Weed Technology

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Investigation of inquiries on weed control efficacy of XtendiMax® herbicide with VaporGrip® technology

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Research Article

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Abstract

Herbicide resistance in weeds significantly threatens crop production in the United States. The introduction of dicamba-resistant soybean and cotton stacked with other herbicide tolerance traits has provided farmers with the flexibility of having multiple herbicide options to diversify their weed management practices and delay resistance evolution. XtendiMax® herbicide with VaporGrip® Technology is a dicamba formulation registered for use on dicamba-resistant soybean and cotton crops by the U.S. Environmental Protection Agency (EPA). One of the terms of its registration includes an evaluation of inquiries on reduced weed control efficacy by growers or users of XtendiMax for suspected weed resistance. A total of 3,555 product performance inquiries (PPIs) were received from 2018 to 2021 regarding reduced weed control efficacy by dicamba. Following the criteria recommended by EPA for screening of suspected resistance in the field, a total of 103 weed accessions from 63 counties in 13 states were collected for greenhouse testing over those 4 yr. Weed accessions for greenhouse testing were collected only in states where resistance to dicamba was not yet confirmed in the weed species under investigation. The accessions, which consisted primarily of waterhemp and Palmer amaranth, were treated with dicamba at rates of 560 g ae ha^{-1} and 1,120 g ae ha^{-1}. All weed accessions, except for one accession each of Palmer amaranth and waterhemp, were controlled by ≥90% with dicamba at 21 d after treatment in the greenhouse.

Introduction

Herbicides are an integral component of weed management in crop production, providing several advantages such as increased productivity, improved quality of produce, and reduced soil erosion due to reduced tillage (Green 2012; Nandula 2019). Additional uses of selective and nonselective herbicides in-crop have been enabled by the introduction of herbicide-tolerant (HT) crop technologies such as glyphosate and glufosinate tolerance in broadacre crops such as maize (*Zea mays* L.), soybean, and cotton. In recent years, the introduction of a dicamba tolerance trait in soybean and cotton and a 2,4-D resistance trait in maize (*Zea mays* L.), soybean, and cotton has provided new uses for dicamba (Anonymous 2017a) and 2,4-D (Anonymous 2014, 2017b), respectively, to control troublesome broadleaf weed species that are resistant to other herbicides, including glyphosate-resistant weeds (De Sanctis et al. 2021; Meyer et al. 2015). The dicamba-resistant crops (trade-named Xtend® crops) commercialized to date are stacked with a glyphosate tolerance trait (trade-named Roundup Ready® Xtend® crops) and may be stacked with a glufosinate tolerance trait (trade-named XtendFlex® crops) to provide more herbicide options for controlling weeds. In-crop (also referred to as over-the-top) application of dicamba on dicamba-resistant soybean and cotton is restricted to certain approved low-volatility formulations of dicamba. These specific dicamba formulations include XtendiMax® herbicide with VaporGrip® technology (hereafter referred to as XtendiMax) developed by Bayer CropScience (Anonymous 2017a, 2022a); Engenia® developed by BASF (Research Triangle Park, NC; Anonymous 2022b), and Tavium® Plus VaporGrip® technology developed by

Syngenta (Greensboro, NC), which contains a mixture of dicamba herbicide with VaporGrip® technology and S-metolachlor (Anonymous 2022c). These three dicamba formulations for over-the-top use on dicamba-resistant soybean and cotton have restrictions that were established by the U.S. Environmental Protection Agency (EPA) when they were registered for use, one of which is that an herbicide resistance management (HRM) plan must be established (US-EPA 2020a, 2020b, 2020c). A component of an HRM plan is field evaluation of weed control efficacy inquiries from growers regarding likely or suspected resistance to dicamba following EPA recommended criteria set forth by Norsworthy et al. (2012) (hereafter referred to as Norsworthy criteria).

The evolution of herbicide-resistant weeds, including resistance to dicamba, is a selection process that is enabled by pre-existing genetic variation within and among weed accessions (Barrett and Schluter 2008; Beckie 2020), and overreliance and repeated use of a herbicide site of action (SOA) (Chahal et al. 2017, 2018). Dyer (2018), Sudheesh et al. (2011), and Tehranchian et al. (2017) have shown that resistance can also be triggered through an accumulation of minor effect genes, particularly in cross-pollinated species, due to sublethal herbicide exposure caused by non-optimal herbicide application practices, such as using reduced rates, applications on larger weeds, and applications during unfavorable weather conditions, any of which may result in insufficient spray coverage.

To date, herbicide resistance has been documented in more than 260 weed species globally, involving 21 known herbicide SOAs (Heap 2023). Four weed species in the United States and 11 species globally are reported to have confirmed resistance to dicamba after more than 60 yr of use (Heap 2023; USDA-ERS 2014). In the United States, confirmed dicamba-resistant weed species include an accession of waterhemp found in maize and soybean production system in Illinois (Bobadilla et al. 2022); an accession of Palmer amaranth found in cotton and soybean production in Tennessee (Foster and Steckel 2022); prickly lettuce (*Lactuca serriola* L.) found in cereals in Washington state; and kochia [*Bassia scoparia* (L.) A. J. Scott] found in Colorado, Idaho, Kansas, Montana, Nebraska, and North Dakota primarily in maize and wheat production systems (Heap 2023).

To slow the spread of herbicide resistance, it is essential to understand weed resistance evolution as early as possible. One of the key elements to better manage herbicide resistance is reporting lack of weed control efficacy by specific herbicides. This targeted monitoring of suspected resistance cases provides an early opportunity to evaluate variability in herbicide sensitivity and resistance development within weed accessions and to assess spray application parameters that can reduce herbicide efficacy after application of the intended herbicide following label requirements.

In this report, we describe the investigation of product performance inquiries (PPIs) over 4 yr (2018 to 2021) related to lack of dicamba, specifically the XtendiMax herbicide efficacy, and follow-up testing of selected weed accessions for suspected resistance to dicamba in a controlled environment, all as part of the post-commercialization HRM plan for dicamba.

Materials and Methods

Field Investigations of Suspected Resistance to Dicamba

As part of the HRM plan for dicamba, a process was established to enable users of the technology to report any reduced weed control efficacy and/or any suspected resistance to dicamba to Bayer CropScience at 1-844-RRXTEND (1-844-779-8363). In addition, Bayer encouraged users to contact local Bayer representatives, extension specialists, or certified crop advisors, and to visit www.xtendimaxapplicationrequirements.com and www.roundupreadyxtend.com to obtain additional herbicide resistance management and/or integrated weed management recommendations for specific crops and weed accessions (Anonymous 2022a). PPIs for dicamba were initiated when a grower or retailer reported reduced weed efficacy following an in-crop application of XtendiMax herbicide to control broadleaf weeds in their dicamba-resistant Xtend soybean and/or cotton fields. Crop fields related to each PPI received in 2018 to 2021 (Figure 1A) were investigated by Bayer's technical representatives in the respective regions using the Norsworthy criteria for suspected resistance evaluation (Norsworthy et al. 2012) according to the terms of the XtendiMax herbicide registration (US-EPA 2020a). In brief, Norsworthy criteria include 1) failure to control a weed species normally controlled by the herbicide at the dose applied, especially if control is achieved on adjacent weeds; or 2) a spreading patch of uncontrolled plants of a particular weed species; or 3) surviving plants mixed with controlled individuals of the same species (Norsworthy et al. 2012). Upon investigating all PPIs, those inquiries that met one or more Norsworthy criteria were identified for further investigation for suspected resistance to dicamba. In states where confirmed resistance to dicamba has not yet been reported for a specific weed species, seeds of that species were collected at physiological maturity from the PPI fields being investigated. A total of 103 accessions comprising 83 waterhemp, 14 Palmer amaranth, two dandelion (*Taraxacum officinale* F.H. Wigg), one kochia, one marestail [*Conyza canadensis* (L.) Cronquist], one velvetleaf (*Abutilon theophrasti* Medic.), and one redroot pigweed (*A. retroflexus* L.) plants were sampled between 2018 and 2021, and the coordinates of each site of sample collection (Figure 1B) were recorded using a Garmin eTrex® 10 handheld system (Garmin International Inc., Olathe, KS) or another similar device. In addition, other factors that affect weed efficacy, such as weed height, herbicide application practices, historical weed management practices followed in the field, and rainfall, were recorded.

Weed Seeds Collection

Seeds were collected from weed accessions that were present in soybean and cotton production fields following sampling methods suggested by Burgos et al (2013). For each selected weed accession, multiple seed heads were collected from 10 to 40 individual plants exclusively within the field following a zig-zag pattern and bulked to form a composite sample. These weed accessions (i.e., test samples) were assigned a unique sample identification (ID) number for the purpose of sample traceability. Seed heads were air-dried completely under shade for approximately 2 wk prior to threshing and cleaning, and the seeds were stored at 4 C in a 50-mL conical centrifuge tube (Corning Incorporated, Corning, NY) until further use.

Plant Growth Conditions in the Controlled Environment for Herbicide Assays

Approximately 1,500 to 5,000 seeds of each test sample were germinated by planting them in 105-cell plug flats (Hummert International, Earth City, MO) containing Premier Pro-mix BX commercial potting medium (Hummert International) in a greenhouse at the Bayer CropScience research facility in Chesterfield, MO. Plug flats were saturated by sub-irrigation prior to planting, covered

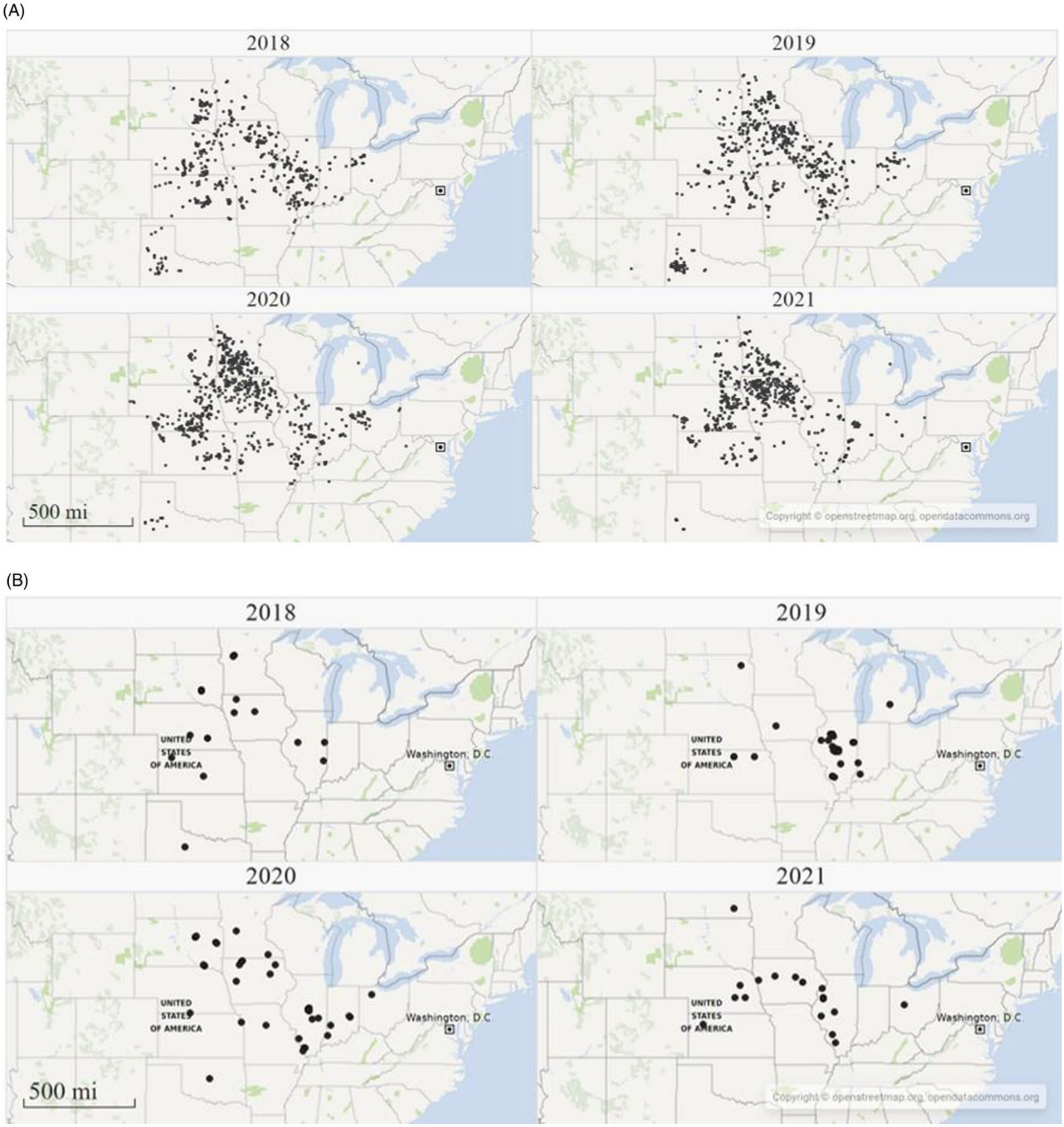

Figure 1. (A) Map depicting the locations of product performance inquiries related to weed control by dicamba from 2018 to 2021. (B) Accessions selected for dicamba weed control efficacy testing in the greenhouse.

with domes, and sub-irrigated as needed or watered with mist spray until seedling emergence. Seedlings were manually thinned to one plant per cell after cotyledons were fully formed. Healthy seedlings were selected 7 to 14 d after planting and individually transplanted into 10-cm-square vacuum deep (SVD) pots (Hummert International) containing the potting medium previously described. Plants were grown in the greenhouse at 29/26 C day/night temperature, relative humidity of 40% to 60% and 16/8-h day/night photoperiods supplemented with sodium halide lamps (560 $\mu mol\ m^{-2}\ s^{-1}$). Prior to transplanting, soil in the SVD pots was thoroughly saturated with water by sub-irrigation and transplanted plants were watered as needed by sub-irrigation or overhead watering. These conditions provided optimal conditions for normal growth of weeds in the controlled environment and were similar to published reports (Bobadilla et al. 2022; Kumar et al 2020). Known dicamba-sensitive accessions of each weed species were grown in the greenhouse together with the test samples as described above to serve as sensitive controls (Table 1). Seeds of these sensitive accessions were increased for several generations in the greenhouse facility at Bayer CropScience.

Table 1. Known dicamba sensitive accessions used as susceptible controls.

Weed species	Accession No.	Site of collection	Year of collection
Waterhemp	11472101	County unknown, Nebraska	2009
Palmer amaranth	11472102	Filmore County, Nebraska	2013
Kochia	11472103	Herbiseed commercial source, United Kingdom	2012
Marestail	11472497	Phillips County, Kansas	2009
Velvetleaf	11493001	Azlin commercial source, Leland, Mississippi	2014
Dandelion	11562237	Unknown location USA	Prior to 2014

Herbicide Treatments

All herbicide assays were conducted in a greenhouse at the Bayer CropScience research facility in Chesterfield, MO. The test and sensitive control accessions were screened with XtendiMax containing VaporGrip technology as volatility reducing agent (Anonymous 2022a) using a custom-built cabinet spray chamber (Bayer Technical Discovery Center, Chesterfield, MO) mounted with a TTI 110015 nozzle (TeeJet Technologies, Springfield, IL, USA). The nozzle was calibrated to deliver 140 L ha^{-1} of spray solution at 276 kPa at an approximate speed of 2.5 km ha^{-1}. Application of dicamba was carried out when the plants were 7 to 10 cm tall. To evaluate suspected resistance to dicamba in each test sample, a tiered herbicide screening approach was followed (Figure 2). Results from each tier were used to make decisions regarding subsequent screening. In the initial screening (i.e., Tier I stage), herbicide sensitivity screening was conducted with a target sample size of 30 plants per accession against the label rate (560 g ae ha^{-1}; hereafter 1× rate) and twice the label rate (1,120 g ae ha^{-1}; hereafter 2× rate) of dicamba. Each treatment included a known sensitive accession as a control susceptible to dicamba. An additional three to five plants were included as untreated checks for each accession. In low weed seed germination cases, plants were divided equally between the two rates with a minimum of 10 plants for each treatment or, in some cases, treated only with the 2× rate due to limited sample size. Based on the results from Tier I screen (Figure 2), the treatments were repeated in the next round of weed sensitivity screening with a larger sample size targeting approximately 100 plants per accession (Tier II stage). Finally, depending on Tier II test results, whole plant dose-response assays were conducted with 0, 1/32×, 1/16×, 1/8×, 1/4×, 1/2×, 1×, and 2× the label rate of 560 g ae ha^{-1} for sensitive control accessions; and 0, 1/16×, 1/8×, 1/4×, 1/2×, 1×, 2×, and 4× the label rate for test accessions, to characterize the level of sensitivity or confirm resistance to dicamba in test accessions (Tier III stage). Individual dose-response experiments included either four or six replications with each replicate consisting of four or six individual plants.

Plant Evaluation for Herbicide Efficacy

Individual test accessions and sensitive control at both the Tier I and Tier II stages were visually evaluated for percent mortality (based on frequency of survivors) and individual plant injury level for any survivors at approximately 21 d after herbicide treatment (DAT). Visual injury was assessed using a scale of 0% (no visible injury) to 100% (no green tissue) compared with untreated checks within the same test or control sample. Plants exhibiting severe injury (>85%) but still showing some green tissues on the older leaves were rated as dead because they had advanced tissue decay (severe stunting, epinasty on meristems, and callus tissues at the base) with no signs of new growth (Figure 3). Based on percent mortality after treatment with dicamba in the controlled environment, each accession was classified as either “sensitive” (mortality ≥90% at 1× rate and 100% at 2× rate) or “less sensitive” (mortality <90% at 1× rate and/or <100% at 2× rate) (Figure 2). Accessions that were categorized as ”less sensitive” at each tier were taken to the next stage of screening. The percent of mortality classification used in this study represents a stringent cut-off because 1) weed seeds were collected from plants that survived the application of dicamba in the field and met one or more of the Norsworthy criteria for suspected resistance (i.e., non-random collection); 2) the survived plants in the field likely represent siblings at least in waterhemp or Palmer amaranth because >95% of cross-pollination in these species happens within 200 meters in an open, non-crop situation (Sarangi et al. 2017; Sosnoskie et al. 2012); and 3) if an accession under investigation was resistant to dicamba, the progeny from these surviving plants was expected to have higher resistance allele frequency and <50% mortality (Gardner et al. 1998; Foster and Steckel 2022; Holmes et al. 2022; Shyam et al. 2022).

For dose-response analysis of accessions that reached Tier III stage, plant assays were conducted in a randomized complete block design and the experiment was replicated over time (Burgos et al. 2013). Individual plants were evaluated for percent injury at 21 DAT. After rating, plants were harvested individually into a paper bag by clipping the aboveground plant parts and dried at approximately 60 C for 2 wk. The dried samples were weighed to estimate their dry biomass using an analytical balance (Mettler-Toledo Inc., Ballwin, MO). Dose-response data were analyzed to determine ER$_{50}$ using the DRC package (Ritz et al. 2015) with R software (R Core Team 2021) and regressed to fit a three-parameter log-logistic model for biomass reduction using the following equation:

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

where Y represents the percent dry biomass, c is the lower limit, d is the upper limit, b is the slope of each curve, x is the herbicide rate, and e is the rate of herbicide required for a 50% reduction in dry biomass.

Results and Discussion

Geographic Distribution of Weed Efficacy PPIs for Dicamba

One of the key components of HRM to delay resistance and/or spread of resistant weeds is early detection and reporting of reduced weed control efficacy against an intended herbicide by growers to registrants of the technology, local extension specialists, and/or certified crop advisors. A total of 3,555 PPIs related to weed control by XtendiMax in Xtend soybean and cotton fields were received and investigated between 2018 and 2021, with a range of 690 to 1,034 PPIs each year (Figure 4). These PPIs included all issues related to weed control efficacy by XtendiMax used in the Xtend crops and not cases of suspected resistance. However, these PPIs do not account for inquiries outside of Bayer’s process or weed control efficacy issues with other dicamba formulations. These PPIs were predominantly received from the North Central and southern regions of the United States, where waterhemp and Palmer amaranth infestations are prevalent (Figure 1A) and

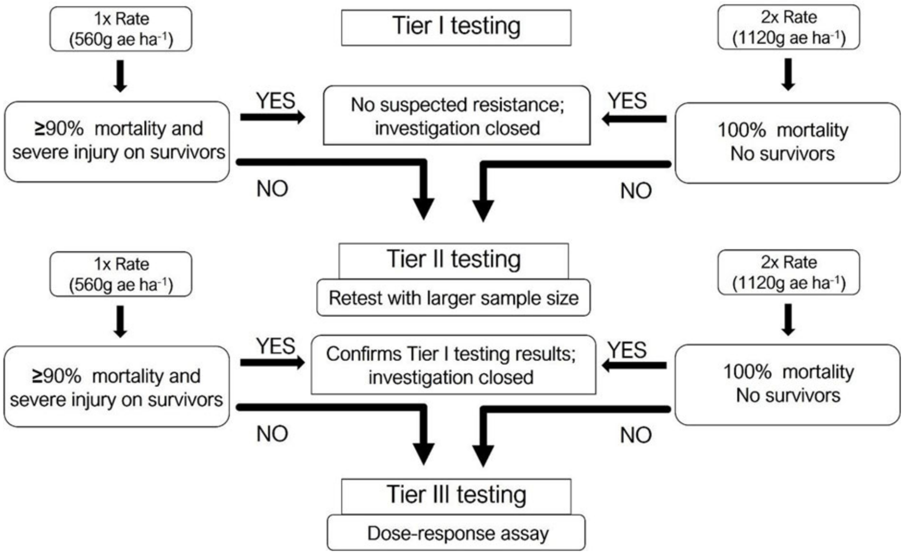

Figure 2. Greenhouse testing decision tree used to evaluate suspected dicamba resistance in weed accessions collected from XtendiMax® product performance inquiries. Any "no" with 1× or 2× rate testing would trigger the next stage of testing.

Figure 3. Example of rating scale for percent injury in waterhemp treated with 560 g ae ha^{-1} of dicamba.

included a total of 396 counties in 27 states over 4 yrs. Of the PPIs received between 2018 and 2021, 79% were from six states: Iowa, Minnesota, Nebraska, Illinois, Kansas, and South Dakota; whereas the remaining 21% of PPIs were received from Texas, Missouri, Indiana, Ohio, and other states (Figure 5). Soybean fields represented most of the PPIs ranging from approximately 87% in 2019 to approximately 98% in 2020 and 2021. In contrast, the number of inquiries from cotton fields ranged from approximately 2% in 2020 and 2021 to approximately 13% in 2019 (Figure 4).

All PPIs were investigated for weed species that are labeled for control with over-the-top applications of dicamba. In terms of the composition of broadleaf weed species represented in PPIs for dicamba, Figure 6 shows the eight most frequently investigated weed species. The majority of inquiries from 2018 to 2021 were related to issues managing a single weed species (64.4%) following dicamba applications, while others included issues with two (27.9%) or more (7.7%) weed species from the same field in a given year. The latter PPIs potentially indicate general herbicide application errors because it would be unlikely for multiple weed species to simultaneously develop resistance or reduced sensitivity to the same herbicide in the same field. Irrespective of the number of weed species exhibiting reduced efficacy after dicamba application in a field, weed seeds were collected from those species that met one or more of the Norsworthy criteria. Among the most common weed species reported for reduced weed control by dicamba, waterhemp triggered the most inquiries (43% to 58%) followed by Palmer

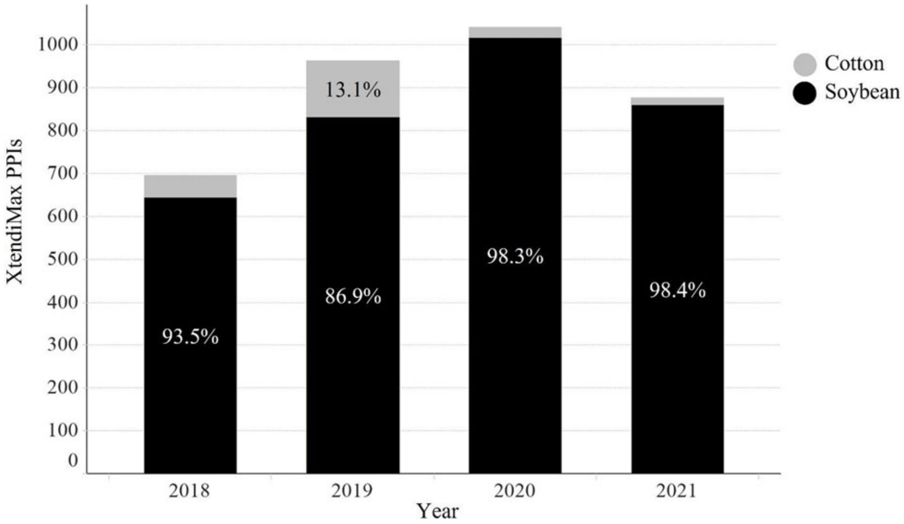

Figure 4. Product performance inquiries (PPIs) received by Bayer related to weed control efficacy by dicamba from 2018 to 2021 in the Xtend® cropping system.

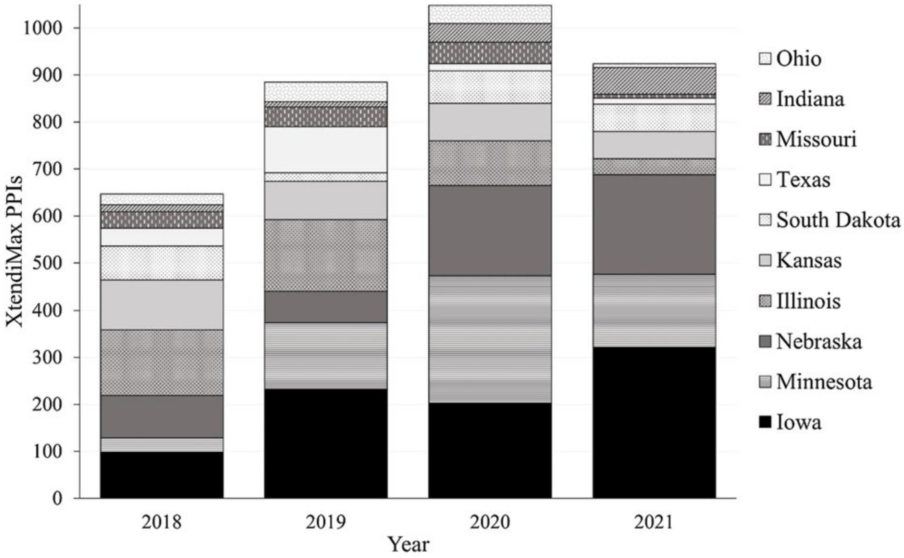

Figure 5. Top 10 states from which product performance inquiries (PPIs) were received by Bayer related to weed control by dicamba from 2018 to 2021. States are listed from most PPIs to fewest PPIs over this 4-yr monitoring period.

amaranth (12% to 21%) across all 4 yrs (2018 to 2021). Other weed species identified across all 4 yr include common lambsquarters (*Chenopodium album* L.; 6% to 12%), kochia (2% to 11%), ragweed (*Ambrosia trifida* L. and *Ambrosia artemisiifolia* L.; 6% to 9%), morning glory [*Ipomoea purpurea* (L.) Roth and *Ipomoea hederacea* (L.) Jacq; 1% to 3%], marestail (4% to 12%), and prickly sida (*Sida spinosa* L.; 1%) (Figure 6). The high frequencies of waterhemp and Palmer amaranth were likely due to their prevalence in soybean and cotton production regions of the United States and corroborates results from annual surveys conducted by the Weed Science Society of America (Van Wychen 2022).

Cases of Suspected Resistance among the XtendiMax PPIs and Follow-Up Investigations

The 3,555 PPIs received between 2018 and 2021 were followed up to assess factors that might be responsible for reduced weed control efficacy and to identify suspected cases of resistance to dicamba. The presence of weeds following an herbicide application is not always an indicator of resistance in a field. Therefore, each PPI was initially assessed to ensure that the grower had followed application requirements according to EPA and state labels for XtendiMax herbicide (www.xtendimaxapplicationrequirements.com). Factors assessed included weed height at the time of dicamba application, herbicide application errors, and weather conditions that could affect herbicide spraying and efficacy. Among the total PPIs, approximately 97% of weed control efficacy issues were attributed to factors known to cause reduced weed control efficacy of dicamba (Figure 7).

Considering the factors that were likely to reduce weed control efficacy, a majority of the PPIs (61%) were due to the presence of larger weeds at application (i.e., weed height >10 cm, which is taller than the recommended height on the label; 13%), poor herbicide coverage (4%), or a combination of the two (44%) (Figure 7). Poor herbicide coverage was a result of several factors including improper use of spray equipment (such as large-orifice

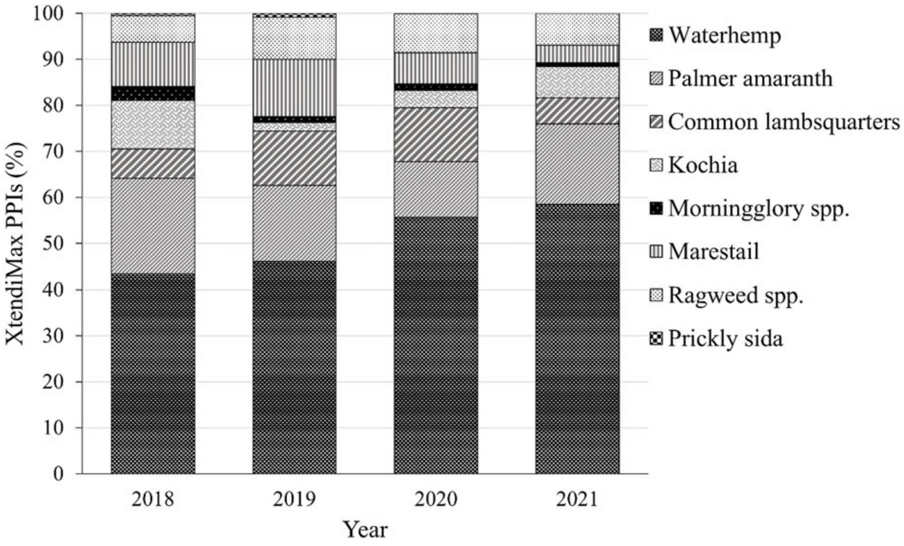

Figure 6. Composition of major broadleaf weed species in the product performance inquiries (PPIs) for dicamba from 2018 to 2021. Species are listed from most to least prevalent in PPIs over the 4-yr monitoring period.

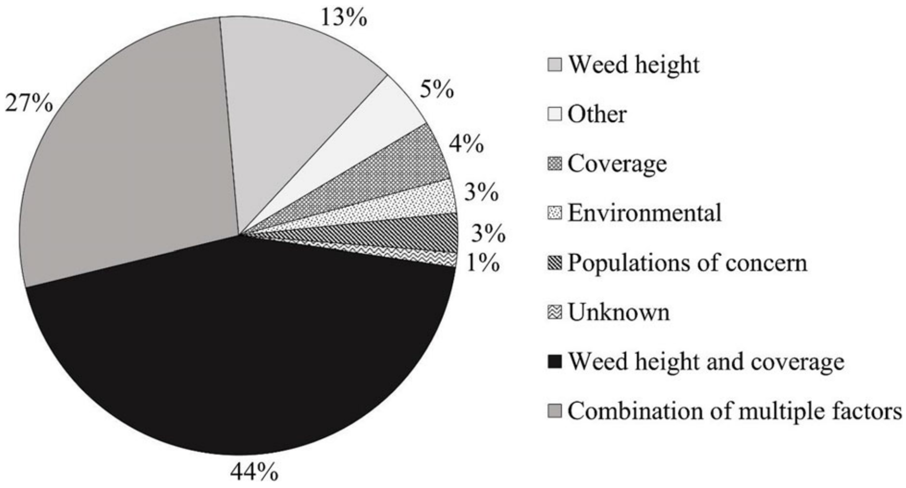

Figure 7. Factors investigated in the field where reduced weed control efficacy to dicamba was reported. Percentages are based on the total number (3,555) of product performance inquiries investigated from 2018 to 2021.

nozzles or incomplete nozzle overlap) and poor spray pattern arising from low pressure range and/or low spray volume. Additionally, complex tank mixtures, use of multiple drift-reducing agents, higher weed pressure due to limited use of residual herbicides and/or dense crop canopy at the time of application might have contributed to poor spray coverage. Several studies have shown that herbicide efficacy can be affected by weed size and density (Kramer and Legleiter 2022; Priess et al. 2022); application techniques such as type and position of nozzles, spray height, spray pressure, and spray volume (Butts et al. 2018; Creech et al. 2015; Dorr et al 2013; Etheridge et al. 1999; Nuyttens et al. 2007); tank contamination and drift-reducing agents (Zaric 2020); and local environment conditions (de Oliveira Rodrigues 2018; Kudsk and Kristensen 1992) at the time of herbicide application. A few cases (5%) were categorized as “other factors” such as when acceptable control was achieved with time, nonlabelled weeds (e.g., volunteer maize) were identified in investigations, or a new flush of weed emergence was detected after the initial dicamba application.

Of the remaining PPIs, 3% (103 out of 3,555) were categorized as identifying accessions of concern, primarily due to the presence of weed accessions that survived dicamba application as per recommended label requirements and met one or more of the three Norsworthy criteria: 1) failure to control a weed species under investigation that is labeled for control at the recommended rate, especially if control is achieved on adjacent weeds; 2) a spreading patch of uncontrolled plants of a particular weed species; and 3) presence of surviving weed plants mixed with controlled plants of the same species. These accessions of concern were further investigated by testing the weed control efficacy of dicamba in controlled environment assays as outlined below.

Weed Control Efficacy Testing of Accessions of Concern in Controlled Environment Assays

The 103 accessions of concern originated from 63 counties in 13 states between 2018 and 2021 (Figure 1B) and were sampled to further test under greenhouse conditions. Eighty-three of these accessions of concern were waterhemp (approximately 81%) followed by 14 Palmer amaranth (approximately 14%), which was similar to the composition of weeds reported in the total set of PPIs. The remaining six accessions consisted of two dandelion accessions and one each of redroot pigweed, velvetleaf, marestail, and kochia. The accessions identified each year were screened for weed control efficacy by dicamba at 1× and 2× rates using a tiered testing approach (Figure 2), as detailed in the Materials and Methods section.

Evaluation of Accessions of Concern Identified in 2018

In 2018, 19 weed accessions were screened in the greenhouse, including 11 accessions of waterhemp, six of Palmer amaranth and one each of kochia and velvetleaf (Figure 8A). All of the Palmer amaranth and velvetleaf accessions were completely controlled at both 1× and 2× rates of dicamba at 21 DAT. Among the 11 waterhemp accessions, all showed >90% control at the 1× rate with seven of them exhibiting 100% mortality, while all 11 accessions exhibited 100% mortality at the 2× rate of dicamba at 21 DAT (Figure 8A). All individual waterhemp plants with less than 100% mortality at the 1× rate exhibited significant injury and/or stunted growth with little or no regrowth at 21 DAT and were considered controlled by dicamba. Similarly, the kochia accession exhibited >90% mortality at the 1× rate with severe injury symptoms at 21 DAT and showed 100% control at the 2× rate of dicamba at 21 DAT.

Evaluation of Accessions of Concern Identified in 2019

Based on field observations, 27 weed accessions were collected in 2019 that met one or more of the Norsworthy criteria, including 24 accessions of waterhemp, two of Palmer amaranth, and one of marestail (Figure 8B). In Tier I stage screening, 24 of the 27 accessions exhibited ≥90% mortality at the 1× rate of dicamba at 21 DAT, while two waterhemp accessions and one Palmer amaranth accession exhibited <90% mortality. However, all 27 accessions exhibited 100% mortality at the 2× rate of dicamba at 21 DAT (Figure 8B). At the 1× rate, two waterhemp accessions that exhibited <90% mortality (approximately 86% mortality) were from South Dakota and Illinois, and a Palmer amaranth accession that exhibited approximately 82% mortality was from Kansas. Moreover, the plants surviving at the 1× rate exhibited substantial injury and/or stunted growth compared with plants that served as checks and showed minimal signs of regrowth. When these three accessions were retested using a larger sample size (approximately 100 plants per accession; Tier II test) at the 1× rate, all three showed >90% mortality (98%, 92%, and 100%, respectively, for the two waterhemp accessions and Palmer amaranth) with survivors exhibiting an average of 75% injury for the two waterhemp accessions. Furthermore, all three accessions exhibited 100% mortality at the 2× rate. Note that a baseline variation in injury levels is expected in *Amaranthus* spp. with auxinic herbicides (dicamba or 2,4-D) as a majority of extension weed management guides published by universities suggest, and as evidence by greenhouse studies carried out by Crespo et al. (2016) and Hamberg et al. (2023). The greenhouse study carried out by Crespo et al. (2016) reported a range of 53% to 77% and 67% to 93% injury, respectively, among 41 waterhemp and 34 Palmer amaranth accessions tested at the 420 g ae ha^{-1} rate of dicamba. Similarly, Hamberg et al. (2023) observed varied susceptibility to dicamba in 103 waterhemp accessions they tested with survival frequencies ranging from 0% to 43% at the label rate. Most of those accessions had survival frequencies of 0%, whereas 10 accessions had survival frequencies >20%, and three accessions had a survival frequency of >30%.

Evaluation of Accessions of Concern Identified in 2020

Of the 37 total accessions of concern identified in 2020, 33 were waterhemp, two were Palmer amaranth, and one each was of dandelion and redroot pigweed. In the Tier I screen, 30 waterhemp accessions and all the Palmer amaranth, dandelion, and redroot pigweed accessions exhibited ≥90% mortality at the 1× rate of dicamba at 21 DAT (Figure 8C), whereas the remaining three waterhemp accessions exhibited <90% mortality at the 1× rate. However, all 37 accessions exhibited 100% mortality when tested at 2× rate.

The three waterhemp accessions with <90% mortality at the 1× rate of dicamba were collected from Illinois (two accessions) and South Dakota, and exhibited 87%, 83%, and 73% mortality, respectively. The two accessions from Illinois that survived the 1× treatment exhibited average injury ratings of 69% and 76% respectively, whereas individual plants in the accession from South Dakota exhibited injury levels that ranged from 65% to 80%. Those three waterhemp accessions were further screened at the Tier II stage with a larger sample size (approximately 100 plants per accession) at 1× and 2× rates. Results from Tier II screening indicated that the two accessions from Illinois exhibited 97% and 100% mortality at the 1× rate of dicamba and 100% mortality at the 2× rate. However, the waterhemp accession from South Dakota exhibited approximately 50% mortality at the 1× rate of dicamba, and one plant survived (approximately 93% mortality) the 2× treatment. Plants that were classified as survivors at the 1× rate had a mean injury of 59%, and the one plant that survived at the 2× rate had 80% injury compared with untreated checks.

Because the waterhemp accession from South Dakota (hereafter SD-AMATA-1) consistently exhibited <90% mortality at the 1× rate of dicamba across two rounds of testing (73% and 50%), and because one plant survived the 2× rate in the Tier II screen, this accession was evaluated in two separate dose-response assays each with eight different rates (Tier III stage) of dicamba to determine the level of reduction in herbicide sensitivity. The ER_{50} (i.e., the effective rate to reduce dry biomass by 50%) values based on the dry biomass from the two dose-response studies indicated an average of 1.37-fold (resistant ÷ susceptible ratio) reduction in herbicide sensitivity against dicamba compared with the sensitive control accession (Figure 9; Table 2). Dose-response analysis based on dry biomass of the F_1-progeny generated among plants that survived at the 1× rate from Tier II stage testing, had a similar fold reduction as the parent population (data not shown). Similar observations of reduced sensitivity to dicamba were previously reported in a Palmer amaranth accession from Texas with 1.7-fold reduction (Garetson et al. 2019), and a waterhemp accession from Texas with 2.2-fold reduction in herbicide sensitivity relative to the sensitive control, however, these accessions were controlled at the 2× rate (Garetson et al. 2019; Singh et al. 2020). In addition, three Palmer amaranth accessions from Tennessee exhibited 1.85-fold, 1.90-fold, and 2.49-fold reductions in herbicide sensitivity to dicamba with 93%, 82%, and 92% control, respectively, at the 2× rate of dicamba (Foster and Steckel 2022). In comparison, a waterhemp accession from Illinois that was confirmed to be resistant to dicamba exhibited a 5-fold to 10-fold reduction in sensitivity (Bobadilla et al. 2022), whereas a Palmer amaranth accession from Tennessee with confirmed resistance to dicamba exhibited a 14.25-fold reduction in sensitivity relative to the sensitive control (Foster and Steckel 2022). Similarly, a Palmer amaranth accession from Kansas that was confirmed to be resistant to 2,4-D herbicide exhibited a 6-fold to 9-fold reduction in herbicide sensitivity relative to the sensitive control (Shyam et al. 2022).

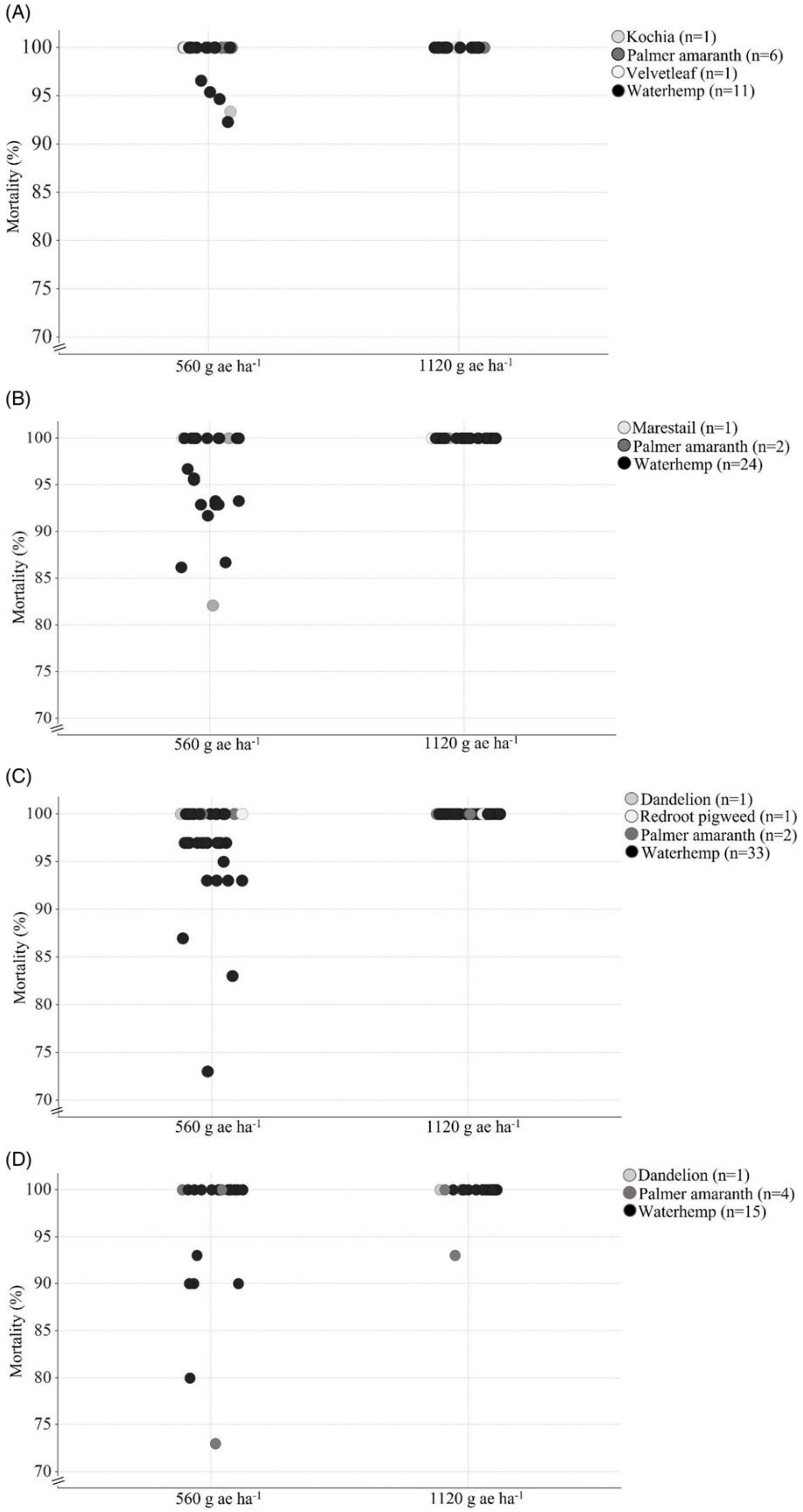

Figure 8. Percent mortality of different weed species screened for suspected resistance to 560 and 1,120 g ae ha^{-1} of dicamba herbicide in the greenhouse in Tier I assays in (A) 2018, (B) 2019, (C) 2020, and (D) 2021. Numbers (n) in the legends represent the number of accessions sampled for each weed species.

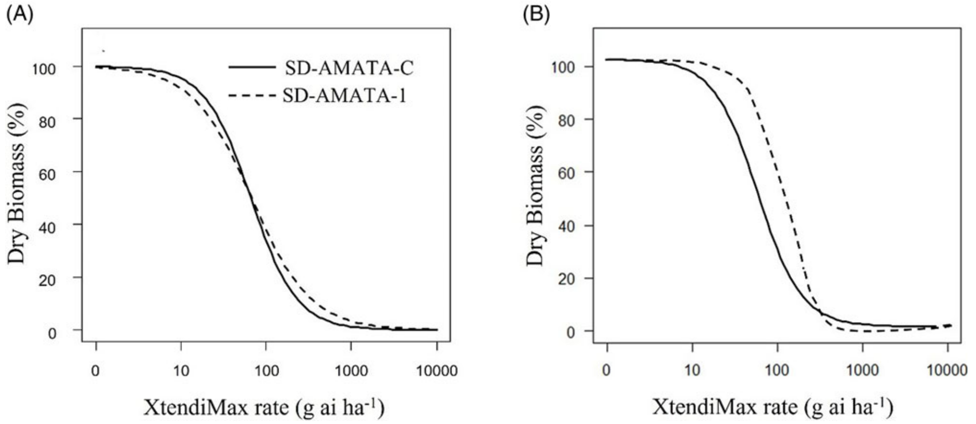

Figure 9. Dose-response analysis of SD-AMATA-1 waterhemp accession to dicamba application (postemergence treatment) from (A) experimental run 1 and (B) experimental run 2. Efficacy was assessed based on the biomass dry weight at 21 d after treatment. Dose-response analysis was conducted using three-parameter log-logistic model using the DRC package with R software (Ritz et al. 2015). SD-AMATA-C is a known dicamba-sensitive waterhemp accession that was collected from Hand County, South Dakota, in 2021.

Table 2. Regression parameter estimates generated from dose–response curves for SD-AMATA-1 waterhemp accession.[a]

	ER_{50}[b]	
	g ae ha^{-1}	
Accession	Experimental run 1	Experimental run 2
SD-AMATA-C	66 (5)	75 (6)
SD-AMATA-1	67 (7)	129 (8)

[a] SD-AMATA-C is a known dicamba-sensitive waterhemp accession from Hand County, South Dakota.

[b] ER_{50} represents the rate required to achieve 50% reduction in biomass. Numbers in parentheses represent the standard error.

Furthermore, the corresponding field site where the SD-AMATA-1 accession was collected, was monitored in the 2021 and 2022 crop seasons following best weed management recommendations in collaboration with the grower, and no weed survivors were found following dicamba applications. The variation in dicamba sensitivity observed in SD-AMATA-1 compared with the sensitive control may be attributed to a reduction in sensitivity, or to a natural variation that exists in weed accessions, or both (Crespo et al. 2016; Hamberg et al. 2023; Kumar et al. 2020; Singh et al. 2020). Furthermore, seasonal variability has been shown to influence the efficacy of synthetic auxins including dicamba and 2,4-D herbicides (Bobadilla et al. 2022; Johnston et al. 2019; Shergill et al. 2018; Shyam et al. 2022). The natural variability, particularly in *Amaranthus* spp. which are known to have a greater propensity for differential herbicide response, may be attributed to high genetic variability and prolific seed production (Assad et al. 2017; Tranel 2021; Ward et al. 2013; Werner et al. 2020).

Evaluation of Accessions of Concern Identified in 2021

In 2021, 20 weed accessions consisting of 15 waterhemp, four Palmer amaranth, and one dandelion were identified as accessions of concern. Based on Tier 1 screening of these accessions in the greenhouse, 18 accessions, including 14 waterhemp, three Palmer amaranth, and the dandelion accession, exhibited ≥90% mortality at the 1× rate of dicamba at 21 DAT (Figure 8D). An accession of waterhemp from Iowa (hereafter IA-AMATA-1) and a Palmer amaranth accession from Kansas (hereafter KS-AMAPA-1) exhibited 80% and 73% mortality, respectively, at the 1× rate of dicamba at 21 DAT. Surviving plants in the waterhemp accession were stunted and exhibited 65% to 80% injury compared with plants that served as untreated checks and showed signs of regrowth at 21 DAT. At the 2× rate treatment, all the accessions exhibited 100% mortality at 21 DAT except for KS-AMAPA-1, which exhibited 93% mortality with surviving plants exhibiting 75% to 80% injury.

Results from Tier II testing using a larger sample size (approximately 100 plants per accession) indicated 74% mortality of IA-AMATA-1 with an average survivor injury of 59% at the 1× rate of dicamba, whereas all plants exhibited 100% mortality at the 2× rate. A resampling of the IA-AMATA-1 accession was carried out in 2022 from the same field after dicamba had been applied, and the greenhouse test result of the resampled accession indicated 100% control at both 1× and 2× rates of dicamba. Further testing is planned to investigate observed variability in dicamba sensitivity in the IA-AMATA-1 accession.

The KS-AMAPA-1 accession in the Tier-II test exhibited 51% mortality with an average survivor injury of 79% at the 1× rate of dicamba and 80% mortality with an average survivor injury rating of 80% at the 2× rate. Further research is needed to characterize the level of sensitivity reduction to dicamba in KS-AMAPA-1 and IA-AMATA-1. Moreover, the field site from where KS-AMAPA-1 and IA-AMATA-1 accessions were collected are closely monitored for Palmer amaranth and waterhemp control.

Weed species that are resistant to dicamba and other auxin herbicides are relatively rare despite the long-term use of these herbicides, possibly due to multiple SOAs and fitness costs in resistant phenotypes (Busi et al. 2018). However, weeds evolve resistance to herbicides because of overreliance on a specific class or family of herbicides without herbicide diversification, which results in strong selection pressure (Peterson et al. 2018), and there are recent and first reports of confirmed resistance to dicamba in an accession each of waterhemp and Palmer amaranth in the United States (Bobadilla et al. 2022; Foster and Steckel 2022).

To maximize the durability of these weed control solutions, targeted monitoring of suspected resistance cases is essential. Such information is critically necessary, particularly for economically damaging weeds such as Palmer amaranth (Klingaman and Oliver 1994; MacRae et al. 2013; Massinga et al. 2001), waterhemp (Hager et al. 2002; Steckel and Sprague 2004), and kochia (Kumar et al. 2019; Sarangi and Jhala 2018), which are known to be troublesome weeds in regions of the United States that produce maize, soybean, and cotton (Van Wychen 2022). Over 4 yrs of targeted monitoring of inquiries related to reduced weed control efficacy by dicamba, a majority of weed control efficacy-related PPIs were attributed to factors known to cause weed escapes such as poor herbicide application coverage, application to larger weeds, inclement weather, and a combinations of these. A small proportion of PPIs where label recommendations were followed (based on the growers' verbal responses), and yet weed control was unsatisfactory, were further investigated for suspected resistance in a controlled environment. Ninety-five of the 103 accessions that were collected in 2018, 2019, 2020, and 2021 demonstrated ≥90% control at the 1× rate of dicamba in the Tier I stage screening. The remaining eight accessions, which included six waterhemp and two Palmer amaranth accessions, exhibited relatively less sensitivity (73% to 87% control) to dicamba at the 1× rate. However, when those eight accessions were retested either at Tier II or Tier III stages, except for one accession each of Palmer amaranth and waterhemp, six accessions were controlled by ≥92% with dicamba at the 1× rate.

This PPI process has demonstrated to be effective in responding to growers' weed control inquiries, determining factors that affect dicamba efficacy, and identifying accessions with suspected resistance to dicamba that require further characterization. Furthermore, as part of Bayer's stewardship practices, the source fields for all accessions that exhibited variable sensitivity to dicamba at the label rate in greenhouse tests were subsequently monitored in collaboration with growers, and Bayer has emphasized the importance of scouting for suspected weed resistance, reporting herbicide efficacy issues, and following resistance management recommendations.

The long-term goal of an HRM plan is to limit seed production from weeds that survive in-season herbicide applications and thus to delay herbicide resistance evolution. Upon investigation of weed control inquiries, growers were offered recommendations for deploying integrated weed management practices that include crop rotation, use of diverse and effective herbicides with different SOAs, scouting fields after herbicide application to detect surviving weeds or shifts in weed species, and reporting any incidence of nonperformance. Bayer emphasizes the importance of using overlapping residual herbicides with multiple effective SOAs for postemergence weed control to ensure timely application on small, actively growing weeds. In addition, an integral element of Bayer's product stewardship practices is to educate all users of XtendiMax on an annual basis in addition to providing recommendations on best weed management practices.

Practical Implications

Reporting and effectively managing weed resistance issues in their initial stages will benefit growers, technology providers, extension specialists and crop advisors in making informed decisions. It allows the deployment of measures that can minimize the weed seedbank in targeted fields and mitigate the further spread of any resistant populations. Bayer has developed and implemented an HRM plan to support XtendiMax product stewardship by monitoring weed control efficacy issues. Investigations of XtendiMax PPIs related to weed control between 2018 and 2021 identified a Palmer amaranth accession in Kansas and a waterhemp accession in Iowa with suspected resistance, which requires further characterization. The results of this study demonstrate the value of PPI process in resistance management and dicamba as one of the valuable tools in diversifying weed management practices. It is important to note that the interpretations of data on herbicide sensitivity offered in this report is limited to the weed control PPI investigations followed for XtendiMax herbicide in dicamba-resistant soybean and cotton and are not to be generalized at the broader landscape level. However, this monitoring has provided valuable information on the factors that affect weed control efficacy and the extent of suspected resistance to dicamba in dicamba-resistant soybean and cotton in the United States. Bayer will continue monitoring the sensitivity of weed accessions to XtendiMax herbicide and partner with growers and extension specialists for sustainable weed management.

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