plots for 48 h were injured, on average, 76, 14, and 5%, respectively (Figure 5). Similar trends were observed for the 0 to

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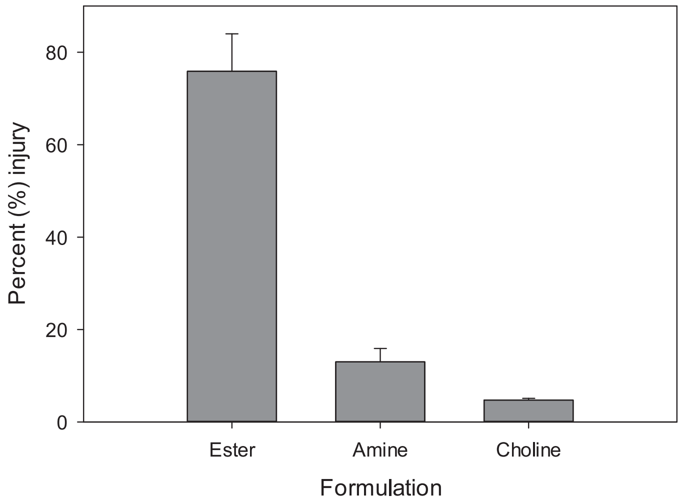

Figure 5. Maximum injury (%) for cotton exposed to 2,4-D volatiles for 48 h under plastic tunnels at 21 to 28 d after application. Significant ($P \leq 0.05$) differences in injury ratings were observed among each of the three formulations.

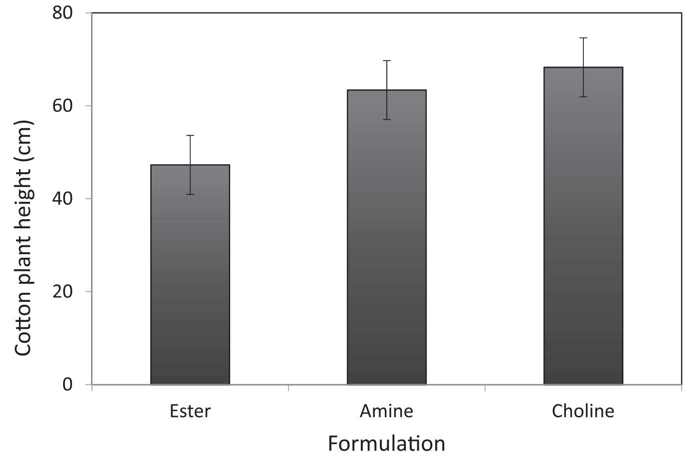

Figure 6. Mean height (cm) of cotton exposed to 2,4-D volatiles for 48 h under plastic tunnels at 28 d after application. Significant ($P \leq 0.05$) differences in plant heights were observed among each of the three formulations.

24 h and 24 to 48 h exposure periods; mean maximum cotton injury was significantly ($P \leq 0.05$) greater for the plants in the ester treatments (72 and 62%) as compared to the amine (4 and < 1%) and the choline (2 and < 1%). Mean plant height was inversely related to plant injury; plants exposed to ester, amine, and choline volatiles for 48 h were, on average, 47, 63, and 68 cm in height, respectively (Figure 6). As mentioned previously, non-treated check plants averaged 70 cm in height. Although there was a tendency towards fewer nodes for cotton plants exposed to concentrated 2,4-D ester volatiles, as compared to the amine and choline, differences among the treatments were not statistically significant for any exposure period (data not shown).

Results from previously published studies have demonstrated that cotton is particularly sensitive to 2,4-D at extremely low concentrations, although plant growth stage at the time of application is likely to affect the degree of symptom development (Egan et al. 2014; Everitt and Keeling 2009; Johnson et al. 2012; Marple et al. 2007, 2008; Robinson et al. 2013; Sciumbato et al. 2004a,b). For example, Everitt and Keeling (2009) reported that 2,4-D amine at 0.0028 kg ai ha^{-1} resulted in an injury estimate of 50% at 14 DAT when the product was applied to cotton at the cotyledon to two-leaf stage, but only 3% when applied to the plant at full bloom. With respect to cotton yields, a meta-analysis performed by Egan et al. (2014) showed that predicted reductions in cotton seed or lint were likely to be more severe from herbicide misapplication (56 g ha^{-1}) and spray drift (5.6 g ha^{-1}), as opposed to volatilization (0.56 g ha^{-1}), regardless of growth stage. However, Egan et al. (2014) also showed that cotton yield response is highly variable in response to 2,4-D and that environmental and agronomic factors can affect both injury and output.

2,4-dichlorophenoxyacetic acid was the first selective herbicide to be widely applied in modern agricultural production (Peterson 1967). Formulations of 2,4-D have been used successfully to control problematic broad-leafed plants that are common to cotton production, such as common cocklebur (*Xanthium strumarium* L.), sicklepod [*Senna obtusifolia* (L.) H. S. Irwin and Barneby], morningglory spp. (*Ipomoea* spp.), and Palmer amaranth (Ferrell and Witt 2002; Lancaster et al. 2005; Norsworthy et al. 2008). The release of cotton resistant to 2,4-D (as well as topical applications of glyphosate and glufosinate) could provide growers with an additional tool for managing these difficult-to-control species, although care must be taken to ensure that the technology is not mishandled so as to avoid nontarget injury and to prevent the evolution of 2,4-D resistance in the target weeds (Egan et al. 2011; Felsot et al. 2010). As with any management strategy, 2,4-D-resistant cotton and 2,4-D should be used as a component of a diversified weed control program.

With respect to the herbicide itself, 2,4-D choline will be formulated with glyphosate and sold as a mixture that is less capable of drifting as compared to tank mixes of currently available glyphosate and 2,4-D formulations (Richburg et al. 2012). In contrast, volatility drift can be more difficult to manage. In addition to vapor pressure, which is a function of a product's chemical formulation, the potential for herbicide volatilization is influenced by numerous external factors, such as the temperature and water content of leaves or soil, the temperature and humidity of the surrounding environment, and the movement of air around treated surfaces (Bauer et al. 1973; Behrens and Lueschen 1979; Burnside and Lavy 1966; Que Hee and Sutherland 1974; Strachan et al. 2010). 2,4-dichlorophenoxyacetic acid esters, which are effective at controlling many agronomically important weed species, are also more volatile than other formulations (Grover 1976; Marple et al. 2007; Marth and Mitchell 1949; Zimmerman et al. 1953). Although not as injurious as the esters, volatilized 2,4-D amine salts can also cause also damage sensitive crops under certain conditions (Sciumbato et al. 2004b). Results from this study suggest that the choline formulation appears to pose a lower threat for off-target movement from volatility than the ester formulation, and is not likely to be more injurious than the amine.

Acknowledgments

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