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Review article

Synthetic auxin herbicides: finding the lock and key to weed resistance

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ABSTRACT

Synthetic auxin herbicides are designed to mimic indole-3-acetic acid (IAA), an integral plant hormone affecting cell growth, development, and tropism. In this review, we explore target site genes in the auxin signaling pathway including $SCF^{TIR1/AFB}$, Aux/IAA, and ARFs that are confirmed or proposed mechanisms for weed resistance to synthetic auxin herbicides. Resistance to auxin herbicides by metabolism, either by enhanced cytochrome P450 detoxification or by loss of pro-herbicide activation, is a major non-target-site resistance pathway. We speculate about potential fitness costs of resistance due to effects of resistance-conferring mutations, provide insight into the role of polyploidy in synthetic auxin resistance evolution, and address the genetic resources available for weeds. This knowledge will be the key to unlock the long-standing questions as to which components of the auxin signaling pathway are most likely to have a role in resistance evolution. We propose that an ambitious research effort into synthetic auxin herbicide/target site interactions is needed to 1) explain why some synthetic auxin chemical families have activity on certain dicot plant families but not others and 2) fully elucidate target-site cross-resistance patterns among synthetic auxin chemical families to guide best practices for resistance management.

1. Introduction to synthetic auxin herbicides

Synthetic auxin herbicides (Weed Science Society of America/Herbicide Resistance Action Committee Group 4/O) are a class of herbicides that mimic the activity of the plant hormone auxin (indole-3-acetic acid, IAA). Synthetic auxins are most commonly used to control broadleaf weeds in small grain cereals, fallow, and rangeland systems, although some are used to control grass and sedge species. On a global scale, synthetic auxin use ranks third behind glyphosate (Group 9/G) and acetolactate synthase inhibitors (Group 2/B) [1]. The two most used synthetic auxins by global treated area are dicamba and 2,4-Dichlorophenoxyacetic acid (2,4-D). Dicamba has been under particular scientific and public scrutiny in the USA in part due to potential off-target movement and damage to neighboring sensitive vegetation when used later in the growing season during hot summer weather on dicamba-resistant soybean [2,3] and cotton [4].

In the USA, the proceedings of the Weed Science Society of America (WSSA) serve as a timeline for the development of important research topics (Fig. 1). Research on synthetic auxins was the second most common topic over the 9 meetings included in the meta-analysis from 2011-2019, accounting for 12% of all submitted abstracts. Recently new crop varieties have been commercialized with stacked transgenic herbicide resistance traits such as Enlist cotton and Enlist E3 soybean (2,4-D, glyphosate, and glufosinate resistant) [5] as well as Roundup Ready 2 Xtend soybean (glyphosate and dicamba resistant) and Roundup Ready 3 XtendFlex cotton (glyphosate, glufosinate, and dicamba) [6] by Corteva Agriscience and Bayer CropScience, respectively. Research from WSSA indicates a surge of studies on these traits and herbicide combinations. Over subsequent years as the adoption of these technologies grew and use of dicamba and 2,4-D increased, WSSA data shows a steady rise in evaluations of crop injury directly related to synthetic auxin use (Fig. 1).

Abbreviations: MOA, Mode of action; SGT1, Suppressor of G2 Allele SKP1; HSP90, Heat Shock Factor 90; $SCF^{TIR1/AFB}$, Skp1-Cullin-TIR1/AFB Ubiquitination Complex; ARFs, Auxin Response Factors; IAA, indole-3-acetic acid; ABCB, ATP Binding Cassette – B; PIN, PIN-formed; WSSA, Weed Science Society of America; β-CAS, beta-cyanoalanine synthase

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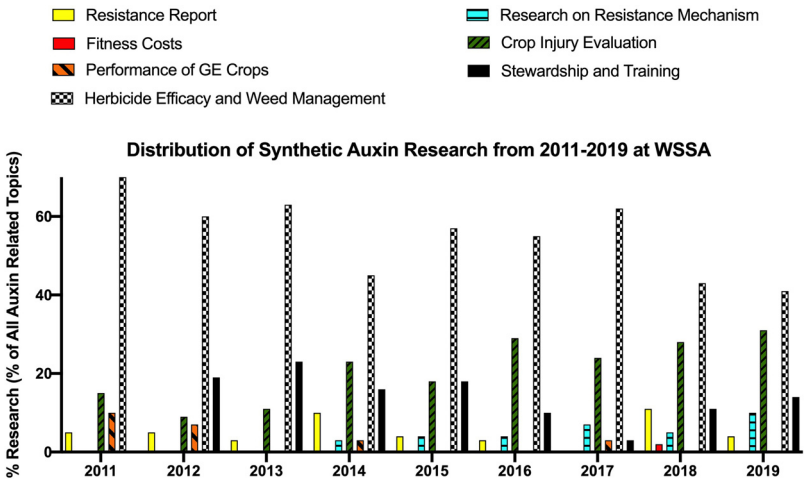

Fig. 1. Research prevalence on topics related to the use of synthetic auxin herbicides. The numbers of abstracts related to synthetic auxin research at the annual Weed Science Society of America (WSSA) conference proceedings from 2011-2019 have been classified into seven topic categories. Data for each topic was normalized as a percent of the total published abstracts on synthetic auxins.

Reports on synthetic auxin resistance mechanisms in weeds have recently increased [1,7, Fig. 1]. New publications reporting synthetic auxin resistance, such as in the important weeds kochia (*Bassia scoparia)* [8] and Palmer amaranth (*Amaranthus palmeri)* [9], have also been increasing in frequency in recent years. Research on efficacy and weed management with synthetic auxin herbicides have been at consistent levels at WSSA over the last 10 years, exemplifying the long-term interest in studying this growing weed science issue.

Since the introduction of 2,4-D as the first synthetic auxin herbicide in 1945, resistance to this class of herbicides has been reported in 41 species, with the first report in 1957 [10]. Despite the importance of this mode of action for weed management, only one molecular resistance mechanism in a weed species has been functionally validated [11]. Due to this lack of information, our scope of understanding of the resistance mechanisms in weedy species for synthetic auxins is relatively poor.

The detailed mechanism of action of synthetic auxin herbicides, specifically the exact genes involved in the phytotoxicity, has long been a mystery locked by the complexity of the auxin signaling pathways. Here we discuss the canonical and non-canonical auxin signaling pathways in model plants and consider potential candidate genes which, if mutated, could be the keys to conferring resistance to synthetic auxins in weeds. Herbicide resistance mechanisms involve mutations and/or changes in expression of target-site genes, as well as changes in expression and/or mutations of genes that reduce the concentration of herbicide at the target-site, known as non-target-site mechanisms [12]. For synthetic auxin herbicides, target-site mutations occur in the auxin perception and signaling complex. Non-target-site mechanisms include changes in herbicide movement and enhanced metabolism to inactive metabolites. We aim to address knowledge gaps in such areas as fitness costs of resistance and effects of ploidy on herbicide resistance. We also identify research needed to understand the differential efficacy of synthetic auxins on different plant families and speculate as to the basis of cross-resistance patterns to chemically dissimilar families of these herbicides.

2. Known and potential resistance mechanisms in the auxin signaling pathway

Auxin signaling involves four major classes of proteins: auxin transporters (influx and efflux: PIN, ABCB, AUX/LAX), transcriptional repressors (Aux/IAAs), auxin response factors (ARFs), and the Skp1-Cullin-F-box TIR1/AFB E3 ubiquitin ligase complex ($SCF^{TIR1/AFB}$). Auxin is transported within and between cells via PIN, ABCB and AUX/LAX transporters. Auxin interacts with the $SCF^{TIR1/AFB}$ complex, which upon creation of the SCF-auxin-Aux/IAA complex, causes ubiquitination of Aux/IAA transcriptional repressors allowing induction of auxin response genes [13,14].

Auxin perception is governed by a molecule of auxin binding to the $SCF^{TIR1/AFB}$ co-receptor complex, which mediates ubiquitination of a family of transcriptional regulators, the Aux/IAA proteins. Functional redundancy exists among the 6 TIR1/AFBs and 29 Aux/IAA proteins in *Arabidopsis*. Some specificity occurs in the interaction of TIR1/AFBs with different auxins and specific Aux/IAA proteins, and the auxin dose dependency of the complexes varies with each specific Aux/IAA protein [15].

Table 1
Assembly of the active $SCF^{TIR1/AFB}$ ubiquitination complex involves several key proteins that affect sensitivity to IAA and synthetic auxins.

Protein	Described insensitivity	Role in auxin pathway	Possible effect on auxin pathway	Source
AXR1 ECR1 RCF1	2,4-D	E1 and E2 ubiquitin-protein ligase complex component	Loss of function could lead to mis/malformation of $SCF^{TIR1/AFB}$ ubiquitination complex	[17,97]
HSP90/SGT1	IAA, 2,4-D, picloram, florpyrauxifen-benzyl	Molecular Chaperone	Loss of function could lead to lack of ubiquitination of Aux/IAAs	[16,98–100]
RUB NEDD8 CAND1	IAA, 2,4-D	Ubiquitin complex modulators	Loss of function could lead to mis/malformation of $SCF^{TIR1/AFB}$ ubiquitination complex	[101,102]
COP9				[103,104]
CUL1		E3 ubiquitin-protein ligase complex component	Missense/loss of function may lead to lowered substrate binding of the $SCF^{TIR1/AFB}$ ubiquitination complex. May lead to incomplete or lowered ubiquitination of the targeted Aux/IAA proteins	[105,106]
TIR1	Dicamba, 2,4-D	Auxin receptor	Lack of auxin perception, lowered posttranslational regulation, leading to lowered/lack of ubiquitination	[18]
AFB5	Dicamba, picloram	Auxin Receptor		

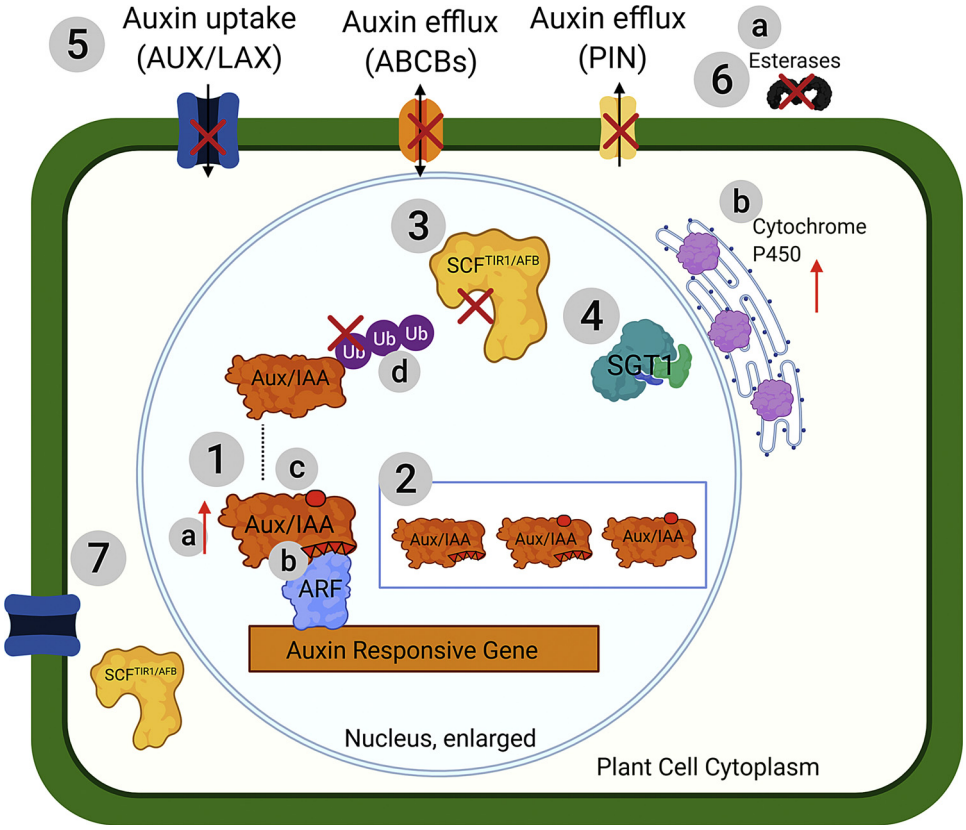

Fig. 2. Confirmed and proposed resistance mechanisms to synthetic auxin herbicides in weeds. Red X indicates loss of function, red up arrow indicates increased expression. 1) Accumulation of Aux/IAA protein dampens the ubiquitination and degradation responses required to release ARF transcription factor for expression of auxin responsive genes due to a, increased expression of Aux/IAA protein (proposed); b, changes to flexibility of regions near the degron of Aux/IAA protein (shown as set of four red triangles, proposed); c, mutation in degron of Aux/IAA protein co-receptor (shown as red dot) to restrict binding to $SCF^{TIR1/AFB}$/auxin complex (confirmed in model systems, Table 2, and in weeds [11]); d, mutations in the regulators of the $SCF^{TIR1/AFB}$ complex restrict ubiquitination of Aux/IAA and subsequent Aux/IAA degradation (proposed). 2) Polyploid containing a mixture of wild-type and resistant mutant Aux/IAA proteins (proposed, mutations in degron and regions near the degron illustrated). 3) Loss of function of $SCF^{TIR1/AFB}$ receptor (confirmed in model systems, proposed in weeds). 4) Loss of function of molecular chaperones such as SGT1 and HSP90 (confirmed in model systems, proposed in weeds). 5) Loss of function of auxin intra-cellular transporters such as AUX/LAX, ABCB transporters, and PIN transporters (proposed). 6) a, loss of activation of pro-herbicide by esterases (proposed); b, enhanced cytochrome P450 metabolism of synthetic auxin herbicides (confirmed in weeds). 7) Changes to fast-acting, non-canonical auxin signaling pathway such as $SCF^{TIR1/AFB}$/auxin interactions at the plasma membrane (proposed) or transmembrane kinases that can stabilize Aux/IAA proteins (proposed in weeds, [42]). Figure created in BioRender (www.biorender.com).

2.1. Mutations in the $SCF^{TIR1/AFB}$

Assembly of the active $SCF^{TIR1/AFB}$ ubiquitination complex involves several key proteins, some of which affect sensitivity to IAA and synthetic auxins (Table 1, Fig. 2). The effects of loss of function mutations or even missense mutation in the genes of the ubiquitination complex would ultimately lead to lack of Aux/IAA degradation and would prompt a resistance response. No reports of mutations in the components of the ubiquitin-conjugating enzymes or ubiquitin ligase have been recorded in weeds, but examples in *Arabidopsis* do illustrate the potential for resistance from these target sites. For example, several mutations in *Arabidopsis* SGT1 chaperone confer resistance to IAA and some synthetic auxin herbicides [16]. Similarly, mutations in proteins that are involved in the modulation of components of the SCF complex such as AXR1 [17] show resistance to IAA and 2,4-D. Critically, mutations in the receptors TIR1 and AFB5 in *Arabidopsis* cause insensitivity to dicamba (*tir1-1* and *afb5*) and 2,4-D (*tir1-1*) [18] or to picloram (*afb5*) [16]. We speculate that due to the lack of observed field-evolved resistance, loss of function mutations in the $SCF^{TIR1/AFB}$ complex may have severe phenotypic consequences and associated fitness costs. Consequently, their initial frequencies in weed populations are likely to be extremely low in the absence of herbicide selection. Alternative resistance mechanisms that are initially more abundant due to a lower fitness penalty may be more easily selected.

2.2. Mutations in Aux/IAAs

Aux/IAAs are transcriptional repressors and auxin co-receptors. Out of 29 Aux/IAA proteins in *Arabidopsis*, many different mutants and expression variants with unique physiological responses have been characterized. Of the four domains present in Aux/IAA proteins, domain II stabilizes interactions with TIR1/AFBs by providing the surface that acts as the auxin co-receptor [19]. Mutations in and around the core motif (GWPPV/I), which is known as the degron, are especially dramatic. Several characterized mutants in *Arabidopsis* showed drastic phenotypic changes such as reduced plant size, leaf morphology, growth, and formation of lateral roots (Table 2). A double base pair mutation within the coding region for domain II of *BsIAA16* in kochia causes an amino acid substitution at a core degron position (Gly127Asn, GWPPV to NWPPV) and confers field-level resistance to dicamba [11]. This is the first Aux/IAA mutation identified to date from natural populations of synthetic auxin resistant weed species (Fig. 2). The authors also suggested that the Gly127Asn mutation in *BsIAA16* conferred cross-resistance to fluroxypyr and 2,4-D; however, greenhouse dose response experiments demonstrated that this kochia line is sensitive to fluroxypyr and 2,4-D [20]. This observation highlights that much more work is needed to fully elucidate patterns of cross-resistance across synthetic auxin chemical families conferred by auxin receptor and co-

Table 2
Variants in Aux/IAA genes. Several characterized mutants in *Arabidopsis* Aux/IAA genes showed severe phenotypic changes in plant size, leaf morphology, growth, and formation of lateral roots. Mutation in the degron of *Aux/IAA16* in *Bassia scoparia* caused dicamba resistance. Auxin insensitivity is also caused by downregulation of Aux/IAA genes in tomato.

Described Mutant	Organism	Source
axr5-1/IAA1	*Arabidopsis thaliana*	[32]
shy2/IAA3	*Arabidopsis thaliana*	[107]
axr2/IAA7	*Arabidopsis thaliana*	[16,108,109]
iaa16	*Arabidopsis thaliana*	[110]
iaa28	*Arabidopsis thaliana*	[111]
SlIAA3	*Solanum lycopersicum*	[112]
SlIAA15	*Solanum lycopersicum*	[113]
SlIAA27	*Solanum lycopersicum*	[114]
Aux/IAA16	*Bassia scoparia*	[11]

receptor mutations as well as possible effects on weed fitness due to these target-site resistance mechanisms, explored below.

In addition to characterized mutations in the degron, variations occurring in the vicinity of the degron [21] could affect auxin-dependent binding to the $SCF^{TIR1/AFB}$ complex, stability of that complex, and/or ubiquitination rate. Increases in expression or half-life of Aux/IAAs could lead to herbicide resistance as such changes would impact the feedback inhibitor response of auxin-induced gene expression (Fig. 2).

2.3. Mutations in auxin response factors

Auxin response factors (ARFs) are transcription factors that bind to auxin response elements on the promoter regions of auxin-regulated genes. A critical auxin-responsive gene regulated by ARFs is 9-*cis*-epoxycarotenoid dioxygenase (NCED), which is the rate-limiting step for abscisic acid (ABA) synthesis. A recent report suggested that ABA synthesis is a key marker of the phytotoxicity response to synthetic auxin herbicide application with a role in suppressing transcription of genes associated with photosynthesis [22]. ARFs comprise four domains, two responsible for DNA binding and regulation, and two for dimerization with Aux/IAAs. The middle region after domain II determines whether the ARF will be a transcriptional activator or repressor [23,24]. In *Arabidopsis*, there are 23 ARFs; 18 are negative regulators of transcription and five are positive regulators. Due to high functional redundancy, many ARF mutants show only modest changes in plant phenotype, although the *arf5* mutant results in extreme loss of body plan and double mutants such as *arf7/arf19* show dramatic changes in lateral rooting responses [25]. The double mutant *nph4-1* (ARF7) *arf19-1* was also highly resistant to 2,4-D and IAA [26]. ARFs 5, 7 and 19 are in the transcriptional activator grouping, and we speculate that it is likely that the reduced redundancy involved with transcriptional activation is more likely to expose auxin insensitivity responses leading to herbicide resistance. However, they will also be more likely to impose fitness costs because there are so few of these activators. No naturally occurring ARF mutants are known to have led to resistant weed populations to date.

2.4. Mutations in Transport Proteins

Polar transport of auxin is conducted by PIN-formed (PIN) efflux carriers and ATP-Binding Cassette class B (ABCB) pump proteins [27–29]. Influx carrier proteins AUX1/LAX contribute to auxin transport [30–33]. All synthetic auxin herbicides are bioavailable as weak acids, thus they will accumulate inside the plant cells due to the anion trap [34]. Some herbicides are not substrates of the AUX1 uptake carrier [35] and must bypass AUX1/LAX influx carriers via passive or low specificity uptake. On the other hand, the phenoxyacetic acids like 2,4-D have high affinities for AUX1. Therefore, the contribution of AUX1/LAX proteins to herbicide transport will vary with each herbicide compound. The *aux1* mutant of Arabidopsis is resistant to 2,4-D [36]. Mutations reducing AUX1/LAX activity are enough to reduce herbicide transport and confer only modest physiological penalty [33,37], and so loss of AUX1/LAX function could be a candidate for synthetic auxin resistance for phenoxyacetic acids (Fig. 2).

Reduced translocation from application point to meristems has been reported for 2,4-D in wild radish (*Raphanus raphanistrum*) [38], prickly lettuce (*Lactuca serriola*) [39], and corn poppy (*Papaver rhoeas*) [40], and for dicamba in kochia [41]. In wild radish, reduced movement of 2,4-D throughout the plant in a 2,4-D resistant line was attributed to loss of cellular transport mediated by ABC transporters [38]. This conclusion was based on the mimicking of resistance in a sensitive line when treated with 1-naphthylphthalamic acid (NPA), which inhibits ABCB and PIN transporter activity. Resistant wild radish populations varied for reduced 2,4-D translocation as well as the increased expression of plasma-membrane associated receptor-like kinases and ABCB19, with no consistent trend across multiple populations for the role of any single mechanism [42,43]. ABCB4 may be a direct herbicidal target of 2,4-D [44,45]. Binding of 2,4-D to ABCB4 results in increased 2,4-D accumulation in *Arabidopsis* root epidermal cells and amplifies herbicidal effects such as swelling and loss of root hairs. It seems that ABCB4 has a role to control auxin concentrations within the cell. Functional redundancy within the ABCB protein family helps to combat loss of function mutations and is a trend seen through the analysis of the auxin signaling pathway; however, the ABCB family may have a degree of specificity for synthetic auxins that is not redundant within auxin transporter gene families. A more common association for ABCBs in resistance mechanisms is with upregulation to rapidly pump compounds out of cells as seen in antibiotic and insecticide resistance for broad-spectrum multi-drug resistance, as opposed to loss of function [46]. Upregulation of one or more ABCBs could readily translate to non-target site resistance to several synthetic auxins. This type of cross-resistance mechanism has not yet been identified in weeds.

3. Herbicide Metabolism

Herbicide metabolism includes (1) activation of a biologically inactive molecule (pro-herbicide) upon entering the plant, and (2) detoxification of the biologically active chemical. In general, metabolic processes work to maintain homeostasis of IAA in cells [47], although IAA homeostasis is destroyed by the arrival of synthetic auxin herbicides. Such disruptions to endogenous auxin pathways contribute to the phytotoxicity of synthetic auxin herbicides, on top of overload to the downstream genetic signaling responses noted above.

Enzymatic activity can contribute to herbicide efficacy through activation of pro-herbicides. An example is the conversion of fluroxypyr meptyl-ester to fluroxypyr acid by esterase enzymes. Several other synthetic auxins are applied as esters including the new aryl-picolinates florpyrauxifen-benzyl and halauxifen-methyl [48]. If the esterase activity is inhibited or reduced, the molecule may not be activated, resulting in no bio-available herbicide to kill the plant. Substantial reduction in metabolic activation of the pro-herbicide triallate to the more toxic triallate sulfoxide confers triallate resistance in wild oats (*Avena fatua*) [49]. Currently no examples of loss of synthetic auxin pro-herbicide activation have been reported in weeds. We predict that loss of function of an esterase gene is a candidate pathway for evolution of resistance to pro-herbicides such as fluroxypyr meptyl-ester (Fig. 2), although such resistance would be recessive, may have fitness costs, and could be impacted by functional redundancy among esterase genes. Another pro-herbicide is 2,4-DB. Legumes such as alfalfa (*Medicago sativa)* do not metabolically activate 2,4-DB to 2,4-D [50], rendering them tolerant to 2,4-DB applications and enabling selective dicot weed control with 2,4-DB in these crops.

Herbicide detoxification can involve one or multiple detoxifying plant enzymes such as glutathione *S*-transferases, cytochrome P450 monooxygenases, esterases, and glucosyl-transferases [51]. Synthetic auxins are subject to various metabolic pathways including reversible amino acid conjugation in dicots and irreversible hydroxylation followed by sugar conjugation in grasses, with variation among species in the specific metabolic processes [52]. Several examples of enhanced metabolic detoxification of synthetic auxins have been reported as resistance mechanisms in dicot weeds [53] (Fig. 2), including hemp nettle (*Galeopsis tetrahit*) resistant to MCPA [54], chickweed (*Stellaria media*) resistant to mecoprop [55], and waterhemp (*Amaranthus tuberculatus*) resistant to 2,4-D [56]. Enhanced metabolism by cytochrome P450-mediated 2,4-D hydroxylation was reversed by the cytochrome P450 inhibitor malathion in *A. tuberculatus* [56]. In some cases, a metabolic resistance gene may confer resistance across chemical families within one mode of action, or even to herbicides from unrelated modes of action [51]. Resistance to non-auxin herbicides mediated via enhanced P450 activity can lead to reduced fitness in the absence of the herbicide [57] and the persistence of such resistance alleles will depend on relative fitness. A similar reduction in fitness may occur for plants

resistant to synthetic auxins through enhanced detoxification.

4. Limitations in Understanding

4.1. Fitness cost of synthetic auxin resistance in weeds

In order for a resistance trait to increase in frequency in a population, the resistance benefit should exceed any fitness cost associated with the resistance trait [58]. Understanding fitness costs linked to synthetic auxin resistance could guide management approaches to exploit fitness costs to decrease the resistance allele frequency. Several studies investigating fitness cost of synthetic auxin resistance mechanisms in weed species have been conducted in kochia [11,59], wild mustard (*Sinapis arvensis*) [60–62], as well as other weedy species [63–65]. Commonly, a fitness cost has been identified. The field-evolved *BsIAA16* mutation endowing resistance to dicamba in kochia has a 75% and 50% fitness cost for reduced seed mass in homozygous and heterozygous resistant plants, respectively [11], possibly related to changes in endogenous IAA signaling as a consequence of the degron mutation in the Aux/IAA16 gene. On the other hand, a recent report [66] showed no measurable fitness cost in several wild radish field populations resistant to 2,4-D after a thorough evaluation of physiological responses and crop competition analysis. We conclude that more understanding of the evolutionary trajectory of synthetic auxin herbicide resistance is needed, and the relative lack of such research, in particular on a greater understanding of fitness costs for various resistance mechanisms [67], is illustrated by the lack of reports on the topic presented at WSSA (Fig. 1).

4.2. Variation in response of broadleaf plant families to different synthetic auxin chemical groups and cross-resistance patterns in weeds

Different chemical families within the synthetic auxins have variable efficacy on certain entire plant families as well as species within the same family. Fluroxypyr, used widely in the USA on rangeland and cereals for broadleaf weed control, is in the pyridine-carboxylic acid group and controls kochia well [68], but has poor control of common lambsquarters (*Chenopodium album*) [69] even though both are members of the *Caryophyllales* family. Conversely, 2,4-D, a member of the phenoxy-carboxylic acid subfamily, has poor control of kochia [68], but controls common lambsquarters well [70]. The picolinic acid herbicide clopyralid is used to control *Asteraceae* and *Fabaceae* weeds in canola, a crop in the naturally clopyralid-tolerant *Brassicaceae* family [71]. Similarly, halauxifen-methyl is used selectively in *Brassicaceae* forage crops to control weeds from several other broadleaf weed families, demonstrating lack of activity on *Brassicaceae* species but a high activity on other families [72].

We propose that an ambitious research effort into synthetic auxin herbicide/target site interactions is needed to 1) explain why some synthetic auxin chemical families have activity on certain dicot plant families but not others and 2) fully elucidate target-site cross-resistance patterns among synthetic auxin chemical families to guide best practices for herbicide rotation and mixture in resistance management. Potential explanations include physiological differences such as wax cuticle thickness and leaf hairiness leading to changes in herbicide translocation to growing points, differential rates of metabolism, and differences in target-site sensitivity among plant species [52]. We propose that the factorial combinations of target-site auxin receptor/co-receptor complexes in key weeds need further characterization with regards to binding of synthetic auxin herbicide from different chemical groups, particularly across the large gene family of Aux/IAA co-receptors. We currently lack a complete understanding of synthetic auxin herbicide-plant interactions across key weed species, though binding efficiency of some of these herbicides to receptors and co-receptors has been characterized. AFB5 was characterized as the preferred $SCF^{TIR1/AFB}$ receptor protein for the picolinate auxin herbicides compared to TIR1 in Arabidopsis, whereas other auxin herbicides preferentially bound to TIR1 [73]. We speculate that different synthetic auxin herbicide families may differ from IAA in their main receptor target/co-receptor complex. Currently, only one receptor (TIR1) has been crystallized [74]. A homology model for AFB5 has been published with picloram bound [15]. A limitation of the TIR1 structure is that it was crystallized with a peptide containing only the degron region that interacts with TIR1 and not the full Aux/IAA co-receptor protein; therefore, questions remain as to how the entire Aux/IAA protein interacts with TIR1. Structures for other auxin signaling proteins have been described, namely ARF5 domain III/IV [75], ARF1 [76], and Aux/IAA17 domain III/IV [77]. Given that at least one auxin herbicide resistance mechanism is based on a mutation in the Aux/IAA co-receptor degron sequence, more structures could help to guide hypotheses regarding mechanisms of resistance, their evolution, and perhaps guide decisions on resistance management using rotations and mixtures among different synthetic auxin chemical families.

4.3. Herbicide interactions with fast acting auxin signaling responses

The auxin signaling pathway via $SCF^{TIR1/AFB}$ (Fig. 2) has been well characterized and is considered the canonical auxin signaling pathway. Changes in abundance of Aux/IAAs have been recorded within minutes of an auxin stimulus. However, other more rapid pathways involving auxin signaling proteins may also exist. Research within the last three years suggests that there may be another role for the long-described $SCF^{TIR1/AFB}$ auxin receptors that acts in seconds, and at the plasma membrane as opposed to the nucleus [reviewed by 78]. This rapid response mechanism affects primary root and root hair growth, potentially valuable traits for herbicides to target, but it is not yet known how many synthetic auxins activate this pathway. Interestingly, these fast root responses required an AUX1 uptake carrier for activity and so the families of synthetic auxins not carried by AUX1 [35] may not engage with this system. A fascinating recent report describes rapid cell death (visible leaf necrosis within 2 hr after 2,4-D application) induced by 2,4-D in 2,4-D resistant *Conyza sumatrensis* [79]. We speculate that this 2,4-D resistance could involve rapid auxin response signaling pathways such as a plasma membrane receptor leading to H_2O_2 production and rapid cell death within 15 min of 2,4-D application, thereby reducing 2,4-D translocation to the apical meristem and causing resistance.

Transmembrane kinases have also been associated with rapid auxin signaling. In the presence of auxin the C-terminus on a transmembrane kinase is cleaved and translocated to the nucleus where it stabilizes specific Aux/IAA proteins [80]. This stabilization regulates auxin response factors, inducing transcription of auxin-induced genes [81]. The transmembrane kinase gene family is composed of four functional overlapping members, *TMK1-4* [82]. Single mutants in these genes have no observable phenotype. Double null mutants (*tmk1; tmk4)* are less sensitive to auxin, and triple mutants (*tmk1; tmk3; tmk4)* completely auxin insensitive and have lower seed production phenotypes and reduced size. Further work is needed to characterize how activity of these transmembrane kinases responses may potentially regulate synthetic auxin herbicide activity and selectivity, as well characterizing a potential role for transmembrane kinases in evolved 2,4-D resistance in weeds [42].

4.4. Resistance to quinclorac in grasses

Quinclorac is a unique synthetic auxin that is primarily used in rice and is selective against annual grasses and broadleaf weeds [83]. Resistance to quinclorac in grass weeds has been a management issue, including quinclorac resistance in smooth crabgrass (*Digitaria ischaemum*) [84] and *Echinochloa* species. One resistance mechanism reported in *Echinochloa* has been increased activity in the enzyme beta-cyanoalanine synthase (β-CAS), the key enzyme in cyanide degradation. The increase in β-CAS activity is proposed to detoxify hydrogen

cyanide, which is produced as a consequence of ethylene biosynthesis following quinclorac application [83,85–88].

Several mutations associated with enhanced activity have been identified in β-CAS. Met-295-Lys was identified in two resistant *Echinochloa crus-galli* var. *zelayensis* lines, which is the sequence present in the same position in naturally quinclorac-tolerant rice [86]. Three mutations in *Echinochloa crus-galli* var. *mitis* (Asn-105-Lys, Gln-195-Glu, and Gly-298-Val) were determined to expand the binding pocket, conferring higher β-CAS activity [88]. However, in other resistant *Echinochloa* lines the same overall effect has been achieved by reducing ethylene synthesis, hence alleviating the source of cyanide production [89,90]. Other mechanisms that reduce the impact of elevated cyanide such as downregulation of genes involved in photosynthesis and electron transport have been reported from *Echinochloa crus-galli* var. *zelayensis*, suggesting a broad array of mechanisms have been selected which allow it to survive quinclorac application [87]. Whether these confer resistance to other synthetic auxins as well as quinclorac is unclear, and further investigation into quinclorac resistance mechanisms is needed. Both target-site and non-target-site mechanisms may be involved in quinclorac resistance in grass weeds.

4.5. Impacts of polyploidy on synthetic auxin resistance

Polyploidy is common throughout the angiosperms, with 30-70% of plant species within families estimated to be polyploid [91]. Of the 41 species that have been reported with resistance to synthetic auxins, 35% are polyploid and/or mixed ploidy [10]. Understanding resistance mechanisms in polyploid weed species can be especially complex as the presence of multiple genomes results in a suite of regulatory mechanisms that are not found in diploid species. Many of the cases of quinclorac resistance in grasses occur in the *Echinochloa* spp., which are frequently polyploid and similar considerations arise. Allele dosage, gene sub-functionalization, silencing and redundancy, inheritance modes, and mutational load all have implications. This may be particularly true for synthetic auxin resistance mechanisms resulting from target-site mutations, such as a mutation in an Aux/IAA gene. In this instance, resistance in a diploid species may be dominant but the same mutation in a polyploid may have less effect, due to the reduced representation of the resistance allele in the total gene expression of a polyploid. By the same measure, high relative fitness may be maintained in a polyploid that is resistant because this plant may express both mutant and non-mutated versions of the affected gene from homoeologous chromosomes, allowing resistant alleles to remain cryptic with minimal apparent fitness penalty until their selective advantage is uncovered by herbicide application. Genome assembly for polyploids is especially challenging. Therefore, polyploidy in weeds presents a considerable challenge when investigating herbicide resistance mechanisms for synthetic auxins given the complex gene families for auxin co-receptors, transporters, and auxin response factors. The inheritance of synthetic auxin resistance traits has been determined mostly in diploids [e.g., 92,93] as well as identification of specific resistance mechanisms [11], but despite these challenges, further investigation is needed to better understand the evolutionary trajectory of synthetic auxin resistance in polyploid weed populations.

5. Concluding thoughts and available resources

Research to unlock the mysteries of resistance to synthetic auxin herbicides and cross-resistance patterns across weed species will require comprehensive sequence data for the genomes involved. The availability of weed genomics resources is expanding, through efforts of individual groups [e.g., 94,95] and the International Weed Genomics Consortium [96]. With better genomic tools, the identification and validation of synthetic auxin resistance genes will improve and help inform the future development and sustainability of synthetic auxin herbicides. The research that has been done to date enables predictions of possible genetic mechanisms for evolved synthetic auxin herbicide resistance (Fig. 2). As we continue to unravel the interactions between synthetic auxin herbicide molecules and auxin signaling pathways, new insights may lead to novel inhibitors that bypass existing resistance mechanisms or enable inhibition of other components of the auxin signaling pathway.

Declaration of Competing Interest

The authors report no declarations of interest.

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