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Mechanistic understanding and sustainable management of non-target site herbicide resistance in modern day agriculture

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Abstract: Background: The evolution of non-target site resistance (NTSR) to herbicides in weeds has made weed management extremely difficult. Weeds can develop NTSR to herbicides because of changes in one or more physiological processes. **Objective**: This review aims to address the complexities of NTSR by investigating the factors influencing the evolution of NTSR in weeds. It explains mechanisms associated with NTSR and examines sustainable management strategies. **Methods**: This review draws upon an extensive examination of existing literature on NTSR. It summarizes NTSR mechanisms in weeds and discusses strategies that delay the evolution of herbicide resistance. **Results**: The analysis reveals that NTSR mechanisms threaten not only the efficacy of existing herbicides but also the utility of new herbicides. The study highlights that crop diversification, improved herbicide use patterns and understanding of weed biology, including fitness costs and gene flow, are crucial for developing sustainable weed management strategies. The study advocates a holistic approach, integrating diverse weed management practices and modern technologies, to effectively address the challenges posed by NTSR and ensure sustainable herbicide resistance management. **Conclusions**: A holistic approach is urgently needed to manage NTSR. Understanding the complex interaction of genetic factors, inheritance patterns, effects of herbicide application patterns, and fitness costs is crucial for managing NTSR. This knowledge informs effective use of technology and conventional tools for effective weed control.

Keywords: Herbicide Resistance; Chemical Control; Integrated Weed Management; Metabolic Resistance

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1. Introduction

Chemical control offers effective, low-cost, quick, and noticeably profitable results in weed management compared to other weed management tools thus most growers still opt for herbicide use (Valverde, 2007). However, non-judicious herbicide use leads to the evolution of resistance. Continuous use of herbicides with the same mode of action increases the selection pressure for resistance development among weed populations (Jugulam, Shyam, 2019). While effective in controlling the present weed population, these herbicides eventually induce strong immunity in surviving weeds against future applications. The two major types of herbicide resistance mechanisms include the target-site and non-target-site resistance (NTRS). Target site resistance (TSR) occurs at the specific site of action and typically involves mutations in the gene encoding the protein target of herbicides (Gaines et al., 2020). These mutations affect herbicide binding at appropriate domains. While the majority of TSR mutations involve non-synonymous single-nucleotide polymorphisms (SNPs), polymorphisms over multiple codons can also happen (Gaines et al., 2020). The complexity of TSR mechanisms increases when herbicides bind to multiple proteins. In contrast, NTSR occurs when one or more plant physiological processes are affected. For example, changes in herbicide absorption, translocation and metabolism are associated with NTSR (Jugulam, Shyam, 2019). The NTSR tend to be more complex than TSR, potentially leading to cross-resistance to various modes of action. Increased activity of certain enzymes such as cytochrome P450 monooxygenases, glucosyl transferases and glutathione S-transferases (GSTs) have been reported to induce NTSR in some weeds (Yu, Powles, 2014).

The evolution of NTSR poses a challenge to weed control since it can confer resistance to a variety of herbicides including some modes of action that are yet to be discovered (Jugulam, Shyam, 2019). It is more difficult to manage NTSR herbicide resistance than TSR because simply switching mode of action may not be effective. The polygenic nature of NTSR mechanisms, conferring resistance across a spectrum of herbicides, especially in the early phases of post-herbicide application, suggests a primary avenue through which weed populations respond to herbicide applications (Kreiner et al., 2018). Polygenetic inheritance characteristic of NTSR has been reported as a common mode of resistance against glyphosate, acetyl-CoA carboxylase

(ACCase) and acetolactate synthase (ALS) inhibitor herbicides and has become very difficult to manage (Kreiner et al., 2018). Moreover, changes in climatic conditions, particularly elevated temperatures, can exert a pronounced impact on the weed control efficacy (Matzrafi et al., 2016). For instance, sensitivity of various grass weeds to ACCase inhibitors was found to reduce under high temperatures (Matzrafi et al., 2016).

Better understanding of NTSR mechanisms can help optimize weed control programs and substantially reduce the selection pressure and consequently herbicide resistance. Therefore, this paper aims to provide a snapshot of NTSR without detailing all instances of this type of resistance in different weed species as this has been comprehensively discussed recently (e.g. Jablonkai, 2015; Jugulam, Shyam, 2019; Suzukawa et al., 2021). Rather, efforts were made to summarize the wide array of resistance mechanisms. Most of the NTSR mechanisms are not fully understood (Baucom, 2019, Goldberg-Cavalleri et al., 2023), however, this review will provide an account of what is known about these mechanisms. The review further discusses how various herbicide resistance management strategies delay resistance evolution, with emphasis on NTSR. The knowledge will provide insights into how a holistic approach to managing NTSR can be achieved in modern agriculture.

2. Mechanisms of NTSR

Prediction of the resistance evolution traits is achieved if the mechanisms are understood. NTSR refers to a combination of different processes that reduce the concentration of active ingredients reaching the target sites as well as coping with herbicide inhibition effects (Gaines et al., 2020). Resistance results from modified plant physiology often leading to reduction in herbicide absorption, vacuolar sequestration, altered membrane transport activity, altered translocation (foliar hypersensitivity and root exudation) and increased metabolism (Jugulam, Shyam, 2019). Each of these physiological processes is discussed below.

2.1 Reduced herbicide absorption

Trans-laminar and systemic herbicide action can only be facilitated when the active ingredient has been successfully absorbed into the plant tissue upon contact (Domínguez-Valenzuela et al., 2021). Systemic herbicides penetrate via the roots or shoots, move through vessels or phloem and can be effective at sites which were not directly sprayed. Resistance due to reduced absorption has emerged in some weeds which is mainly driven by decreased translocation of herbicide molecules to the target site. This mechanism should not be confused with inherent tolerance, which is a natural phenomenon in plants. Reduced absorption is a mechanism developed in initially susceptible weed species (Powles, Yu 2010).

Foliar altered absorption has been observed in several problematic species like *Amaranthus tuberculatus* (Moq.) J. D. Sauer (Nandula et al., 2013), *Lolium multiflorum* Lam. (Michitte et al., 2007) and *Sorghum halepense* L. (Vila-Aiub et al., 2012), but no evolved altered absorption resistance has been reported in roots yet. Favorable conditions for soil-applied herbicide movement and herbicide characteristics tend to increase chemical absorption. Important edaphic factors influencing herbicide absorption can include soil type, pH, moisture, and cation exchange capacity. Any evolved plant root physiology conferring resistance is not significant because plant attributes have little influence on root herbicide absorption compared to environmental and herbicide factors, whereas plant leaf characteristics are equally important to other factors in influencing the herbicide efficacy when applied on plant canopy (Gaines et al., 2020).

Cellular uptake of herbicide droplets occurs after the herbicide overcomes several leaf surface barriers and passively diffuses through the cell membrane (Suzukawa et al., 2021). This process depends on the interaction between herbicide chemical properties and leaf surface characteristics. Herbicide-resistant weeds develop cuticle characteristics that result in herbicide penetration reduction. Cuticle encompasses the continuous complex matrix of extracellular wax layer, the cutin, and pectin that coats the epidermis of leaves (Schuster, 2016). Development of a thick waxy layer and thick upper leaf surface can decrease cuticle herbicide retention and subsequently their absorption. For example, leaf cuticle of a resistant biotype of *Lolium rigidum* L. found to have higher epicuticular wax density than that of a susceptible biotype, while cell wall did not exhibit hindrance in cellular uptake of herbicide (Prado et al., 2005). The mechanism responsible for increasing the epicuticular wax density is not fully understood, however, studies have suggested that transposable elements (TEs) contribute to plant adaptive evolution after exposure to stressful environmental conditions (González, Petrov, 2009; Franco-Ortega et al., 2021). TEs are short DNA sequences that can change positions within a sequence and can often have phenotypic variations by generating a wide variety of mutations (Franco-Ortega et al., 2021). A type of TE classified in the long terminal repeat retrotransposons was observed to influence cuticle chitin catabolic processes (Franco-Ortega et al., 2021). In future, weed genomics research can help es to identify possible roles of TEs in NTSR reduced absorption mechanism.

The presence of dense pubescence covering the surface of plant leaves or stems can lead to reduced herbicide absorption post-application (Zhang et al., 2020). Dense pubescence refers to a high concentration or abundance of fine, short, soft hairs, also known as trichomes, which often serve as the primary line of defense for plants (Zhang et al., 2020). A recent study has found a positive correlation between branched trichomes and glyphosate resistance in *Abutilon theophrasti* Medik., indicating that branched trichomes function as a trait enabling herbicide

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resistance (Johnson, Baucom, 2023). The research further demonstrated that continuous exposure to herbicides could result in adaptive evolution of *A. theophrasti* from single branched to a multi-branched trichome phenotype and potential compartmentalization of herbicides into the cell walls and vacuoles of branched trichomes contributing to herbicide resistance in *A. theophrasti* (Baucom, 2019; Johnson, Baucom, 2023). Although reduced absorption is not widespread, resistant populations of noxious weeds like *Glechoma hederacea* L. and *Lactuca serriola* L. are known to exhibit this process (Kohler et al., 2004; Riar et al., 2011).

2.2 Vacuolar Sequestration

Vacuolar sequestration refers to mechanisms in which weeds sequester applied herbicide within plant vacuoles or leaf trichomes to inhibit its translocation (Yu et al., 2010). This affects herbicide efficacy since its effectiveness relies on reaching a specific target site localized within organelles. Environmental conditions, especially temperature directly influence the vacuolar sequestration mechanism associated with increased expression of ATP-binding cassettes (ABC) transporters (Suzukawa et al., 2021). For example, vacuolar sequestration of herbicide in a resistant population of *Lolium Perenne* L. was found to substantially increase with increasing temperature (Ghanizadeh et al., 2015). Vacuolar sequestration of glyphosate has been extensively documented in various weed species including *Eleusine indica* (L.) Gaertn. (Lee, Ngim, 2000), *L. rigidium*, and *Conyza canadensis* (L.) Cronquist (Moretti, Hanson, 2017). In *C. canadensis*, sequestration of glyphosate was rapid, showing 85% fractional vacuole occupancy in resistant biotypes 24 hours after application, whereas susceptible vacuole tissues showed glyphosate occupancy of 15% after the same time.

Vacuole sequestration of glyphosate not only reduces the amount of herbicide translocated from source to sink but also reduces the available glyphosate in the cytoplasmic pool as observed in *C. canadensis* (Moretti, Hanson, 2017). In Australia, paraquat resistance in weedy *Hordeum spp.* and *L. rigidum* biotypes was also linked to vacuolar sequestration imparting NTSR (Preston et al., 1992, Yu et al., 2010). According to recent study, ABC transporters played an important role in translocating metabolites across membranes through ATP hydrolysis and were additionally involved in the compartmentation and detoxification of herbicides in *Alopecurus myosuroides* Huds. as confirmed by transcriptomic analysis (Goldberg-Cavalleri et al., 2023).

2.3 Altered translocation

Systemic herbicides are designed to be absorbed by plant tissues and then translocated from the site of application to their target site (Domínguez-Valenzuela et al., 2021). This transport involves active and or passive diffusion processes. Resistant weeds develop mechanisms that sequester herbicides in the source or prevent normal movement by altering the activity of membrane transporters (Gaines et al., 2020). These sequestration mechanisms are discussed below.

2.3.1 *Altered membrane transporters activity*

Most herbicides are transported by the plant cell via a non-facilitated diffusion process, however, hydrophilic herbicides such as 2,4-D and paraquat are absorbed via an active transport mechanism where molecules move against a concentration gradient (Jarzyniak, Jasiński, 2014). This energy-dependent mechanism utilizes a membrane protein carrier to transport the herbicide across the membrane. Certain plants that can alter the activity of these transporters can reduce herbicide effectiveness. An account of this mechanism has been reported on *Raphanus raphanistrum* L. resistance to 2,4-D (Goggin et al., 2016). The resistant *R. raphanistrum* plants altered a plasma membrane ATP-binding cassette auxin transporter in *Arabidopsis* (Goggin et al., 2016). The biotypes then developed ABCB-type mutants displaying low affinity for 2,4-D and, thus reduced the amount of translocated herbicide into the cell (Ghanizadeh, Harrington, 2017a). These mutants were a result of alterations in functioning of protein carriers enabling 2,4-D transport (Goggin et al., 2016).

Similarly, resistance to glyphosate and MCPA in *L. rigidium* and *R. raphanistrum*, respectively was attributed to a root exudation process caused by rapid herbicide translocation and, eventually, reduced accumulation in root tissues (Jugulam, Shyam, 2019; Lorraine-Colwill et al., 2001). The overactive or overexpressed ABC and related transporters promote this resistance mechanism by releasing herbicide compounds from the treated plant into the rhizosphere.

2.3.2 *Foliar hypersensitivity*

Foliar hypersensitivity is a process in which mature leaves undergo severe necrosis and die, thereby preventing herbicide translocation to developing foliage and apical meristems (Ghanizadeh, Harrington, 2017a). The term hypersensitivity is employed when cells die without observed defence to the applied herbicide. Physiology of this mechanism remains unclear, however, studies on resistant biotypes of *Ambrosia trifida* L. (Moretti et al., 2018) and *Conyza sumatrensis* (Retz.) E.Walker (Queiroz et al., 2020) have denoted rapid necrosis to NTSR. Initiation of foliar hypersensitivity relies on increased synthesis of reactive oxygen species and nitric oxide defense response (Queiroz et al., 2020). This triggered sacrificial defense is accounted for by evolved resistant proteins, however, no studies determining herbicide mechanisms have been conducted yet. Resistant phenotypes show injury of mature leaves within hours after application resulting in efficient containment of herbicide (Queiroz et al., 2020).

2.4 Herbicide metabolism

The NTSR mechanism facilitated by enhanced metabolism of herbicide molecules enables plants to withstand herbicide inhibition at the target site by detoxifying the herbicide (Jablonkai, 2015). The metabolism of herbicides occurs in three phases which include redox reactions to increase its hydrophilicity forming free amino, hydroxyl, or carboxylic acid groups (phase I), further reduction of phase I products to water conjugates by directly conjugating herbicides or conjugating a sugar molecule to a variety of lipophilic molecules including xenobiotics (phase II), and storage of metabolized compounds (phase III) (Jablonkai, 2015; Gaines et al., 2020). Membrane-bound metabolizing enzymes such as GSTs and P450s located in the endoplasmic reticulum are responsible for detoxifying herbicides and have been well-documented in crop plants (Baerg et al., 1996; Han et al., 2013; Liu et al., 2018). P450 are heme-iron-dependent proteins which mainly facilitate the oxidation, hydroxylation, deamination or dealkylation of herbicides to produce less mobile, hydrophilic metabolites in phase I (Rigon et al., 2020). Primary metabolism in phase I leads to crop selectivity (Riechers et al., 2010). In phase II, directly conjugate herbicides by glycosylating specific functional groups of lipophilic molecules, forming water-soluble plant metabolites (Yuan et al., 2007). These intermediates lose their herbicidal phytotoxicity and are then compartmentalized into the vacuole or cell wall in phase III. The incorporation of metabolites is mediated by ABC transporters, however, few studies link ABC genes to herbicide resistance (Rigon et al., 2020).

Enhanced metabolism NTSR is most common and well-studied mechanism accounting for *Alopecurus japonicus* Steud. and *Echinochloa crusgalli* (L.) Beauv. resistance to ACCase inhibitors, *A. tuberculatus* and *Amaranthus palmeri* S.Watson resistance to photosystem II (PS-II)-Inhibitors (Jugulam, Shyam, 2019). Enhanced metabolism NTSR mechanism via GST-atrazine conjugation-resistant *A. palmeri* was found to be inherent (Nakka et al., 2017). A weighted gene co-expression network analysis (WGCNA) on transcriptome identified key genes that promoted core mechanisms (Franco-Ortega et al., 2021). Similarly, different GST proteins accounted for enhanced metabolism of chlorotoluron and fenoxaprop-P-ethyl in *Alopecurus myosuroides* Huds. Their study also confirmed that TEs were linked with herbicide metabolism. The WGCNA provides a great opportunity to advance research on gene expression and mechanisms conferring NTSR.

Even though NTSR mechanisms are not fully understood, Table below summarizes known NTSR mechanisms on different modes of action.

3. Sustainable management of NTSR

Resistance management is guided by Harper's observation which encourages lowering the severity of selection and preventing the surviving, resistant weeds from reproducing (Norsworthy et al., 2012). Factors influencing the selection pressure in weeds relate to cropping systems, weed features and herbicide characteristics. These factors determine the persistence of certain weeds and eventually the survival of resistant biotypes. Previously, researchers focused more on TSR and partially neglected looking into NTSR mechanism, however, next-generation sequencing technologies and transcriptomics are now being used in ongoing research to uncover underlying molecular and genetic regulations of NTSR (Gaines et al., 2020). It is now evident that the NTSR mechanisms can be expressed for several herbicides with different sites of action if they share a common degradation route. As such, the NTSR requires integrated diversification of chemical and nonchemical

Table - A list of known non-target site resistance mechanisms for different herbicide modes of action

Non-target site mechanism	Mode of action	Reference
Reduced absorption	· Synthetic auxinic herbicides · Photosystem II (PS-II) inhibitors · Acetyl-Coenzyme A carboxylase (ACC-ase) inhibitors	· (Kohler et al., 2004) · (Svyantek et al., 2016) · (De Prado et al., 2005)
Altered translocation	· Synthetic auxinic herbicides · Photosystem I (PS-I) inhibitors · 5-Enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors	· (Dang et al., 2018) · (Yu et al., 2010) · (Nandula et al., 2013)
Foliar hypersensitivity	· Acetoacetate synthase (ALS) inhibitors · EPSPS inhibitors · Photosystem II (PS-II) inhibitors	· (Queiroz et al., 2020) · (Moretti et al., 2018) · (Burnet et al., 1993)
Enhanced metabolism	· ACC-ase inhibitors · ALS inhibitors · EPSPS inhibitors · Synthetic auxinic herbicides · PS-II inhibitors · Protoporphyrinogen Oxidase (PPO) inhibitors · 4-Hydroxyphenylpyruvate Dioxygenase (HPPD) inhibitors	· (Zhao et al., 2019) · (Tranel, Wright, 2002) · (Dominguez-Valenzuela et al., 2017) · (Figueiredo et al., 2018) · (Anderson, Gronwald, 1991) · (Varanasi et al., 2019) · (Nakka et al., 2017)

weed control approaches (Jugulam, Shyam, 2019). Although applied generally as best practice to manage any type of herbicide resistance, the following approaches are crucial for the sustainable management of NTSR in weeds.

3.1 Crop diversification

Crop diversification is the most popular traditional weed control strategy, as well as a very effective and sustainable method of reducing resistance occurrence (Rouhi, 2013). Increased soil nutrient recycling, improved soil structure, improved water use efficiency, and effective crop pollination are some additional benefits of crop diversification (Sharma et al., 2021). Strategic use of crop rotation, intercropping and cover crops can play a vital role. Crop rotation is growing different crops in a specified sequence in a field over a specific period to provide temporal variability (Ball et al., 2021). As a result, weed cycles are naturally broken. However, the best results are observed if the rotated crops do not favor the establishment of the same weed species. While specific studies that directly link crop diversification effects to NTSR are limited, Davis et al. (2012) reported that longer rotations (four years) required ten times less herbicide use than shorter (two years) rotations, which also meant lower selection pressure resistance. Crop rotations result in the use of alternative management strategies, patterns, and timings, which help diversify weed selection pressure. Diverse rotations can affect weed density processes, but they may not strongly inhibit established weed seedling growth (Ball et al., 2021).

Other strategies like using competitive crop genotypes could exert a more substantial influence on reducing weed biomass (Bajwa et al., 2017). Intercropping is a practice of growing more than one crops concomitantly in the same field while cover crops are grown between growing seasons of main cash crops (Sharma et al., 2021). These approaches increase crop competitive advantage over weeds for available resources as crops exhibit different resource capture strategies. For instance, parasitic weed, *Striga* spp. was effectively controlled by intercropping. Suicidal germination of this weed was stimulated when non-host intercrops acted as trap crops (Zwanenburg et al., 2016). The non-host catch crops and germinated *Striga* spp. seedlings were then destroyed using tillage implements or non-selective herbicides (Kountche et al., 2019). Relay intercropping systems and cover crops can also help deplete weed seedbank and aid weed suppression, thereby reducing selection pressure (Sharma et al., 2021).

Due to the complexity of farming systems, model-based exploration tools are frequently employed to optimize traditional crop diversification programs. These models assist growers in making cropping plans and crop rotation decisions (Dury et al., 2012). The development of modelling approaches is challenging often due to lack of empirical data and geographic variability. The available information is limited to selected cases and surveys. However, software tools like ROTAT (Dogliotti, 2003), can be used to generate possible crop rotation plans for various crops based on provided agronomic parameters by the user. The use of these crop planning tools in collaboration with risk herbicide resistance calculators (Lawrence et al., 2020) can help advance herbicide resistance management on a sustainable basis.

3.2 Improved herbicide use patterns

Herbicide resistance management also requires novel modes of action, yet no significant development has happened in the last three decades when compared to the preceding era (Sharma et al., 2021). It is therefore necessary to use the available herbicides in rotations or mixtures to delay resistance. Herbicide rotation involves using chemicals from different modes of action in different parts of the crops rotation within a field, while a herbicide mixture application is the application of two or more herbicides mixed in a tank. Tank mixtures can either include herbicides with different modes of action or herbicides in the same family (Jhala et al., 2013). Repeated use of a chemicals targeting a single site of action leads to rapid evolution of herbicide resistance (Beckie, 2006; Mallory-Smith, Retzinger, 2003). Recently, a range of pre-packaged mixtures have been introduced into the market providing an opportunity to employ these best practices.

Computer models have been used to compare the effectiveness and resistance evolution potential of single herbicide, mixtures and rotated herbicides (Birch, Shaw, 1997). Simulations have confirmed that simple herbicide rotations have little influence on delaying resistance, while mixing or rotating reduced the resistance occurrence by 2–3 times (Gressel, Segel, 1990; Birch, Shaw, 1997). Using this technology and optimizing its insights can result in the development of tools that can forecast herbicide evolution based on a production practice, as well as recommendations for complex herbicide rotations and/or mixtures to apply.

Actual and perceived environmental and health concerns related herbicides have intensified pressure on farmers to reduce pesticide use, thus they opt to reduce herbicide dosage applications (Kudsk, 2008). In some European countries, the use of below label rate is getting popular. However, this practice could have a potential role in herbicide resistance evolution rates since it increases the risk of developing minor herbicide resistance traits, which, over time, could contribute to the rapid evolution of NTSR (Ofosu et al., 2023). Low dosage rate applications reduce mortality rate, thereby increasing the rate of selection of resistant biotypes and facilitating their accumulation in cross-pollinating weeds (Jugulam, Shyam, 2019). Mild stress due to low dosage rate applications or herbicide hormesis can precondition plants or their offspring to tolerate subsequent exposure (Ofosu et al., 2023). A study on *L. rigidum* reported that the resistant traits selected by low rates were NTSR (Manalil et al., 2011). suggested that resistance evolution after low-dose applications was a result of surviving plants that initially possessed weak resistant traits. These traits were enriched through recurrent selection and accumulated through cross-pollination (Manali et al., 2011).

Similarly, effective herbicides tend to impose higher selection pressure on resistant individuals in that population (Hansen et al., 2014). A small number of high seed producing resistant individuals drive inheritance of resistance in subsequent generations leading to population scale, wide-spread resistance (Hansen et al., 2014). Unfortunately, effective herbicides eliminate all the susceptible individuals leaving the most resistant ones to cross-pollinate and produce more dominant species. This process can be delayed by rotating herbicides and applying them in mixtures since herbicide effectiveness reduces selection heterogeneity, thus reducing the chances of survival of resistant biotypes (Gressel, Segel, 1990; Beckie, Reboud, 2009). If practiced in collaboration with herbicide rotations, selection is eliminated, and resistant biotypes can be preserved at a low frequency (Busi et al., 2020).

The ability to produce resilient phenotypes hastens the evolution of NTSR (Belz, 2020; Mollaee et al., 2020). Although researchers still encourage adhering to label rates to ensure significant weed mortality, there is ongoing pressure to minimize the quantity of chemicals used in field applications (Manalil et al., 2011). Models to address this conundrum have been developed to determine optimal doses taking herbicide resistance into consideration. These simulations can assist in reducing herbicide use while applying the most effective dosage rate that minimizes environmental impacts. Variable-rate technology is also an important tool for reducing the application of pesticides (Ahmad, Mahdi, 2018). It can adapt parameters on herbicide applicator machine according to the exact variation in plant growth or environmental factors and apply adjusted rates (Genna et al., 2021). Weed maps have also been used to demonstrate how "mix and rotate" approach can manage the evolution of metabolic NTSR by integrating both phenotypic and genetics-based herbicide-resistant assays (Comont, Neve, 2021). The image sensors have been successfully used to quantify the maximum quantum efficiency of PS-II (Fv/Fm) of susceptible and resistant *Papaver rhoeas* L. and *Stellaria media* (L) Vill. populations in response to ALS inhibitor herbicides (Linn et al., 2019). Their investigation managed to distinguish susceptible and resistant plants 3 days after treatment with 62–100% accuracy (Linn et al., 2019). Linn et al. (2019) confirmed that imaging can help advance the monitoring process of susceptible and resistant weeds, enabling the early detection of potential threats of both NTSR and TSR threats.

3.3 Better understanding of weed biology

Understanding weed emergence patterns, growth patterns, fecundity and their persistence is essential for developing effective and lasting management strategies (MacLaren et al., 2020). Effective weed control strategies can be implemented when the weeds are more susceptible. The following aspects of weed biology play a significant role in NTSR evolution and management:

3.3.1 *Better understanding of plant characteristics*

Several plant factors influence how herbicides are taken up and metabolised by weed plants even under optimal environmental conditions (Petersen, Hurle, 2000). The plant size, cuticle thickness, leaf angle, and leaf area are some of the most important biological features and Knowledge of their impact on herbicide efficacy is important in planning herbicide applications (Varanasi et al., 2016). For example, opening of stomata and the presence of thinner leaves with a greater specific area contribute to increased surface coverage, thereby enhancing herbicide penetration in plant foliage (Mohr et al., 2007; Varanasi et al., 2016). Additionally, high plant branching casts shadows on lower parts of the plant, thus preventing effective herbicide application (Mohr et al., 2007). Stomatal regulation has been linked with the uptake and effectiveness of glyphosate and overall resistance dynamics (Baccin et al., 2023). The study showed that glyphosate resistant *Conyza bonariensis* exhibited altered adaxial (leaf upper side) morphology, characterized by increased number of trichomes and modified stomatal density compared to the susceptible biotypes (Baccin et al., 2023). Similar modifications in leaf morphology were documented in glyphosate-resistant *L multiflorum* and paraquat-resistant *C. sumatrensis* populations (Galvani et al., 2012; Pereira, 2019).

Temporal variations can also play a significant role in regulating certain plant traits which have a significant impact on herbicide efficacy. Variables such as the time of day or early-season scheduling should be carefully considered, as they can significantly influence herbicide control (Mohr et al., 2007). The link between application timing and NTSR evolution is unclear and warrant more investigation (Metzger et al., 2019). Model-based simulations can potentially be used to predict herbicide response. Coupled with hyperspectral images, modeling can be used to track the efficacy of herbicides after application and potential resistance evolution. Although not fully explored in weed management, Ali et al. (2022) used hyperspectral images to monitor the efficacy of bentazone in a soybean (*Glycine max* L.) crop. They assessed the physiological parameters after the chemical application. Using the visual score system for efficacy and differences in normalized difference vegetation index (NDVI) cultivars tolerant or sensitive to bentazone were identified. This method can be potentially used to observe the influence on weed selection pressure and provide early indication into herbicide resistance occurrences, especially in the case of NTSR.

3.3.2 *Unraveling reproduction and fitness penalty*

Plant fitness is defined as the propensity of its reproductive success. After herbicide treatment, the fitness penalty is expressed in the progeny of surviving weed populations through reduced relative growth, photosynthetic activity, resource competition, and fecundity (Hawkins, Fraaije, 2018). Certain management options like herbicide rotation or the use of crop competition may have a greater influence in reducing resistance when a significant fitness penalty is present (Preston et al., 2009). This is because, in the absence of herbicide selection, a significant fitness penalty tends to preserve resistance genes at low frequency in populations and will eventually be diluted over time if the same herbicide is not used (Preston et al., 2009). It has been shown that glyphosate resistance carries a large fitness penalty and can be exploited by implementing integrated weed management programs Pedersen et al., 2007; Preston, Wakelin 2008; Preston et al., 2009). The fitness penalty is the main reason for prolonged resistance evolution to glyphosate despite its widespread use in cropping systems (Preston et al., 2009).

In a classic example of NTSR to glyphosate, smaller plant size and tolerance to low resource availability was reported in *L. rigidum* (Vila-Aiub et al., 2009). Another instance of direct fitness penalty has been reported in triazine NTRS-resistant plants. The substitution of serine to glycine resulted in reduced electron transfer to PS-II, consequently reducing photosynthesis rate and negatively affecting vegetative and reproductive growth (Devine, Shukla, 2000). Resistant mutations arising from either the subtle pleiotropic effects or adaptive response to management practices in agroecosystem can coevolve with increased seed dormancy and altered germination conditions (Vila-Aiub, 2019). These co-evolutions maximize the fitness of NTSR weed populations and promote the spread of resistance mutations (Vila-Aiub, 2019).

While herbicide-resistant weeds' fitness has been well studied, early-stage NTSR evolution and adaptive systems developed by herbicide-resistant plants during the selection process remain understudied (Ghanizadeh, Harrington, 2017b). Additionally, gaining meaningful estimates of fitness for NTSR polygenic resistance can be problematic due to the impact of multiple and typically unknown alleles (Comont et al., 2022). The widely accepted understanding highlights that phenotype fitness can be influenced by alleles governing physiological functions in plants (Orr, 2003). Frequency of such alleles may change over generations because of selective pressures, leading to physiological adaptations that allocate resources towards specific organs or functions, ultimately affecting fitness (Ghanizadeh, Harrington, 2017b). Although there is limited understanding of how the frequency of allele changes during the selection process to influence individual plant fitness, many traits influencing phenotype fitness are physiological responses driven by specific gene expression at developmental stages or under specific environmental conditions (Orr, 2003; Délye, 2013). Understanding the connections between genetic and non-genetic factors, such as phenotypic variability, epigenetics, and allele interactions, can offer further insights into how the fitness of resistant phenotypes is influenced (Ghanizadeh, Harrington, 2017b).

3.3.3 *Navigating the gene flow*

Genes define the weeds' morphological, physiological, and biochemical characteristics. Gene flow is defined as the process in which genes are transferred through gametes, diasporas, or individuals from one place to another (Golenburg, 1986). Gene flow by pollen or seed dispersal promotes genetic diversity, allows for gene mobility, and enables rapid transfer of evolutionary dynamics (Busi, 2011). Laforest et al. (2021) reported upregulated genes governing transmembrane transport and altered expression of genes linked to oxidative stress after trifloxysulfuron treatment in *Poa annua* L. ALS resistant biotypes were ABC-2 type transporter and a P450. Accumulation of these upregulated genes likely increased the chances of *P. annua* survival when exposed to different modes of action, hence, even in the presence of a target-site mutation, NTSR mechanisms remain a concern, as it can lead to cross-resistance (Laforest et al., 2021). Dispersal of herbicide-resistant genes can introduce herbicide-resistant alleles in previously herbicide susceptible populations (Busi, 2011).

Research on gene flow within agricultural ecosystems increased after the introduction of glyphosate-tolerant crops. While the adoption of these crops had numerous benefits, flow of herbicide resistance genes to wild/weedy species have been a major setback requiring strict environmental risk evaluations and stewardship (Beckie et al., 2016; Beckie et al., 2019). Busi (2011) suggested that actively preventing the selection of resistant weeds by implementing diverse weed control methods such as crop diversification and herbicide rotations can address the widespread evolution of resistance through inter-specific gene flow. Continued rise in herbicide resistance justifies the need to quantify the influence of gene flow on the rate and intensity of the spread of herbicide resistance (Busi, 2011; Laforest et al., 2021). The omics approach which involves the genomics, transcriptomics, proteomics, and metabolomics fields is used to identify NTSR genes responsible for cross-resistance patterns across different modes of action. It is encouraging to see that genomes of problematic herbicide resistant weeds like *A. tuberculatus*, *C. canadensis*, and *R. raphanistrum* have been obtained (Gaines et al., 2020). These high-quality genomes can be used to identify resistant genes through genetic mapping even if they are not over-expressed (Jamann et al., 2015).

4. A holistic approach to manage NTSR

The key areas that need to be studied to achieve a holistic approach to herbicide resistance include current weed management strategies practiced and how they influence weeds selection, exploration of alternative resistant weed control methods and the use of population models or modern technology to assess sustainable weed control strategies (Liu et al., 2018). NTSR mechanisms can evolve on herbicides that share a common degradation route (Jugulam, Shyam, 2019). NTSR may involve the accumulation of genes contributing to partial resistance levels; however, most mechanisms are not fully understood (González, Petrov, 2009; Franco-Ortega et al., 2021). Investigations on regulatory transcriptional networks in transcriptome datasets, which also aid in the identifying NTSR-endowing genes and the rate of evolution are a necessity (Preston et al., 2009; Busi, 2011; Franco-Ortega et al., 2021). If not proactively managed, the excessive use of herbicides will result in widespread resistance evolution to all modes of action in most weeds (Gage et al., 2019). Integrated weed management promotes a multi-faceted approach to weed control that includes prevention, seed bank management, modern technology, different cropping strategies, biological control, and guided herbicide use methods (Sharma et al., 2021; Westwood et al., 2018; Gage et al., 2019).

Although NTSR development involves complex processes, a full understanding of NTSR mechanisms allows the detection of genes that can be used as biotechnological tools to develop herbicide-resistant (HR) and parasitic weeds-resistant crops (Gill, Nestares, 2019). Modern omics-based technologies can play a vital role in mining of NTSR genes. Identification of NTSR alleles procedure is achieved in three main stages; i) conducting controlled crosses of selected weed species and segregating resistant plants from sensitive progeny ii) using a combination of time course and omics analysis to identify phenotype-related differences in gene expression and iii) using NTSR markers and functional characterization methods to validate the candidate alleles (Gill, Nestares, 2019). Recently genome acquisitions enabled the identification of NTSR genes through genetic mapping (Gaines et al., 2020; Jamann et al., 2015). However, omics research in weed science is challenging due to the management of complex datasets for many species with a diversity of biological and evolutionary traits (Maroli et al., 2018). These challenges are being addressed by exploiting next-generation sequencing data analysis which can be performed through public online platforms (Patterson et al., 2019). The prospect of developing tolerant crops from the NTSR mechanism needs extra precaution in assessing gene flow from crops to wild species (Beckie, 2020). Managing gene flow of resistant novel transgenic combinations requires the implementation of IWM strategies, thus, the ultimate herbicide resistance management principle lies in the adoption of sustainable guidelines.

Although the trend in weed management is shifting towards non-chemical control, chemical control cannot be fully ignored. It is therefore important to develop systems that can track pesticide performance and monitor the evolution of resistance. High-resolution aerial imagery and agronomic machine learning models are currently being used to manage herbicide resistance by predicting herbicide efficacy and its influence on weed selection pressure (Linn et al., 2019). Additionally, imaging can help in the early detection of potential NTSR threats (Linn et al., 2019). These models can be employed in conjunction with vegetation indices derived from hyperspectral imaging (Ali et al., 2022). A recent study successfully used NDVI and weed spectral resistance index (WSRI) to identify herbicide resistant biotypes (Xia et al., 2022). Additionally, soil maps and yield maps created from aerial imagery data are currently being employed to determine the variance in the field, based on soil health, plant health, vigor, and end-of-season production (Nackley et al., 2021). The digital geo-referenced zonal maps use an algorithm to develop a site-specific prescription map, which includes variable rate applications for each management zone. The precise application of herbicide dosage rates ensures effective control of weeds and, sequentially, lowering the severity of selection by preventing the survival of resistance biotypes and delaying NTSR (Nackely et al., 2021).

The development of system-oriented decision support systems aimed at reducing weed resistant population numbers while also limiting the huge number of herbicides being applied is now believed to be necessary (Van Ittersum et al., 2008; Beckie et al., 2019). Using decision support systems based on image analysis can be a great opportunity to digitalize the process, replace manual scouting and increase precision (Riemens et al., 2022). Model simulations have been developed to assist in drawing up simple or complex systems (Dogliotti, 2003; Manalil et al., 2011; Metzger et al., 2019; Lawrence et al., 2020). However, most of these models are normative and based on assumptions that the cropping systems are predictable. New crop planning decision models need to shift from a single normative approach to the use of biophysical cropping plans which can address the complexity of cropping systems (Van Ittersum et al., 2008; Gill, Nestares, 2019). A transdisciplinary approach to herbicide resistance management is urgently needed since it builds a dynamic scientific framework that integrates biological, economic, environmental, and social systems on weeds exhibiting high evolution rates. This approach can be catalyzed by integrating knowledge from key stakeholders including the farming, academic, industry and government sectors (Beckie et al., 2019). A rational, designed integrated SEAMLESS framework is illustrated by Van Ittersum et al. (2008), and shows that integrative assessment tools address sustainability issues in agricultural systems. Providing farmers with highly detailed frameworks and precise guidelines on cropping systems can increase the adoption of diverse models and precision technologies in weed NTSR resistance management.

5. Conclusions

Crop production systems that heavily rely on chemical weed control are vulnerable to the rapid evolution and spread of NTSR. Knowledge of mechanisms can assist in predicting anticipated herbicide evolution, however, the study on NTSR mechanisms has not been prioritized since they are more complex to decipher than TSR. More attention needs to be given to the study and modelling of gene mutations and gene flow as this directly influences the frequency of HR alleles in a population. Herbicide resistance management requires a diverse approach that integrates cultural, mechanical, physical, chemical, and biological strategies while leveraging modern technologies. Farmers are generally risk averse; hence the presence of proper cropping knowledge and guidelines will provide compelling insights into the economic benefits of adopting complex herbicide resistance management techniques on a large scale. The adoption of stewardship practices and optimal management strategies that disrupt the biology of weedy species will be vital in keeping pace with evolving NTSR herbicide problems.

Authors' contributions

All authors read and agreed to the accepted version of the manuscript. TM, JR, and AAB: conceptualization of the manuscript and development of the methodology. TM, and JR: writing the original draft of the manuscript. TM, JR, and AAB: writing, review, and editing.

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References

Ahmad L, Mahdi SS. Variable rate technology and variable rate application. In: Ahmad L, Mahdi SS, editors. Satellite farming: an information and technology based agriculture. Cham: Springer; 2018 [access September 22, 2023]. Available from: https://doi.org/10.1007/978-3-030-03448-1_5

Ali L, Jo H, Choi SM, Kim Y, Song JT, Lee JD. Comparison of hyperspectral imagery and physiological characteristics of bentazone-tolerant and -susceptible soybean cultivars. Agronomy. 2022;12(10):1-13. https://doi.org/10.3390/agronomy12102241

Anderson MP, Gronwald JW. Atrazine resistance in a velvetleaf (*Abutilon theophrasti*) biotype due to enhanced glutathione S-Transferase activity. Plant Physiol. 1991;96(1):104-9. Available from: https://doi.org/10.1104/pp.96.1.104

Baccin LC, Albrecht AJP, Pedroso RM, Araújo LS, Dotta MA, Victoria Filho R. Glyphosate-resistant hairy fleabane (*Conyza bonariensis*) exhibits a larger number of trichomes and altered stomatal density relative to the susceptible counterpart. Biosci J. 2023;(39):1-12. Available from: https://doi.org/10.14393/BJ-v39n0a2023-64343

Baerg RJ, Barrett M, Polge ND. Insecticide and insecticide metabolite interactions with cytochrome P450 mediated activities in maize. Pest Biochem Physiol. 1996;55(1):10-20. Available from: https://doi.org/10.1006/pest.1996.0030

Bajwa AA, Walsh M, Chauhan BS. Weed management using crop competition in Australia. Crop Prot. 2017;95:8-13. Available from: https://doi.org/10.1016/j.cropro.2016.08.021

Ball BC, Bingham I, Rees RM, Watson CA, Litterick A. The role of crop rotations in determining soil structure and crop growth conditions. Can J Soil Sci. 2005;85(5):557-77. Available from: https://doi.org/10.4141/S04-078

Baucom RS. Evolutionary and ecological insights from herbicide-resistant weeds: what have we learned about plant adaptation, and what is left to uncover? New Phytol. 2019;223(1):68-82. Available from: https://doi.org/10.1111/nph.15723

Beckie HJ, Blackshaw RE, Hall LM, Johnson EN. Pollen- and seed-mediated gene flow in Kochia (*Kochia scoparia*). Weed Sci. 2016;64(4):624-33. https://doi.org/10.1614/WS-D-16-00038.1

Beckie HJ, Busi R, Bagavathiannan V, Martin SL. Herbicide resistance gene flow in weeds: under-estimated and under-appreciated. Agric Ecosys Env. 2019;283. Available from: https://doi.org/10.1016/j.agee.2019.06.005

Beckie HJ, Reboud X. Selecting for weed resistance: herbicide rotation and mixture. Weed Technol. 2009;23(3):363-70. Available from: https://doi.org/10.1614/WT-09-008.1.

Beckie HJ. Herbicide resistance in plants. Plants. 2020;9(4):1-4. Available from: https://doi.org/10.3390/plants9040435

Beckie HJ. Herbicide-resistant weeds: management tactics and practices. Weed Technol. 2006;20(3):793-814. Available from: https://doi.org/10.1614/WT-05-084R1.1

Belz RG. Low herbicide doses can change the responses of weeds to subsequent treatments in the next generation: metamitron exposed PSII-target-site resistant *Chenopodium album* as a case study. Pest Manag Sci. 2020;76(9):3056-65. Available from: https://doi.org/10.1002/ps.5856

Birch CPD, Shaw MW. When can reduced doses and pesticide mixtures delay the build-up of pesticide resistance? A mathematical model. J App Ecol. 1997;34(4):1032-42. Available from: https://doi.org/10.2307/2405292

Burnet MWM, Loveys BR, Holtum JAM, Powles SB. Increased detoxification is a mechanism of simazine resistance in *Lolium rigidum*. Pest Bioch Physiol. 1993;46(3):207-18. Available from: https://doi.org/10.1006/pest.1993.1052

Busi R, Powles SB, Beckie HJ, Renton M. Rotations and mixtures of soil-applied herbicides delay resistance. Pest Manag Sci. 2020;76(2):487-96. Available from: https://doi.org/10.1002/ps.5534

Busi R. Gene flow increases the initial frequency of herbicide resistance alleles in unselected *Lolium rigidum* populations. Agric Ecosys Env. 2011;142(3-4):403-9. Available from: https://doi.org/10.1016/j.agee.2011.06.012

Comont D, MacGregor DR, Crook L, Hull R, Nguyen L, Freckleton RP et al. Dissecting weed adaptation: Fitness and trait correlations in herbicide-resistant *Alopecurus myosuroides*. Pest Manag Sci. 2022;78(7):3039-50. Available from: https://doi.org/10.1002/ps.6930

Comont D, Neve P. Adopting epidemiological approaches for herbicide resistance monitoring and management. Weed Res. 2021;61(2):81-7. Available from: https://doi. org/10.1111/wre.12420

Dang HT, Malone JM, Boutsalis P, Krishnan M, Gill G, Preston C. Reduced translocation in 2,4-D-resistant oriental mustard populations (*Sisymbrium orientale* L.) from Australia. Pest Manag Sci. 2018;74(6):1524-32. Available from: https://doi.org/10.1002/ps.4845

Davis AS, Hill JD, Chase CA, Johanns AM, Liebman M. Increasing cropping system diversity balances productivity, profitability and environmental health. PLoS ONE. 2012;7(10):1-8. Available from: https://doi.org/10.1371/journal.pone.0047149

Délye C. Unravelling the genetic bases of non-target-site-based resistance (NTSR) to herbicides: a major challenge for weed science in the forthcoming decade. Pest Manag Sci. 2013;69(2):176-87. Available from: https://doi.org/10.1002/ps.3318

Devine MD, Shukla A. Altered target sites as a mechanism of herbicide resistance. Crop Protect. 2000;19(8-10):881-9. Available from: https://doi.org/10.1016/S0261-2194(00)00123-X

Dogliotti S. ROTAT, a tool for systematically generating crop rotations. Euro J Agron. 2003;19(2):239-50. Available from: https://doi.org/10.1016/S1161-0301(02)00047-3

Domínguez-Valenzuela JA, Alcántara-de la Cruz R, Palma-Bautista C, Vázquez-García JG, Cruz-Hipolito HE, Prado R. Non-target site mechanisms endow resistance to glyphosate in saltmarsh aster (*Aster squamatus*). Plants. 2021;10(9):1-12. Available from: https://doi.org/10.3390/plants10091970

Dominguez-Valenzuela JA, Gherekhloo J, Fernandez-Moreno PT, Cruz-Hipolito HE, Cruz RAL, Sanchez-Gonalez E et al. First confirmation and characterization of target and non-target site resistance to glyphosate in Palmer amaranth (*Amaranthus palmeri*) from Mexico. Plant Physiol Biochem. 2017;115:212-8. Available from: https://doi.org/10.1016/j.plaphy.2017.03.022

Dury J, Schaller N, Garcia F, Reynaud A, Bergez J. Models to support cropping plan and crop rotation decisions: a review. Agron Sust Dev. 2012;32:567-80. Available from: https://doi.org/10.1007/s13593-011-0037-x

Figueiredo MR, Leibhart LJ, Reicher ZJ, Tranel PJ, Nissen SJ, Westra P et al. Metabolism of 2,4-dichlorophenoxyacetic acid contributes to resistance in a common waterhemp (*Amaranthus tuberculatus*) population. Pest Manag Sci. 2018;74(10):2356-62. Available from: https://doi.org/10.1002/ps.4811

Franco-Ortega S, Goldberg-Cavalleri A, Walker A, Brazier-Hicks M, Onkokesung N, Edwards R. Non-target site herbicide resistance is conferred by two distinct mechanisms in black-grass (*Alopecurus myosuroides*). Front Plant Sci. 2021;3:1-16. Available from: https://doi.org/10.3389/fpls.2021.636652

Gage KL, Krausz RF, Walters SA. Emerging challenges for weed management in herbicide-resistant crops. Agriculture. 2019;9(8):1-11. Available from: https://doi.org/10.3390/agriculture9080180

Gaines TA, Duke ST, Morran S, Rigon CAG, Tranel PJ, Kupper A, Dayan FE. Mechanisms of evolved herbicide resistance. J Biol Chem. 2020;295(30):10307-30. Available from: https://doi.org/10.1074/jbc.REV120.013572

Galvani J, Rizzardi MA, Carneiro CM, Bianchi MA. Leaf anatomy of *Lolium multiflorum* sensitive and resistant to glyphosate. Planta Daninha. 2012;30(2):407-13. Available from: https://doi.org/10.1590/S0100-83582012000200021

Genna NG, Gourlie JA, Barroso J. Herbicide efficacy of spot spraying systems in fallow and postharvest in the pacific northwest dryland wheat production region. Plants. 2021;10(12):1-14. Available from: https://doi.org/10.3390/plants10122725

Ghanizadeh H, Harrington KC, James TK. Glyphosate-resistant population of *Lolium perenne* loses resistance at winter temperatures. New Zealand J Agric Res. 2015;58(4):423-31. Available from: https://doi.org/10.1080/00288233.2015.1076490.

Ghanizadeh H, Harrington KC. Non-target site mechanisms of resistance to herbicides. Crit Rev Plant Sci. 2017a;36(1):24-34, Available from: https://doi.org/10.1080/07352689.2017.1316134

Ghanizadeh H, Harrington KC. Perspectives on non-target site mechanisms of herbicide resistance in weedy plant species using evolutionary physiology. AoB Plants. 2017b;9(5):1-5. Available from: https://doi.org/10.1093/aobpla/plx035

Gill M, Nestares G. Decoding non-target-site herbicide resistance in sunflower: the beginning of the story. Helia. 2019;42(70):1-16. https://doi.org/10.1515/helia-2019-0002

Goggin DE, Cawthray GR, Powles SB. 2,4-D resistance in wild radish: reduced herbicide translocation via inhibition of cellular transport. J Exp Bot. 2016;67(11):3223-35. Available from: https://doi.org/10.1093/jxb/erw120

Goldberg-Cavalleri A, Onkokesung N, Franco-Ortega S, Edwards R. ABC transporters linked to multiple herbicide resistance in blackgrass (*Alopecurus Myosuroides*). Front Plant Sci. 2023;(14):1-18. Available from: https://doi.org/10.3389/fpls.2023.1082761

Golenburg EM. Estimation of gene flow and genetic neighbourhood size by indirect methods in selfing annual *Triticum dicoccoides*. Evolution. 1986;41(6):1326-34. Available from: https://doi.org/10.2307/2409097

González J, Petrov DA. The adaptive role of transposable elements in the Drosophila genome. Gene. 2009;448(2):124-33. Available from: https://doi.org/10.1016/j.gene.2009.06.008

Gressel J, Segel L. Modelling the effectiveness of herbicide rotations and mixtures as strategies to delay or preclude resistance. Weed Technol. 1990;4(1):186-98. Available from: https://doi.org/10.1017/S0890037X00025215

Han H, Yu Q, Cawthray GR, Powles SB. Enhanced herbicide metabolism induced by 2,4-D in herbicide susceptible *Lolium rigidum* provides protection against diclofop-methyl. Pest Manag Sci. 2013;69(9):996-1000. Available from: https://doi.org/10.1002/ps.3552

Hawkins NJ, Fraaije BA. Fitness penalties in the evolution of fungicide resistance. Ann Rev Phyto. 2018;56:339-60. Available from: https://doi.org/10.1146/annurev-phyto-080417-050012

Jablonkai I. Herbicide metabolism in weeds: selectivity and herbicide resistance. In: Price A, Kelton J, Sarunaite L, editors. Herbicides, physiology of action, and safety. London: InTechOpen; 2015[access September 22, 2023. Available from: https://doi.org/10.5772/61674.

Jamann TM, Balint-Kurti PJ, Holland JB. QTL mapping using high-throughput sequencing. In: Alonso J, Stepanova A, editors. Plant functional genomics: methods in molecular biology. New York: Humana; 2015[access January 4, 2024]. p. 257-85. Available from: https://doi.org/10.1007/978-1-4939-2444-8_13

Jarzyniak KM, Jasiński M. Membrane transporters and drought resistance: a complex issue. Front Plant Sci. 2014;5:1-15. Available from: https://doi.org/10.3389/fpls.2014.00687

Jhala AJ, Ramirez AHM, Knezevic SZ, Van Damme P, Singh M. Herbicide tank mixtures for broad-spectrum weed control in Florida citrus. Weed Technol. 2013;27(1):129-37. Available from: https://doi.org/10.1614/WT-D-12-00105.1

Johnson NM, Baucom RS. The double life of trichomes: understanding their dual role in herbivory and herbicide resistance. BioRxiv. 2023;1-27. Available from: https://doi.org/10.1101/2023.04.28.538721

Jugulam M, Shyam C. Non-target-site resistance to herbicides: recent developments. Plants. 2019;8(10):1-16. Available from: https://doi.org/10.3390/plants8100417

Kohler EA, Throssell CS, Reicher ZJ. 2,4-D rate response, absorption, and translocation of two ground ivy (*Glechoma hederacea*) populations. Weed Technol. 2004;18(4):917-23. Available from: https://doi.org/10.1614/WT-03-089R1

Kountche BA, Jamil M, Yonli D, Nikiema MP, Blanco-Ania D, Asami T et al. Suicidal germination as a control strategy for *Striga hermonthica* (Benth.) in smallholder farms of sub-Saharan Africa. Plant People Planet. 2019;1(2):107-18. Available from: https://doi.org/10.1002/ppp3.32

Kreiner JM, Stinchcombe JR, Wright SI. Population genomics of herbicide resistance: adaptation via evolutionary rescue. Ann Rev Plant Biol. 2018;69:611-35. Available from: https://doi.org/10.1146/annurev-arplant-042817-040038

Kudsk P. Optimising herbicide dose: a straightforward approach to reduce the risk of side effects of herbicides. Environmentalist. 2008;28:49-55. Available from: https://doi.org/10.1007/s10669-007-9041-8

Laforest M, Soufiane B, Patterson EL, Vargas JJ, Boggess SL, Houston LC et al. Differential expression of genes associated with non-target site resistance in *Poa annua* with target site resistance to acetolactate synthase inhibitors. Pest Manag Sci. 2021;77(11):4993-5000. Available from: https://doi.org/10.1002/ps.6541

Lawrence N, Adjesiwor A, Kniss A. Herbicide resistance risk calculator: a new resistance management tool. CropWatch. Oct 29, 2020[access March 13, 2023]. Available from: https://cropwatch.unl.edu/2020/herbicide-resistance-risk-calculator-new-resistance-management-tool

Lee LJ, Ngim J. A first report of glyphosate-resistant goosegrass (*Eleusine indica* (L) Gaertn) in Malaysia. Pest Manag Sci. 2000;56(4):336-9. Available from: https://doi.org/10.1002/(SICI)1526-4998(200004)56:4<336::AID-PS123>3.0.CO;2-8

Linn A, Mink R, Peteinatos G, Gerhards R. In-field classification of herbicide-resistant *Papaver rhoeas* and *Stellaria media* using an imaging sensor of the maximum quantum efficiency of photosystem II. Weed Res. 2019;59(5):357-66. Available from: https://doi.org/10.1111/wre.12374

Liu W, Bai S, Zhao S, Li W, Zhang L, Wang J. Non-target site-based resistance to tribenuron-methyl and essential involved genes in *Myosoton aquaticum* (L.). BMC Plant Biol. 2018;18:1-14. Available from: https://doi.org/10.1186/s12870-018-1451-x

Lorraine-Colwill D, Powles S, Hawkes T, Preston C. Inheritance of evolved glyphosate resistance in *Lolium rigidum* (Gaud.). Theor Appl Gen. 2001;102:545-50. Available from: https://doi.org/10.1007/s001220051680

MacLaren C, Storkey J, Menegat A, Helen Metcalfe H, Dehnen-Schmutz K. An ecological future for weed science to sustain crop production and the environment: a review. Agron Sustain Develop. 2020;24(40):1-29. Available from: https://doi.org/10.1007/s13593-020-00631-6

Mallory-Smith CA, Retzinger Jr EJ. Revised classification of herbicides by site of action for weed resistance management strategies. Weed Technol. 2003;17(3):605-19.

Manalil S, Busi R, Renton M, Powles S. Rapid evolution of herbicide resistance by low herbicide dosages. Weed Sci. 2011;59(2):210-7. Available from: https://doi.org/10.1614/WS-D-10-00111.1

Maroli AS, Gaines TA, Foley ME, Duke SO, Doğramacı M, Anderson JV et al. Omics in weed science: a perspective from genomics, transcriptomics, and metabolomics approaches. Weed Sci. 2017;66(6):681-95. Available from: https://doi.org/10.1017/wsc.2018.33

Matzrafi M, Seiwert B, Reemtsma T, Rubin B, Peleg Z. Climate change increases the risk of herbicide-resistant weeds due to enhanced detoxification. Planta. 2016;244:1217-27. Available from: https://doi.org/10.1007/s00425-016-2577-4

Metzger BA, Soltani N, Raeder AJ, Hooker DC, Robinson DE, Sikkema PH. Influence of application timing and herbicide rate on the efficacy of tolpyralate plus atrazine. Weed Technol. 2019;33(3):448-58. Available from: https://doi.org/10.1017/wet.2019.25

Michitte P, Prado R, Espinoza N, Ruiz-Santaella JP, Gauvrit C. Mechanisms of resistance to glyphosate in a ryegrass (*Lolium multiflorum*) biotype from Chile. Weed Sci. 2007;55(5):435-40. Available from: https://doi.org/10.1614/WS-06-167.1

Mohr K, Sellers BA, Smeda RJ. Application time of day influences glyphosate efficacy. Weed Technol. 2007;21(1):7-13. Available from: https://doi.org/10.1614/WT-04-251.1

Mollaee M, Matloob A, Mobli A, Thompson M, Chauhan BS. Response of glyphosate-resistant and susceptible biotypes of *Echinochloa colona* to low doses of glyphosate in different soil moisture conditions. PLoS ONE. 2020;15(5):1-16. Available from: https://doi.org/10.1371/journal.pone.0233428

Moretti ML, Hanson BD. Reduced translocation is involved in resistance to glyphosate and paraquat in *Conyza bonariensis* and *Conyza canadensis* from California. Weed Res. 2017;57(1):25-34. Available from: https://doi.org/10.1111/wre.12230

Moretti ML, Van Horn CR, Robertson R, Segobye K, Weller SC, Young BG et al. Glyphosate resistance in *Ambrosia trifida*: part 2: rapid response physiology and non-target-site resistance. Pest Manag Sci. 2018;74(5):1079-88. Available from: https://doi.org/10.1002/ps.4569

Nackley LL, Warneke B, Fessler L, Pscheidt JW, Lockwood D, Wright WC et al. Variable-rate spray technology optimizes pesticide application by adjusting for seasonal shifts in deciduous perennial crops. Hort Technol. 2021;31(4),479-89. Available from: https://doi.org/10.21273/HORTTECH04794-21

Nakka S, Godar AS, Thompson CR, Peterson DE, Jugulam M. Rapid detoxification via glutathione S-transferase (GST) conjugation confers a high level of atrazine resistance in Palmer amaranth (*Amaranthus palmeri*). Pest Manag Sci. 2017;73(11):2236-43. Available from: https://doi.org/10.1002/ps.4615

Nandula VK, Ray JD, Ribeiro DN, Pan Z, Reddy KN. Glyphosate resistance in tall waterhemp (*Amaranthus tuberculatus*) from Mississippi is due to both altered target-site and nontarget-site mechanisms. Weed Sci. 2013;61(3):374-83. Available from: https://doi.org/10.1614/WS-D-12-00155.1

Norsworthy J, Ward S, Shaw D, Llewellyn R, Nichols R, Webster T et al. Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci. 2012;60(sp1):31-62. Available from: https://doi.org/10.1614/WS-D-11-00155.1

Ofosu R, Agyemang ED, Márton A, Pásztor G, Taller J, Kazinczi G. Herbicide resistance: managing weeds in a changing world. Agronomy. 2023;13(6):1-16. Available from: https://doi.org/10.3390/agronomy13061595

Orr HA. The distribution of fitness effects among beneficial mutations. Genetics. 2003;4(163):1519-26. Available from: https://doi.org/10.1093/genetics/163.4.1519

Patterson EL, Saski C, Küpper A, Beffa R, Gaines TA. Omics potential in herbicide-resistant weed management. Plants. 2019;8(12):1-14. Available from: https://doi.org/10.3390/plants8120607

Pedersen BP, Neve P, Andreasen C, Powles SB. Ecological fitness of a glyphosate-resistant *Lolium rigidum* population: growth and seed production along a competition gradient. Basic Appl Ecol. 2007;8(3):258-68. Available from: https://doi.org/10.1016/j.baae.2006.01.002

Pereira VGC. Characterization of *Conyza sumatrensis* resistance to paraquat herbicide [thesis]. Botucatu: Universidade Estadual Paulista; 2019.

Petersen J, Hurle K. Influence of climatic conditions and plant physiology on glufosinate ammonium efficacy. Weed Res. 2000;41(1):31-9. Available from: https://doi.org/10.1046/j.1365-3180.2001.00214.x

Powles SB, Matthews JM. Multiple herbicide resistance in annual ryegrass (*Lolium rigidum*): a driving force for the adoption of integrated weed management. In: Denholm I, Devonshire AL, Hollomon DW, editors. Resistance '91: achievements and developments in combating pesticide resistance. Cham: Springer; 1992 [access March 13, 2023]. Available from: https://doi.org/10.1007/978-94-011-2862-9_7

Powles SB, Yu Q. Evolution in action: plants resistant to herbicides. Ann Rev Plant Biol. 2010;61:317-47. Available from: https://doi.org/10.1146/annurev-arplant-042809-112119

Prado JL, Osuna MD, Heredia A, Prado R. *Lolium rigidum*, a pool of resistance mechanisms to ACCase inhibitor herbicides. J Agric Food Chem. 2005;53(6):2185-91. Available from: https://doi.org/10.1021/jf049481m

Preston C, Wakelin AM, Dolman FC, Bostamam Y, Boutsalis P. A decade of glyphosate-resistant *Lolium* around the world: mechanisms, genes, fitness, and agronomic management. Weed Sci. 2009;57(4):435-41. Available from: https://doi.org/10.1614/WS-08-181.1

Preston C, Wakelin AM. Resistance to glyphosate from altered herbicide translocation patterns. Pest Manag Sci. 2008;64(4):372-6. Available from: https://doi.org/10.1002/ps.1489

Preston CM, Holtum JA, Powles SB. On the mechanism of resistance to paraquat in *Hordeum glaucum* and *H. Leporinum*: delayed inhibition of photosynthetic O_2 evolution after paraquat application. Plant Physiol. 1992;100(2):630-6. Available from: https://doi.org/10.1104/pp.100.2.630

Queiroz A, Delatorre C, Lucio F, Rossi C, Zobiole L, Merotto A. Rapid necrosis: a novel plant resistance mechanism to 2,4-D. Weed Sci. 2020;68(1):6-18. Available from: https://doi.org/10.1017/wsc.2019.65

Riar DS, Burke IC, Yenish JP, Bell J, Gill K. Inheritance and physiological basis for 2,4-D resistance in prickly lettuce (*Lactuca serriola* L.). J Agric Food Chem. 2011;59(17):9417-23. Available from: https://doi.org/10.1021/jf2019616

Riechers DE, Kreuz K, Zhang Q. Detoxification without intoxication: herbicide safeners activate plant defense gene expression. Plant Physiol. 2010;153(1):3-13. Available from: https://doi.org/10.1104/pp.110.153601

Riemens M, Sønderskov M, Moonen A, Storkey J, Kudsk P. An integrated weed management framework: a pan-european perspective. Eur J Agron. 2022;133. Available from: https://doi.org/10.1016/j.eja.2021.126443

Rigon C, Gaines T, Kuepper A, Dayan F. Metabolism-based herbicide resistance, the major threat among the non-target site resistance mechanisms. Out Pest Manag. 2020;31(4):162-8. Available from: https://doi.org/10.1564/v31_aug_04

Rouhi H. Suitable crop rotation results in effective weed control in potato field. Ann Biol Res. 2013;4:318-26.

Schuster AC. Chemical and functional analyses of the plant cuticle as leaf transpiration barrier [thesis]. Würzburg: Julius-Maximilians-Universität Würzburg; 2016 [access June 22, 2017]. Available from: https://d-nb.info/111188868X/34

Sharma G, Shrestha S, Kunwar S, Tseng T-M. Crop diversification for improved weed management: a review. Agriculture. 2021;11(5):1-17. Available from: https://doi.org/10.3390/agriculture11050461

Suzukawa AK, Bobadilla LK, Mallory-Smith C, Brunharo CACG. Non-target-site resistance in *Lolium* spp. globally: a review. Front Plant Sci. 2021;11:1-17. Available from: https://doi.org/10.3389/fpls.2020.609209

Svyantek A, Aldahir P, Chen S, Flessner M, McCullough P, Sidhu S et al. Target and nontarget resistance mechanisms induce annual bluegrass (*Poa annua*) resistance to atrazine, amicarbazone, and diuron. Weed Technol. 2016;30(3):773-82. Available from: https://doi.org/10.1614/WT-D-15-00173.1

Tranel P, Wright TR. Resistance of weeds to ALS-Inhibiting herbicides: what have we learned? Weed Sci. 2002;50(6):700-12. Available from: https://doi.org/10.1614/0043-1745(2002)050[0700:RROWTA]2.0.CO;2

Valverde BE. Status and management of grass-weed herbicide resistance in Latin America. Weed Technol. 2007;21(2):310-23. Available from: https://doi.org/10.1614/WT-06-097.1

Van Ittersum MK, Ewert F, Heckelei T, Wery J, Olsson JA, Andersen E et al. Integrated assessment of agricultural systems: a component-based framework for the European Union (Seamless). Agricult Syst. 2008;96(1-3):150-65. Available from: https://doi.org/10.1016/j.agsy.2007.07.009

Varanasi A, Prasad PVV, Jugulam M. Impact of climate change factors on weeds and herbicide efficacy. Adv Agric. 2016;135:107-46. Available from: https://doi.org/10.1016/bs.agron.2015.09.002

Varanasi V, Brabham C, Korres N, Norsworthy J. Nontarget site resistance in Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] confers cross-resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Technol. 2019;33(2):349-54. Available from: https://doi.org/10.1017/wet.2019.12

Vila-Aiub MM, Balbi MC, Distéfano AJ, Fernandez L, Hopp HE, Yu Q et al. Glyphosate resistance in perennial *Sorghum halepense* (Johnsongrass), endowed by reduced glyphosate translocation and leaf uptake. Pest Manag Sci. 2012;68(3):430-6. Available from: https://doi.org/10.1002/ps.2286

Vila-Aiub MM, Neve P, Powles SB. Evidence for an ecological cost of enhanced herbicide metabolism in *Lolium rigidum*. J Ecol. 2009;97(4):772-80. https://doi.org/10.1111/j.1365-2745.2009.01511.x

Vila-Aiub MM. Fitness of herbicide-resistant weeds: current knowledge and implications for management. Plants. 2019;8(11):1-11. Available from: https://doi.org/10.3390/plants8110469

Westwood JH, Charudattan R, Duke SO, Fennimore SA, Marrone P, Slaughter DC et al. Weed Management in 2050: perspectives on the future of weed science. Weed Sci. 2018;66(3):275-85. Available from: https://doi.org/10.1017/wsc.2017.78

Xia F, Longzhe Q, Zhaoxia L, Deng S, Hailong L, Xiaolan L. Identification and comprehensive evaluation of resistant weeds using unmanned aerial vehicle-based multispectral imagery. Front Plant Sci. 2022;13:1-15. Available from: https://doi.org/10.3389/fpls.2022.938604

Yu Q, Huang S, Powles S. Direct measurement of paraquat in leaf protoplasts indicates vacuolar paraquat sequestration as a resistance mechanism in *Lolium rigidum*. Pest Biochem Physiol. 2010;98(1):104-9. Available from: https://doi.org/10.1016/j.pestbp.2010.05.007

Yu Q, Powles S. Metabolism-based herbicide resistance and cross-resistance in crop weeds: a threat to herbicide sustainability and global crop production. Plant Physiol. 2014;166(3):1106-18. Available from: https://doi.org/10.1104/pp.114.242750

Yuan JS, Tranel PJ, Stewart JR. Non-target-site herbicide resistance: a family business. Trends Plant Sci. 2007;12(1):6-13. Available from: https://doi.org/10.1016/j.tplants.2006.11.001

Zhang Y, Song H, Wang X, Zhou X, Zhang K, Chen X et al. The roles of different types of trichomes in tomato resistance to cold, drought, whiteflies, and botrytis. Agronomy. 2020;10(3):1-16. Available from: https://doi.org/10.3390/agronomy10030411

Zhao N, Yan Y, Ge L, Zhu B, Liu W, Wang J. Target site mutations and cytochrome P450s confer resistance to fenoxaprop-P-ethyl and mesosulfuron-methyl in *Alopecurus aequalis*. Pest Manag Sci. 2019;75(1):204-14. Available from: https://doi.org/10.1002/ps.5089

Zwanenburg B, Mwakaboko AS, Kannan C. Suicidal germination for parasitic weed control. Pest Manag Sci. 2016;72(11):2016-25. Available from: https://doi.org/10.1002/ps.4222